U.V. spectroscopy technique for analysis to clarithromycin for gastric pH sensitive formulation

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ABSTRACT

The present study describes a simple, specific, sensitive, rapid and economical U.V. spectroscopy method for estimation clarithromycin concentration for gastric pH sensitive formulation. The absorption maxima or λmax of the drug was found to be 353 nm. A linear response was observed in the range of 2-40 µg/ml in simulated buffer solution (pH 1.2) and phthalate buffer solution (pH 3.4) with a regression coefficient (r²) of 0.9812 and 0.9808 respectively. The characteristics analyzed in simulated gastric fluid like slope, intercept, correlation coefficient(r), standard deviation, LOD, and LOQ, were found to be 0.63, 0.053, 0.9822, 0.261, 1.242 µg/ml and 4.14 µg/ml respectively whereas in phthalate buffer (pH 3.4) the value of slope, intercept, correlation coefficient, standard deviation, LOD, and LOQ were found to be 0.62, 0.045, 0.9295, 0.198, 0.958 µg/ml and 3.19 µg/ml respectively. The low values of LOQ and LOD of the proposed method can be used for the determination of amoxicillin trihydrate in quality control of acidic pH sensitive formulation without interference of the excipients.

Keywords: pH sensitive, intercept, Limit of detection, Limit of quantification, U.V.VIS spectroscopy.

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INTRODUCTION

Oral pH sensitive drug delivery systems are gaining importance as these systems deliver the drug at specific part of the GI as per the pH of gastrointestine, resulting in improved patient therapeutic efficacy and compliance. The pH range of fluids in various segments of the GI may provide environmental stimuli for the response drug release [2]. Oral gastro retentive pH sensitive systems like floating and bioadhesive drug delivery systems would be improve the targeting of gastric and duodenum infection of *Helicobacter pylori* [1-4].

Clarithromycin (CL) a semisynthetic macrolide antibiotic derived from erythromycin, inhibits bacterial protein synthesis by binding to the bacterial 50S ribosomal subunit. Binding inhibits peptidyl transferase activity and interferes with amino acid translocation during the translation and protein assembly process. CL may be bacteriostatic or bactericidal depending on the organism and drug concentration. CL is first metabolized to 14-OH, which is active and works synergistically with its parent compound. Like other macrolides, it then penetrates bacterial cell wall and reversibly binds to domain of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, blocking translocation of aminoacyl transfer-RNA and polypeptide synthesis [5].

Validation of an analytical procedure is suitable for its intended use and to show that the results generated by a particular analytical procedure are reliable and accurate. Analytical methods development of validation plays important roles in the discovery, development and manufacture of Pharmaceutical. The official test methods that result from those processes are used by quality control laboratories to ensure the identity, purity, potency and

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performance of drug product [6]. Clarithromycin is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method . Regression characteristics like slope, intercept, correlation coefficient, limit of detection (LOD), and the limit of quantification (LOQ), correlation coefficients (r), standard deviation are summarized in .The scope of developing and validating analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise the main objective for that is to improve the condition and parameter, which should be followed in the development and validation CL has been the foremost anti-infective agent used in clinical medicine for more than a century. The purpose of this analytical method validation is to demonstrate that this method is suitable for the intended purpose and capable of giving reproducible results.

EXPERIMENTAL SECTION

Reference standard of amoxicillin trihydrate was kind gift from Ranbaxy Laboratory Dawash, India. All other ingredients used were of analytical grade. Instruments: UV-Visible spectro-photometer (Shimadzu UV-1601 PC and UV-1700), Electric balance (Type AUY 120 Shimadzu).

METHOD DEVELOPMENT
Solubility Test
Phase solubility studies were performed according to the method reported by Higuchi and Connors, 1965 [7]. The solubility of the drug was analyzed in distilled water, ethanol, chloroform, acetone, stimulated gastric fluid (pH=1.2) and phthalate buffer solution (pH 3.4). The excess amounts of drug (100 mg) were transferred in to 10 ml stopper conical flask. The mixture was diluted by the solvent with stirring up to the mark. The mixture was shaken in thermostatic shaker bath for 24 hr at 37 ± 5 ^{0}C .The aliquots of 5 ml were withdrawn and filter through a 0.45-µm Whatman filter paper for determining drug concentration by U.V. spectrophotometer at 334.5 nm. All assays were performed in triplicate (Table 1). However, stimulated gastric fluid (SGF) and phthalate buffer solution (PBS) were chosen as a solvent for developing the method. The solvents used *in vitro* drug release in gastric stimuli sensitive formulation [1, 4].

Table 1 Solubility of clarithromycin in aqueous and organic solvents

S.N.	Name of solvent	Solubility (µg/ml)[a]
1.	Distilled water	37±1.2
2.	Ethanol	35±1.4
3.	Chloroform	39±1.3
4.	Acetone	38±1.5
5.	Simulated gastric fluid (pH=1.2	35±1.4
6.	Phthalate buffer solution (pH 3.4)	33±1.6

a = mean ±SD (n=3)

Preparation of stock solution
Standard stock solution of CL was prepared by dissolving 100 mg of CL in 100 ml of the solvent to produce a concentration of 1 mg/ml.

Preparation of working standard solution
One ml was pippetted into a 100 ml volumetric flask and the volume was made up to mark and final to produce the solution of 10 µg/ml**.** Then 10 µg/ml sample was scanned in UV-VIS spectrophotometer in the range 400-200nm using the respective solvent as a blank. The wavelength corresponding to maximum absorbance (λmax) was found to be 353nm.

Preparation of calibration curve
Clarithromycin (100 mg) was dissolved in simulated buffer solutions (pH 1.2)/phthalate buffer solution (pH 3.4) and volume was made to 100 ml in volumetric flask. The solutions were diluted to obtain in concentration range 2 µg/ml to 35 µg/ml. The content of each flask was then filtered through Whatman filter paper. Absorbance of each of the solution was measured at 353 nm (Table 2) using shimadzu UV-1700 spectrometer. The experiment was performed in triplicate and based on average absorbance; the calibration curve was obtained by plotting absorbance versus concentration data (Figure 1 & Figure 2).
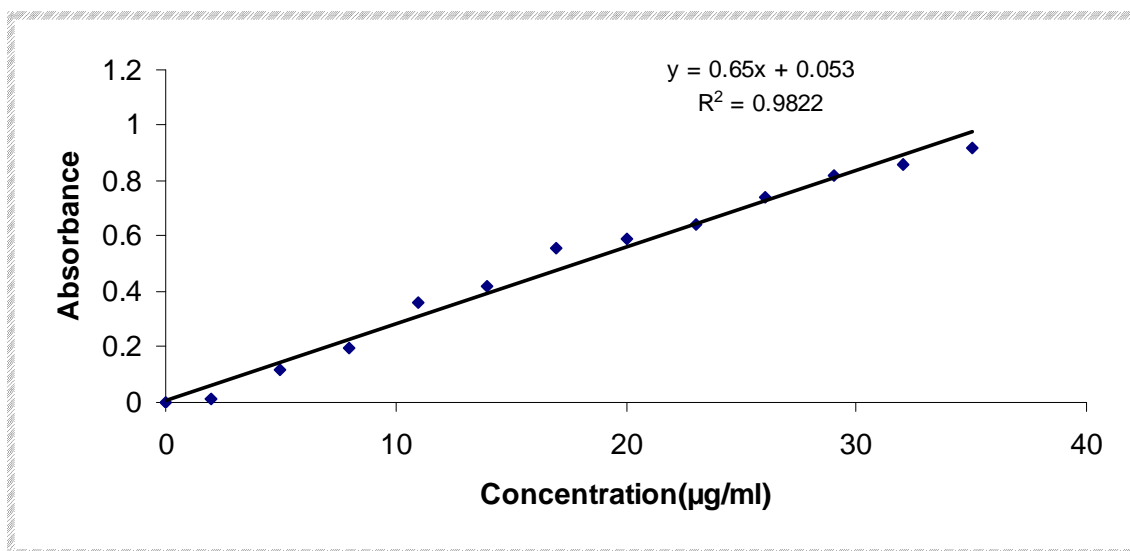
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Figure 1 Calibration curve of clarithromycin in simulated gastric fluid (1.2 pH)
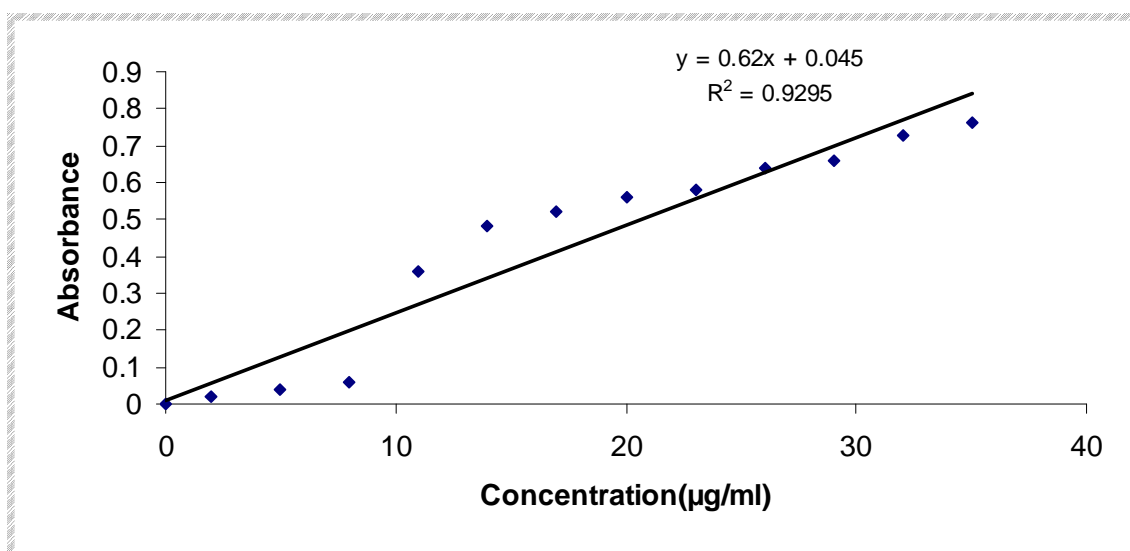
Figure 2 Calibration curve of clarithromycin in phthalate buffer solution (3.4 pH)

Method of validation [8]
Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Specificity , Limit of Detection (LOD) and Limit of Quantification (LOQ).

Regression characteristics like slope, intercept, correlation coefficient, limit of detection (LOD), and the limit of quantification (LOQ), correlation coefficients (r), standard deviation are summarized in (Table 2).The limit of detection is defied as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. The limit of quantification is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision, and variability. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following eq 1 and eq 2.

LOD = 3 S/M   ………………… (1)

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LOQ = 10 S/M   ………………… (2)

Where S, the noise of estimate is the standard deviation of the absorbance of the sample and M is the slope of the related calibrations graph.

Linearity: Various aliquots were prepared form the stock solution (1 mg/ml) ranging from 2- 35 µg/ml (Table 3 and Figure 1 , Figure 2).

Table 2 Absorbance of clarithromycin for preparation of standard calibration curve in simulated gastric fluid (pH 1.2) and phthalate buffers solution (pH 3.4)

S.N.	Concentration and absorbance of clarithromycin in simulated gastric fluid (1.2 pH)			Concentration and absorbance of clarithromycin in phthalate buffer (3.4 pH)		
	Concentration (µg/ml)	Absorbance (average)	Standard deviation (n=3)	Concentration (µg/ml	Absorbance (average)	Standard deviation (n=3)
1.	2	0.02	±0.04	2	0.02	±0.02
2.	5	0.12	±0.05	5	0.04	±0.08
3.	8	0.24	±0.03	8	0.14	±0.04
4.	11	0.36	±0.08	11	0.36	±0.03
5.	14	0.42	±0.02	14	0.48	±0.04
6.	17	0.56	±0.05	17	0.52	±0.05
7.	20	0.59	±0.02	20	0.58	±0.07
8.	23	0.64	±0.08	23	0.56	±0.03
9.	26	0.74	±0.02	26	0.64	±0.08
10.	29	0.82	±0.03	29	0.66	±0.04
11.	32	0.86	±0.02	32	0.73	±0.03
13.	35	0.92	±0.08	35	0.76	±0.02

Table 3 Regression data of the calibration lines for quantitative determination of clarithromycin by UV method

S.N.	Parameters	Values (simulated gastric fluid ;1.2 pH)	Values (phthalate buffer ; 3.4pH)
1.	Λmax (nm)	353 nm	353 nm
2.	Beer's law limits (µg/ml)	2-35	2-35
3.	Regression equation	Y = 0.63x + 0.053	y = 0.62x + 0.045
4.	Slope	0.63	0.62
5.	Y-Intercept	0.053	0.045
6.	Correlation coefficient (r)	0.9822	0.9295
7.	Standard deviation (SD ±)	0.261	0.198
8.	Limit of Detection (LOD; µg/ml)	1.242	0.958
9.	Limit of Quantification (LOQ; µg/ml)	4.14	3.19

RESULTS AND DISCUSSION

The analytical method development recommends the quality, purity and specificity of the drug during the manufacturing process and hence the standard of the drug may not vary, which produce the desirable therapeutic effect. The drug obeyed Beer's law in the concentration range of 2-35 μg/ml in acidic buffer. The characteristics analysed in simulated gastric fluid like slope, intercept, correlation coefficient(r), standard deviation, LOD, and LOQ, were found to be 0.63, 0.053, 0.9822, 0.261, 1.242 µg/ml and 4.14 µg/ml respectively whereas in phthalate buffer (pH 3.4) the value of slope, intercept, correlation coefficient, standard deviation, LOD, and LOQ were found to be 0.62, 0.045, 0.9295, 0.198, 0.958 µg/ml and 3.19 µg/ml respectively.

The validated method was found to be simple, economical, sensitive, accurate, precise, and reproducible. The linearity of CL solution in the concentration range 2 to 35 µg/ml was satisfactory with absorbance maximum at 353 nm . The low values of standard error established the precision of the proposed method. The low values of LOQ and LOD of the proposed method signify that it can be adopted for routine quality testing and analysis of CL in the prepared formulation. The analytical method of validation of CL   in SGF and PBS solution was shows the best elution of the peak. The specificity test studies shows that the analyte chromatographic peak is not attributable to more than one component. The linearity of calibration curve shows linear response over the range of concentration used. Therefore the data of the study clearly indicated that all validation parameters meet the predetermined

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acceptance criteria. Thus it has been concluded that the method is validated for the analysis of CL in acidic pH sensitive delivery system.

CONCLUSION

The proposed method for estimation of AM was found to be simple, accurate, precise, economical and rapid. LOD values of calibration curves indicates the lowest concentration of analyte in a sample that can be detected under a stated experimental conditions and LOQ values of calibration curves indicates the lowest concentration of analyte(s) in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Hence, it can be employed for routine analysis in quality control in gastric pH sensitive delivery system.

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