Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2014, 5(1):29-33

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

U.V. Spectroscopy technique for analysis of amoxcillin trihydrate in pH stimuli sensitive formulation

Girish Kumar Tripathi, Satyawan Singh and Menu Gupta

Nanoparticulate Analytical Laboratory, Saroj Institute of Technology & Management Lucknow, India

---

ABSTRACT

The present study describes a simple, specific, sensitive, rapid and economical u.v. spectroscopy method for estimation amoxicillin trihydrate concentration in pH sensitive formulation. The absorption maxima or λmax of the drug was found to be 334.5 nm. A linear response was observed in the range of 2-50 µg/ml in simulated buffer solution (pH 1.2) and phthalate buffer solution (pH 3.4) with a regression coefficient (r^2) of 0.9812 and 0.9808 respectively. The method was then validated like slope, intercept , standard deviation , limit of detection (LOD), and the limit of quantification (LOQ) were found to be 0.075 , 0.069 , 0.042, 1.68 µg/ml , 5.6 µg/ml respectively whereas in phthalate buffer (pH 3.4) and value of slope, intercept, correlation coefficient, standard deviation , LOD and LOQ were found to be 0.084 , 0.032 , 0.046 , 1.642 µg/ml, 5.47 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, UV-VIS spectroscopy.

INTRODUCTION

Despite tremendous advancements in the drug delivery research, oral route remains the preferred route of administration of drugs because of low cost of therapy, ease of administration, patient compliance etc. Stimuli-sensitive polymers are generally used in the delivery system for the targeting of specific segments of gastrointestine (GI). A small change in environmental condition like pH and temperature etc shows a sharp change in their properties, this behavior of the polymer can be utilized for the preparation of smart drug delivery system [1]. 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* [3].

Amoxcillin trihydrate (AM) is a β-lactam antibiotic and semisynthetic penicillin of the aminopenicillin group that has a broad *in vitro* spectrum against gram negative and gram positive bacteria [4] . It acts through the inhibition of mucopeptide synthesis in the bacterial cell wall. AM diffuses readily into most body tissues and fluids. Various hydrated forms of AM, including monohydrate, dihydrate, and trihydrate have been reported and amoxicillin which the trihydrate is the most stable form. AM is not highly protein bound and its elimination half-life ranges from 0.7 to

1.4 hours in patients with normal renal function. AM is partially metabolized to microbiologically inactive metabolites and both are rapidly excreted from the urine [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 performance of drug product [6]. 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 AM has been the foremost anti-infective agent used in clinical medicine for more than a century[7-9]. 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.

MATERIALS AND METHODS

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

Solubility test for AM was performed by using various solvents.

Phase solubility studies were performed according to the method reported by Higuchi and Connors, 1965 [10]. 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 UV - 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 of the developed pH sensitive formulation [11] .

Table 1 Solubility of amoxicillin trihydrate in aqueous and organic solvents

S.N.	Name of solvent	Solubility (µg/ml)[a]
1.	Distilled water	52±1.2
2.	Ethanol	58±1.3
3.	Chloroform	56±1.4
4.	Acetone	55±1.5
5.	Simulated gastric fluid (pH=1.2	50±1.8
6.	Phthalate buffer solution (pH 3.4)	51±1.4

a = mean ±SD (n=3)

Determination of λmax

Preparation of stock solution

Standard stock solution of AM was prepared by dissolving 100 mg of AM 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 334.5 nm.

Preparation of calibration curve

Amoxicillin trihydrate (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 40 µg/ml. The content of each flask was then filtered through Whatman filter paper. Absorbance of each of the solution was measured at 334.5 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 (Fig. 1 & Fig. 2).

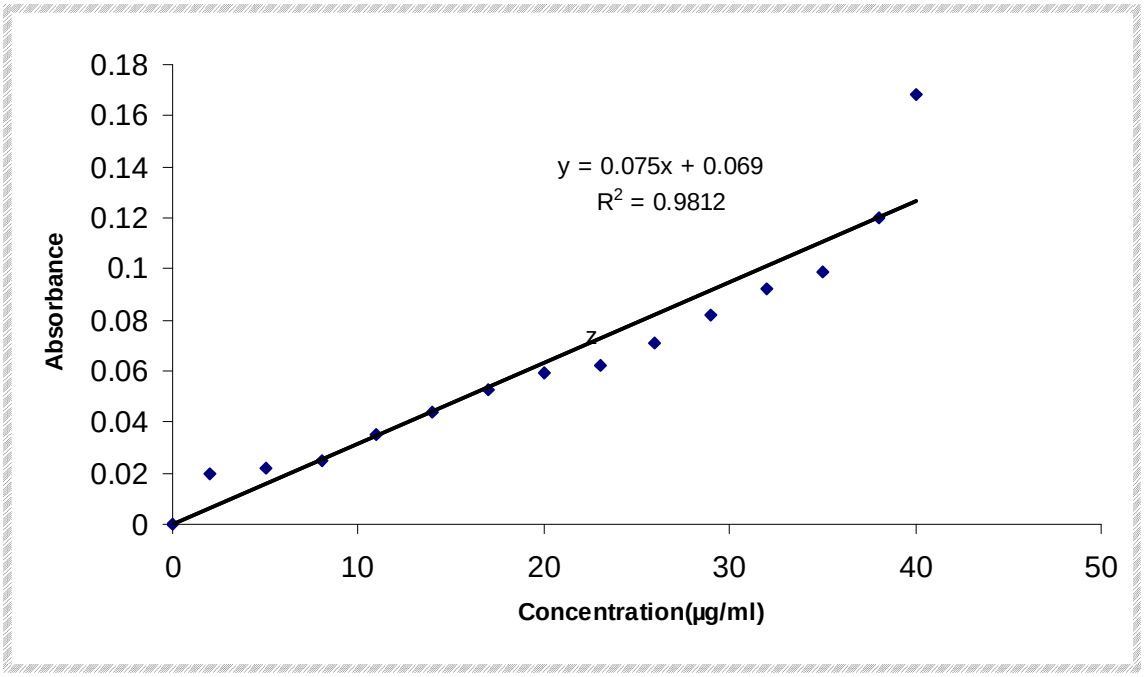

Fig. 1 Calibration curve of amoxicillin trihydrate in simulated gastric fluid (1.2 pH)

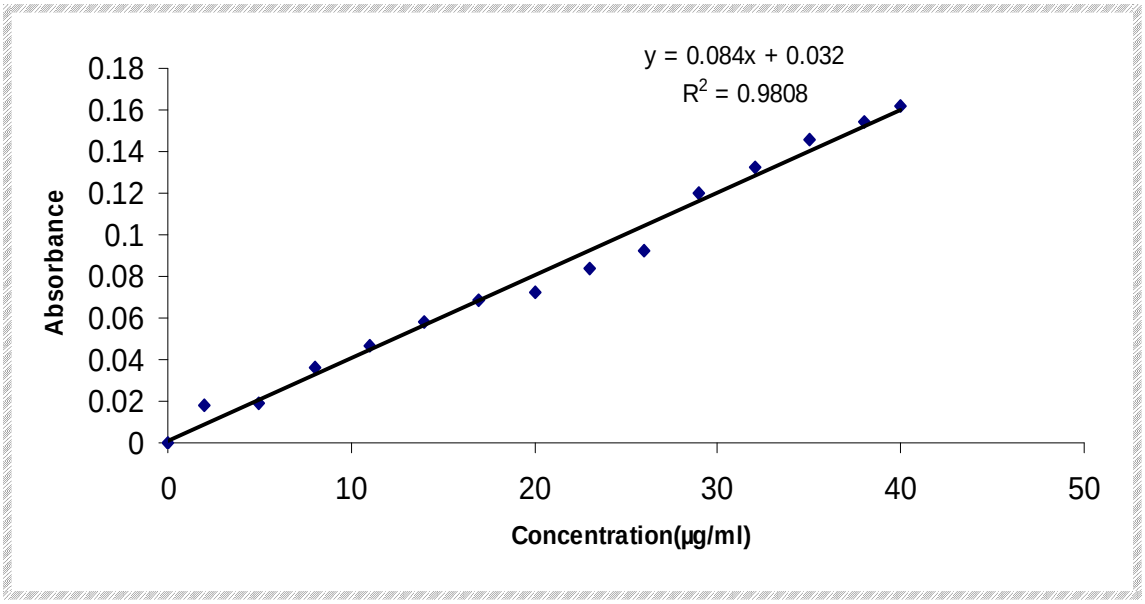

Fig. 2 Calibration curve of amoxicillin trihydrate in phthalate buffer solution (3.4 pH)

METHOD VALIDATION [12]

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)

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- 40 µg/ml (Table 3 and Figure 1 , Figure 2).

Table 1 Absorbance of amoxicillin trihydrate 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 amoxicillin trihydrate in simulated gastric fluid (1.2 pH)			Concentration and absorbance of amoxicillin trihydrate 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	0.020	±0.034	2	0.018	±0.0053
2.	5	0.022	±0.0058	5	0.019	±0.0068
3.	8	0.025	±0.0034	8	0.036	±0.0059
4.	11	0.035	±0.0038	11	0.047	±0.0046
5.	14	0.044	±0.0042	14	0.058	±0.0093
6.	17	0.053	±0.0058	17	0.069	±0.0067
7.	20	0.059	±0.0072	20	0.072	±0.0059
8.	23	0.062	±0.0038	23	0.084	±0.0065
9.	26	0.071	±0.0028	26	0.092	±0.0039
10.	29	0.082	±0.0034	29	0.124	±0.0046
11.	32	0.092	±0.0023	32	0.132	±0.0057
12.	35	0.099	±0.0028	35	0.146	±0.0059
13.	38	0.124	±0.0056	38	0.154	±0.0033
14.	40	0.168	±0.0048	40	0.162	±0.0078

Table 2 Regression data of the calibration lines for quantitative determination of amoxicillin trihydrate by UV method

S.N.	Parameters	Values (Simulated gastric fluid;1.2 pH)	Values (Phthalate buffer; 3.4 pH)
1.	Amax (nm)	334.5	334.5
2.	Beer's law limits (µg/ml)	2-40	2-40
3.	Regression equation	Y = 0.075x +0.069	y = 0.084x + 0.032
4.	Slope	0.075	0.084
5.	Y-Intercept	0.069	0.032
6.	Correlation coefficient (r)	0.9812	0.9808
7.	Standard deviation (SD ±)	0.042	0.046
8.	Limit of Detection (LOD; µg/ml)	1.68	1.64
9.	Limit of Quantification (LOQ; µg/ml)	5.60	5.47

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 2-40 µg/ml in acidic buffer. The characteristics analyzed in simulated gastric fluid like slope, intercept, correlation coefficient(r) , standard deviation , LOD, and LOQ, were found to be 0.075 , 0.069 , 0.9812 , 0.042, 1.68 µg/ml , 5.6 µg/ml respectively whereas in phthalate buffer (pH 3.4) and value of slope, intercept, correlation coefficient, standard deviation , LOD and LOQ were found to be 0.084 , 0.032 , 0.9808 ,0.046 , 1.642 µg/ml, 5.47 µg/ml respectively.

The validated method was found to be simple, economical, sensitive, accurate, precise, and reproducible. The linearity of amoxicillin trihydrate solution in the concentration range 2 to 40 µg/ml was satisfactory with absorbance maximum at 334.5 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 AM in the prepared formulation. The analytical method of validation of AM 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 acceptance criteria. Thus it has been concluded that the method is validated for the analysis of AM in acidic pH sensitive delivery system.

CONCLUSION

The proposed method for estimation of amoxicillin trihydrate was found to be simple, accurate, precise, economical and rapid. LOD values of calibration curves indicates the lowest Concentration of analyte(s) 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 ph stimuli sensitive delivery system.

Acknowledgements
The authors are thankful to Director Pharmacy, Saroj Institute of Technology and Management Lucknow India for providing laboratories facilities. Thanks also go to Dr. P.R. Misra, Scientist, division of Pharmaceutics Central Drug Research Institute Lucknow for providing support.

REFERENCES

[1] Tripathi G.K. and Singh S., **2010**. *Daru*, 18, 4, 247-53.
[2] Miyazaki, S., Kawasaki, N., Kudo, W. and Attwood, D., **2001**. *International Journal Pharmaceutical*, 220 , 161–168.
[3] Tripathi , G. K. and Singh S., **2010**. *Tropical Journal of Pharmaceutical Research*, 9 6, 533-539.
[4] Lindhe J, Warring T and Lang NP. Clinical Periodontology and Implant Dentistry. 4th edition, Blackwell Munsgaard, Oxford, U.K. **2000**; 499.
[5] Lindhe J, Warring T and Lang NP. Clinical Periodontology and Implant Dentistry. 4th edition, Blackwell Munsgaard, Oxford, U.K. **2000**; 499.
[6] Allen L.V., Popovich, N.G., Ansel, H.C. (**2005**). Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. (8th ed., pp. 260-275). Lippincott Williams & Wilkin.
[7] Trinath M., Banerjee S.K., Hari Hara Teja. D and Bonde C.G., *Der Pharmacia Sinica*, **2010**, 1(1), 36-41.
[8] Shilpa P. Chaudhari, M. P. Ratmaparkhi, Neeraj R. Kotian.., *Der Pharmacia Sinica*, **2012**, 3 (4):427-432
[9] Adhikari I.,Moitra S., Murthy P.N. and Mishra U. ., *Der Pharmacia Sinica*, **2012**,3(2),170 176.
[10] Higuchi T, Connors K. , *Adv. Anal Chem. Instrum*. **1965** , 4, 117–123
[11] Tripathi, G.K. , Singh, S., *Der Pharmacia Lettre*, **2010** , 2, 2, 131-138.
[12] ICH, Q2 (R1) validation of analytical procedures: text and methodology, International conference on harmonization; Nov.**1996**.