Development and Validation of RP-HPLC Method for Quantitation of Clarithromycin in Matrix Tablet Dosage Form

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ABSTRACT: This study was aimed to develop a simple, sensitive and rapid procedure for the analysis of clarithromycin in pure as well as in matrix tablet dosage form by using RP-HPLC method. The chromatographic separation was achieved by a reversed phase C_{18} column (150 mm length × 4.6 mm i.d., 5 µm particle size) in an isocratic mode with mobile phase comprising of acetonitrile and 0.035 M potassium dihydrogen phosphate (pH 4.4 ± 0.017) in a ratio of (55: 45, v/v). The eluent was pumped at a flow rate of 0.6 ml/min and the effluent was monitored using UV detector at 210 nm. The method was validated according to the ICH guidelines with respect to linearity, precision, accuracy, selectivity, specificity, ruggedness and robustness. It was found to be linear over the concentration range of 320- 480 µg/ml (R^2= 0.9993) with detection limit of 0.04 µg/mL. Considering the specifications of this method, the system was found to be suitable for rapid and routine analysis of clarithromycin in pure and matrix tablet dosage form.

Key words: Clarithromycin, chromatographic procedure, RP-HPLC, validation

INTRODUCTION

Clarithromycin is a semi-synthetic macrolide antibiotic derived from erythromycin A. It consists of a 14 membered lactone ring as well as cladinose and desosamine residues at positions 3 and 5 of the ring, respectively. Like erythromycin, it has no conjugated double bond in the lactone ring, hence significant UV absorbance is only obtained at wavelengths below 210 nm.[1-2] It is white or almost white crystalline powder, practically insoluble in water, slightly soluble in alcohol and acetonitrile and freely soluble in acetone. It may be bacteriostatic or bactericidal depending on the organism and drug concentration and it mainly acts by inhibiting bacterial protein synthesis. The drug is used to treat pharyngitis, tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, pneumonia, skin infections, etc.[3]

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Literature survey revealed that various methods have been developed for the estimation of clarithromycin from laboratory prepared mixtures, pharmaceutical preparations and biological matrices (such as human plasma) through automated solid phase extraction and electrochemical detection, liquid chromatographic electrospray tandem mass spectrometry and high performance liquid chromatography.[4-7] But most of these reported methods were cumbersome due to poor resolution, troublesome mobile phase (buffers) preparation and lengthy run time, hence difficult for routine analysis. Therefore, the aim of the present work was to establish a rapid, simple, precise, specific, accurate, and cost-effective RP-HPLC method according to ICH guidelines Q2 (R1) for routine analysis of clarithromycin in pharmaceutical formulations.

MATERIALS AND METHODS

Chemicals and reagents. Clarithromycin powder was provided as generous gift from Incepta

Pharmaceuticals Limited, Bangladesh. HPMC K100M CR, HPMC K15M CR, and avicel PH 102 were purchased from Colorcon Asia Pvt. Ltd. (USA). Magnesium stearate and talc were collected from Wilfrid Smith Ltd. (UK). HPLC grade acetonitrile was procured from RCI Labscan Ltd. (Thailand). Analytical grade potassium dihydrogen phosphate was purchased from BDH Chemicals (England). HPLC grade water was prepared from Millipore Milli-Q water purification system from Bedford, MA, USA. All other materials and reagents were of analytical grade.

Preparation of clarithromycin matrix tablets and placebo tablets. For manufacturing the matrix tablets, drug (clarithromycin 500 mg as API), polymer and other excipients were weighed separately for 30 tablets. The active ingredient, polymer, avicel PH 102 and magnesium stearate were blended properly for 15 minutes. Then aerosil 200 was added to the mixture and blended again for about 1 minute, and the mass thus obtained was passed through a no. 40 sized mesh. Then by direct compression the tablet was prepared in a single punch mini compress machine using "D"- tooling of punch size 11.1 mm in diameter (round shape) and was adjusted to get the desired weight of the tablet (1100 mg). Placebo tablets were prepared by following the same order and technology as mentioned above excluding the active drug.

HPLC instrumentation and chromatographic parameters. The chromatographic system used for the investigation was Shimadzu LC- 20AT (Kyoto, Japan), equipped with UV/visible detector (Shimadzu SPD- 20A), degasser (Shimadzu DGU- 20A3), manual injector (Rheodyne, USA) and software (LC solution). The chromatographic analysis was performed in an isocratic separation mode by a Capcell Pak C_{18} column (150 mm × 4.6 mmi.d., 5 µm particle size). The mobile phase was a homogenous mixture of acetonitrile and potassium dihydrogen phosphate (0.035 M) in the ratio of (55: 45, v/v) at pH 4.4 ± 0.017, pumped at a flow rate of 0.6 ml/min and the effluent was monitored at wavelength 210 nm. The injection volume was 20 µl and the run time was about 6 min as the retention time of clarithromycin was found about 4.1 min.

Preparation of solutions

Preparation of mobile phase. Accurately measured 4.76 gm of potassium dihydrogen phosphate (0.035 M) was taken into a 1000 ml volumetric flask and diluted with distilled water up to the mark. Then 450 ml of this solution was mixed with 550 ml of HPLC grade acetonitrile and mixed well. The resulting solution was filtered using 0.45 µm filter.

Preparation of stock solution. 100 mg reference standard of clarithromycin was properly weighed and transferred to a clean and dry 25 ml volumetric flask. About 15 ml of mobile phase was added and sonicated for 5 minutes for complete dissolution of the drug. The solution was allowed to cool at room temperature and then the volume was made up to the mark with the mobile phase. Thus the concentration of resulting standard solution was 4000 µg/ml. The five different concentrations (320, 360, 400, 440 and 480 µg/ml) of standard solution were prepared with proper dilution with the mobile phase.

Preparation of sample solution. To prepare assay solution, powdered sample equivalent to 100 mg of Clarithromycin was weighed properly and prepared in the same manner as the preparation procedure of stock solution.

Validation of the test procedure

Method validation study was performed based on the current pharmaceutical regulatory guidelines.[8-10]

System suitability. For the evaluation of system suitability, the peak area, tailing factor, theoretical plate and retention time of six replicate injections of working standard solution of clarithromycin (400 µg/ml) were used and % RSD values were calculated for each.

Linearity. Five different concentrations of standard solution ranging 320 - 480 µg/ml were analyzed. Calibration curve was made and the regression line was calculated as $Y = mX + c$, where

X was the concentration of standard and Y was the response (peak area expressed as AU).

Sensitivity. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated according to ICH Q2 (R1) recommendations considering signal to noise ratio of chromatogram. To perform this, base line parameter was checked and diluted standard solutions of clarithromycin were analysed. The concentration where the peak area of the chromatograms were about 3.3 times and 10 times higher than the signal to noise ratio were considered as the LOD and LOQ, respectively.

Accuracy (recovery test). Recovery test was done by analyzing standard solutions of drug substance and sample and the percent recoveries (mean ± % RSD of six replicates) were calculated.

Precision. Repeatability (intra-day precision) and intermediate precision (inter-day precision) of the methods were determined by using the solution of standard clarithromycin (400 µg/ml) and the solutions were analyzed in six replicates on the same day (intra-day precision) and daily for six times over a period of three days (inter-day precision).

Ruggedness. Ruggedness of the method was determined by analyzing six solutions of standard clarithromycin (400 µg/ml) by two analysts in two different laboratories to check the reproducibility of the test results. The percentage recovery and % RSD were calculated in both cases.

Robustness. To determine the robustness of the method, different flow rate and composition of the mobile phase and the pH of the buffer solution were introduced. The % RSD of robustness testing under these conditions was calculated in all cases.

RESULTS AND DISCUSSION

Validation of the method

System suitability. The results (mean ± %RSD of six replicates) of the chromatographic parameters are shown in table 1 indicating the good performance of the system.

Table 1. System suitability parameters.

Parameters	Value (Mean ± % RSD)
Peak area	952473.6 ± 0.027
Tailing factor	1.206 ± 0.837
Theoretical plate	5820 ± 0.189
Retention time	4.100 ± 0.074

Specificity. A good resolution was obtained between the drug substance and the drug product through the developed chromatographic method (Figures 2, 3 and 4).

Linearity. The calibration curve (figure 5) was obtained using the linear least square regression procedure at five different concentration levels from 320- 480 µg/ml (80- 120% of the nominal concentration of 400 µg/ml) by plotting average peak area against the concentrations. The representative linear equation was Y = 2411.8X – 13879 and the correlation coefficient (R^2) value was found to be 0.9993.

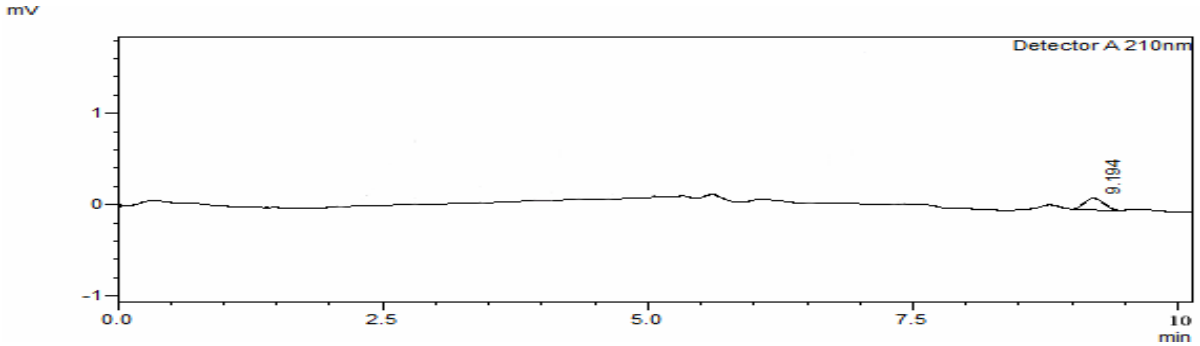

Figure 2. Chromatogram of placebo matrix tablet of clarithromycin

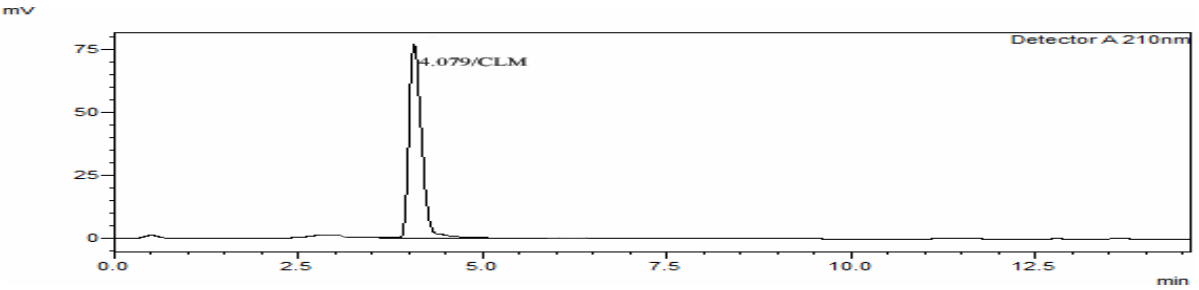

Figure 3. Chromatogram of standard solution of clarithromycin (400 µg/ml)

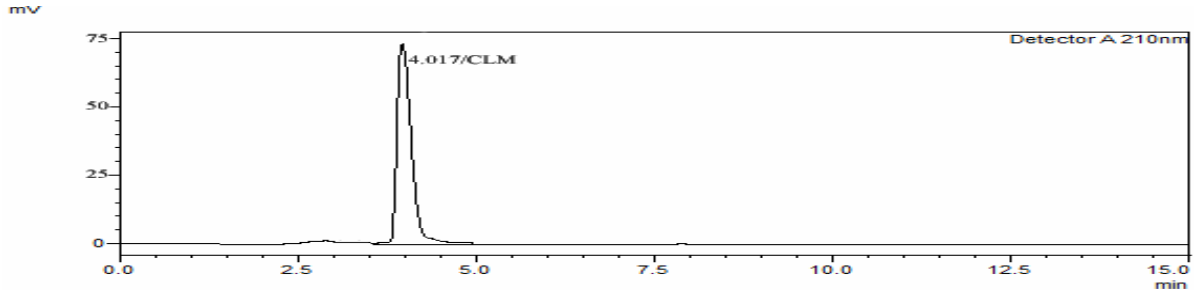

Figure 4. Chromatogram of sample solution of clarithromycin matrix tablet (400 µg/ml)

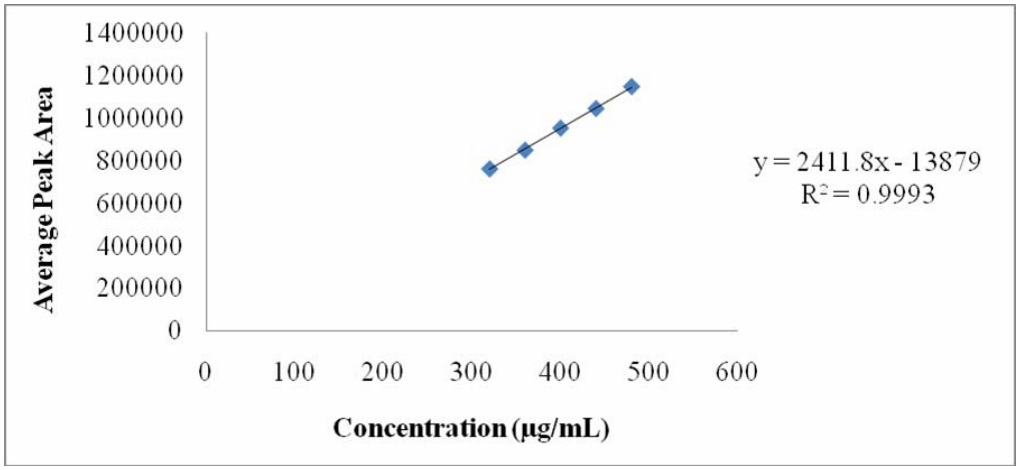

Figure 5. Calibration curve of clarithromycin standard solution

Accuracy. Accuracy of the method was studied by recovery test. The overall results of percent recoveries (mean ± % RSD; n=3) of clarithromycin in pure and in drug-matrix solutions are shown in table 2 indicating good accuracy of the proposed method. The calculated recovery values of clarithromycin ranged from 99.430% (± 0.058%) to 100.482% (± 0.128%) in pure and from 99.643% (± 0.174%) to 101.199% (± 0.122%) in drug-matrix solutions.

Precision. The results obtained from repeatability (intra-day) and intermediate (inter-day) precision analyses are listed in table 3 as mean recovery (%) which indicates that there was no significant differences between assay results of both standard and sample solutions either within day or between days, implying that the precision of the proposed method was good (% RSD less than 1%).

Sensitivity. The limit of detection (LOD) and limit of quantitation (LOQ) of clarithromycin by the

proposed method were found to be 0.04 µg/ml and 0.2 µg/ml, respectively. Figure 5, 6 and 7 show the sensitivity of the current method.

Ruggedness. The results (% of recovery ± % RSD of six assay samples) given in table 4 indicate the ruggedness of the current method.

Table 2. Result of accuracy study.

Type of solution	Concentration (µg/ml)	% Recovery (Mean ± % RSD)
Standard solution	320	100.482 ± 0.128
	360	99.430 ± 0.058
	400	100.173 ± 0.043
	440	99.684 ± 0.082
	480	100.252 ± 0.084
Drug-matrix solution	320	100.199 ± 0.122
	360	99.643 ± 0.174
	400	100.032 ± 0.100
	440	99.730 ± 0.066
	480	100.098 ± 0.116

Table 3. Intra-day and inter-day precision data for clarithromycin (n = 6).

Type of solution	Spike level (%)	Intra-day % recovery (Mean ± % RSD)	Inter-day (% Recovery ± % RSD)			Inter-day % recovery (Mean ± % RSD)
			Day 1	Day 2	Day 3	
Standard solution	100	100.157 ± 0.079	100.021 ± 0.037	99.917 ±0.043	99.873 ± 0.056	9.937 ± 0.062
Drug-matrix solution	100	100.291 ± 0.052	100.155 ± 0.073	99.899 ± 0.088	99.896 ± 0.072	99.983 ± 0.122

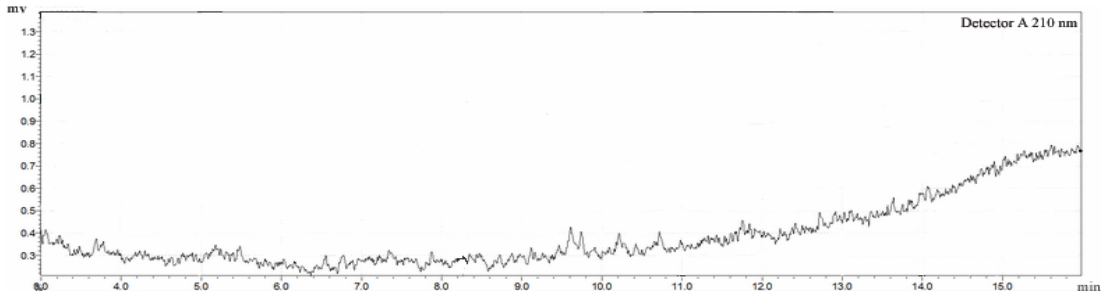

Figure 5. Chromatogram of base line parameter check

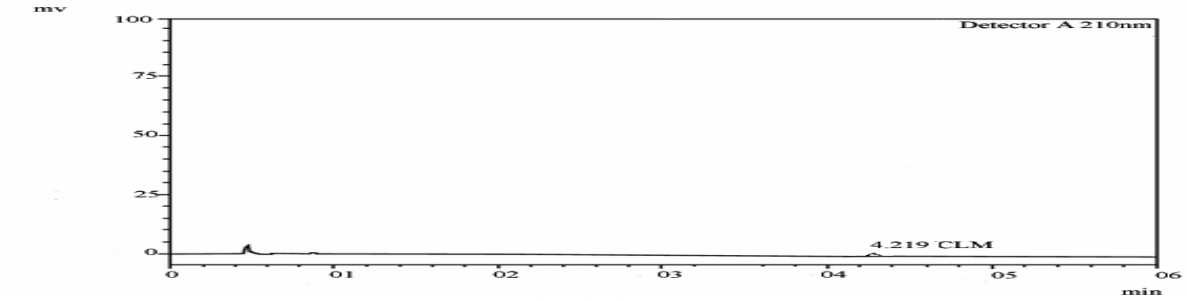

Figure 6. Chromatogram of LOD of clarithromycin

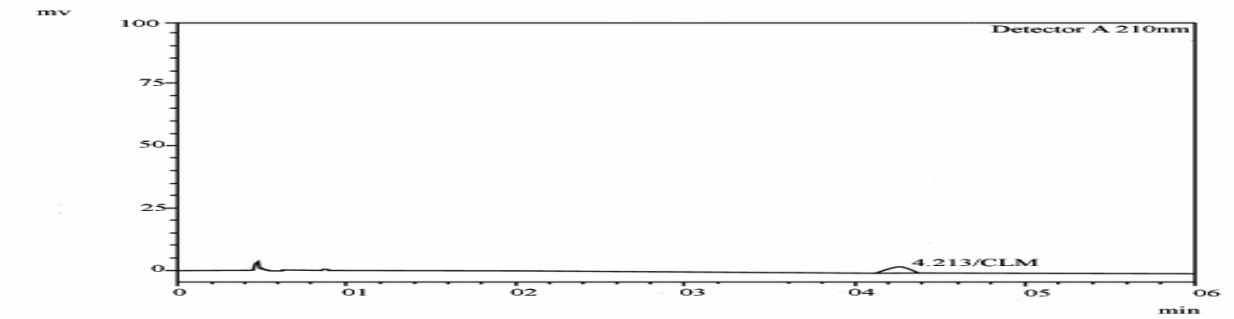

Figure 7. Chromatogram of LOQ of clarithromycin

Table 4. Result of ruggedness study.

Type of solution	Concentration (µg/ml)	Type of ruggedness	% Recovery (Mean ± % RSD)
Drug-matrix solution	400	Analyst 1; Lab 1	100.260 ± 0.049
		Analyst 1; Lab 2	100.195 ±0.063
		Analyst 2; Lab 1	99.984 ± 0.082
		Analyst 2; Lab 2	99.818 ± 0.059

Lab 1: Instrument room (HPLC Lab), Department of pharmacy, University of Dhaka
Lab 2: Centre for Advanced Research in Sciences (CARS), University of Dhaka,

Table 5. Result of robustness study (n = 3).

Parameters	Variations	Concentration (µg/ml)	% Recovery (Mean ± % RSD)
Flow rate (ml/min)	0.5	400	100.03 ± 0.052
	0.6	400	100.182 ± 0.025
	0.7	400	99.837 ± 0.026
Mobile phase composition (% acetonitrile : % phosphate buffer)	53 : 47	400	99.899 ± 0.03
	55 : 45	400	100.170 ± 0.024
	57 : 43	400	99.791 ± 0.025
Mobile phase pH	4.3	400	99.928 ± 0.048
	4.4	400	100.172 ± 0.017
	4.5	400	100.059 ± 0.035

Robustness. The % RSD of robustness study under different altered conditions given in table 5 indicates that the proposed method was robust.

It was possible to achieve elution of clarithromycin within a shorter period of time with the developed method i.e. at about 4.1 min whereas method developed by Srnivasu *et al.*[2] found it at 34.334 min, which represents rapid identification capacity of the newly developed method. Also this method works through an isocratic separation mode compared to the gradient mode, therefore, our developed method is easy to operate and less vulnerable to troublesome noise. Moreover, our method is free from complex mobile phase with the added advantage of a less consumption of organic solvent i.e. 55% of acetonitrile in mobile phase composition. Whereas, Gangishetty *et al.*[7] used a more complex mobile phase containing monobasic phosphate buffer (0.05 M) along with 1-octane sulphonic acid sodium salt monohydrate (pH 3.2) with orthophosphoric acid and acetonitrile.

Moreover, the method also showed higher sensitivity with a LOD and LOQ value of 0.04 and 0.2 µg/ml, respectively, than the respective values found by Srnivasu *et al.*[2] and Gangishetty *et al.*[7] which are 0.9, 1.236 and 5.23, 15.849 µg/ml respectively.

CONCLUSION

The main purpose of this research work was to develop a simple, sensitive and rapid RP-HPLC method for the quantitation of clarithromycin in pure as well as in matrix tablet dosage form. The developed method was found to be versatile, accurate, precise, specific, selective, and obviously less time consuming. Hence it is recommended for routine quality control studies on clarithromycin in bulk as well as in tablet formulations.

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