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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMPICILLIN AND CLOXACILLIN CAPSULES BY USING RP-HPLC

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ABSTRACT

A simple, Accurate, Precise and rapid reverse phase high performance liquid chromatographic isocratic method has been developed for the quantification of method AMPICILLIN and CLOXACILLIN Capsule (oral dosage form). The chormatograpic separation was attained on P.Hypersob C18 (250mm x 4.6, 5 µm,)with precolumn as the stationary phase at ambient temperature .The Mobile phase was composed of Acetonitrile and 0.002 M phosphate buffer pH 3.0, 35:65 with a flow rate of 1.8 ml/min, and UV detection at 225 nm. The run time was 10 minutes. The injection volume was 20. The retention time of ampiciiln and cloxacillin were 1.3 minutes and 6.0 minutes respectively. The linearity in the Ampicillin drug concentration range of 0.1824µg/mL to 0.2736µg/mL and Cloxacillin the drug concentration range is 0.1728 µg/mL to 0.2594µg/mL. The correlation coefficient were greater than 0.99. The mean percentage recovery of Ampicilln & Cloxacillin at 80%, 100%, and 120% concentration levels were for 105.53%, 103.88%, 101.46 & 105.91%, 105.37%, and 108.79% respectively. The results obtained show a method that is simple, rapid and precise. It can be used for the estimation of Ampicillin, Cloxacillin in single as well as combination pharmaceutical dosage forms.

KEYWORDS: Ampicillin, Cloxacillin, RP-HPLC, Validation.

INTRODUCTION

Analysis of drugs present in combined pharmaceutical dosage forms is a quite challenging problem and hence attempts were made to develop analytical methods for relative substances of drugs containing ampicillin and cloxacillin in capsule dosage forms. Chemically ampicillin [1] IUPAC name is (2S, 5R, 6R)-6-[(2R)-2-amino-2 phenylacetamido]-3, 3-dimethyl-7-oxo-4-thia-1- azabicyclo [3.2.0] heptane-2 carboxylic acid. Chemical structure of ampicillin trihydrate is depicted in Figure 1(Audumbar Digambar Mali et al., 2015). Ampicillin is a β-lactam antibiotic. It can sometimes result in allergic reactions that range in severity from a rash to potentially lethal anaphylaxis. Ampicillin is able to penetrate grampositive and some gram-negative bacteria.

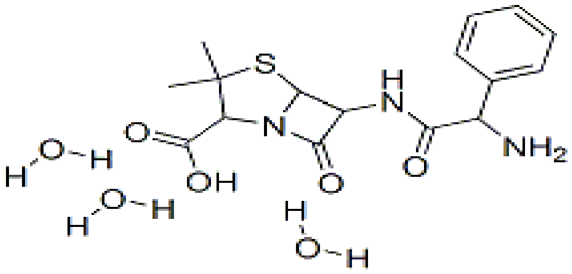


Figure 1: **Structure of Ampicillin.**

Cloxacillin, chemically known as monosodium(2S,5R,6R)-6-[o-(2-chlorophenyl)-5- methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4- thia-1-azabicyclo-[3.2.0]-heptane-2-carboxylate monohydrate, is a semi-synthetic antibiotic in the same class as penicillin. It used against staphylococci that produce β-lactamase. The chemical structure of cloxacillin sodium is depicted in Figure 2.

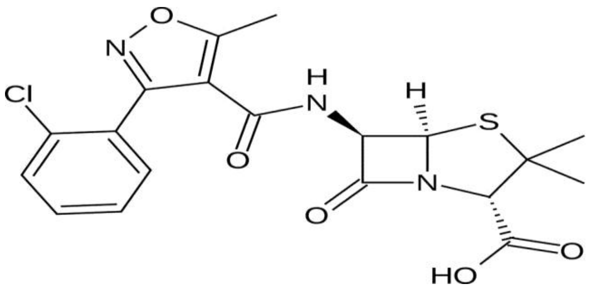


Figure 2: structure of Cloxacillin.

Analysis of drugs present in combined pharmaceutical dosage forms is a quite challenging problem and hence attempts were made to develop analytical methods for relative substances of drugs in ampicillin and cloxacillin dosage forms. All the proposed methods are simple, selective, reproducible, sensitive and accurate with good precision.

According to literature survey, various analytical methods were reported for the estimation of Atenolol[1] and Nifedipine[2] either alone or combination with other drugs. More over various analytical methods like UV,[3] HPLC[4-6], were reported for the simultaneous analysis of ampicillin and cloxacillin. Some RP-HPLC methods were not economical in terms of mobile phase composition, column dimensions and run times. Hence there is need for the development of newer method for estimation of ampicillin and cloxacillin present in tablet to overcome above discussed hurdles. So it is felt worthwhile to develop a simple, rapid, accurate, precise and more economical stability indicating high performance liquid chromatographic method for ampicillin and cloxacillin combination in bulk and its combined dosage form. Hence the present work is aimed to develop and validate the stability indicating assay method for ampicillin and cloxacillin using RP-HPLC technique. The present study was planned to develop a faster isocratic elution for estimation of ampicillin and cloxacillin. The developed method was planned to validate as per ICH guidelines in terms of accuracy, precision, specificity, limit of detection, limit of quantification, linearity, range and robustness.

2. MATERIALS AND METHODS

Chemicals and reagents

Ampicillin and Cloxacillin were obtained from SANZYME (P) Limited, Hyderabad, India, as gift samples. Acetonitrile (HPLC grade), water (HPLC grade) and methanol (HPLC grade) were purchased from E. Merck (India) Ltd., Mumbai, India; while sodium dihydrogen orthophosphate (AR grade) and ortho phosphoric acid (AR grade) were purchased from Rankem, India.

Instrumentation and chromatographic conditions

The liquid chromatographic system consisted of Shimadzu HPLC model with LC software containing variable wave length programmable UV detector. Shimadzu analytical balance, model (Unibloc) was used for weighing purpose. Chromatographic analysis was performed on P.Hypersob C18 (150 x 4.6 mm, 5 µ particle size) column. The separation was carried at 30 °C using a mobile phase consisting of phosphate buffer (pH 3.0) and acetonitrile in the ratio of 35: 65 % v/v, at a flow rate of 1.0 ml/min. The UV detection wavelength was 225 nm, injection volume was 20 µl and runtime was 10 min. Mixture of acetonitrile and water in the ratio of 45: 55 % v/v was used as diluent.

Standard Preparation

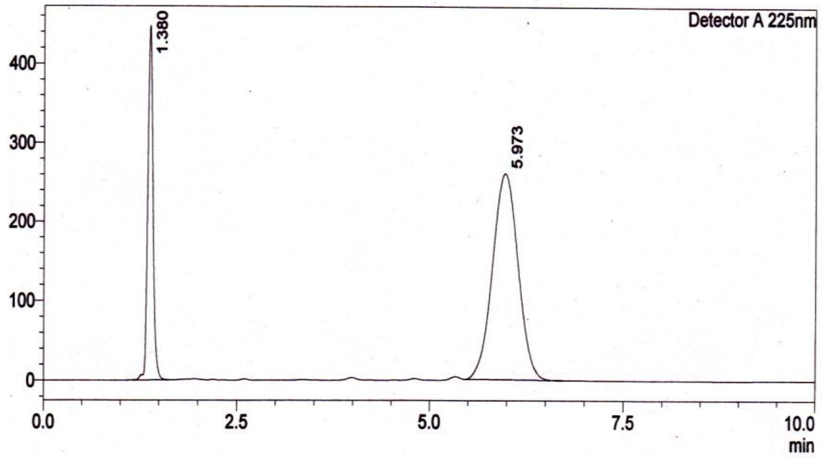
Accurately weighed 57.77 mg of Ampicillin and 54.70mg of cloxacillin and transferred to 50ml volumetric flaskadd sufficient amount of mobile phase and sonicate and for 15 minutes and make upto the mark. dilute 5ml of the solution to 25 ml with mobile phase.

Preparation of sample solution

Twenty capsules powder was taken, 113.15mg was weighed and transferred into a 50mLcalibrated volumetric flask and dissolved using mobile phase. This mixture was sonicated for 20 mns and makes up the volume upto the mark with mobile phase. Dilute 5ml with 25ml of mobile phase.

Separately inject both the standard (5 injections) and sample preparation (6injections) into the chromatographic system and record the peak area responses.

$$\%\ Assay = \frac{\text{sample area}}{\text{standard area}} \text{x} \frac{\text{standard weight}}{\text{sample weight}} \text{x} \frac{\text{potency}}{100} \text{x100}$$



Fugure 3: Chromatogram for assay.

3. RESULTS AND DISCUSSION

Method validation[7]

After method development, the validation of the proposed method has been performed in accordance with ICH guidelines which include accuracy, precision, specificity, linearity, limit of detection, limit of quantification, robustness and ruggedness.

System suitability

System suitability was studied by injecting six replicates of the standard solution of 10 µl into the HPLC system after equilibrating the column with the mobile phase for 30 min prior to injection. The system suitability parameters were evaluated from standard chromatograms by observing theoretical plates, retention time, tailing factor and calculating % RSD of peak area and results obtained were within acceptable limits (Tailing factor below 2.0 and Theoretical plates > 2000) as shown in Table 1.

Table 1: System suitability parameters for Ampicillin And Cloxacillin.

Parameters	**Obtained Values**	
	Amp	**Clox**
Theoretical plates (N)	5727	5727
Tailing factor	1.21	1.05
Resolution	10.197	

Specificity

By comparing the chromatograms of blank, placebo, standard and sample, it was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies by injecting six replicates of standard and sample solutions solution in to the chromatographic system and the response of drug peak and % RSD were calculated as shown in Table 2

Table2: system precision for Ampicillin and Cloxacillin.

System Precision					
Peak response(intra)			**Retention time(inter)**		
Injections	**Ampicillin**	**Cloxacillin**	**Injections**	**Ampicillin**	**Cloxacillin**
Injection- 1	1751702	5154127	Injection -1	1.385	5.991
Injection- 2	1754530	5139735	Injection -2	1.382	5.961
Injection- 3	1755692	5117376	Injection -3	1.387	5.922
Injection- 4	1772564	5163704	Injection -4	1.391	6.103
Injection -5	1753397	4976086	Injection -5	1.381	6.005
Injection- 6	1753210	4991977	Injection -6	1.381	6.069
Mean	1756849	5090499	**Average**	1.38	6.01
Standard deviation	78114.45	84086.08	**Standard deviation**	0	0.07
% RSD	0.44	1.64	**% RSD**	0.28	1.12

Linearity

Six point calibration graphs was constructed in the concentration range of 0.1824-0.2736 μg/ml for Ampicillin and 0.1728-0.2594 μg/ml for cloxacillin by diluting aliquots of 0.25-1.50 ml of standard stock solutions into 10 ml volumetric flasks separately with the diluent.

10 µl of the standard solutions were injected into the HPLC system and analyzed. Linear relationship between the peak area response and the corresponding concentration was observed as shown in Fig.4 and Fig.5. The correlation coefficient, standard deviation of the slope and intercept were calculated as shown in Table 3.

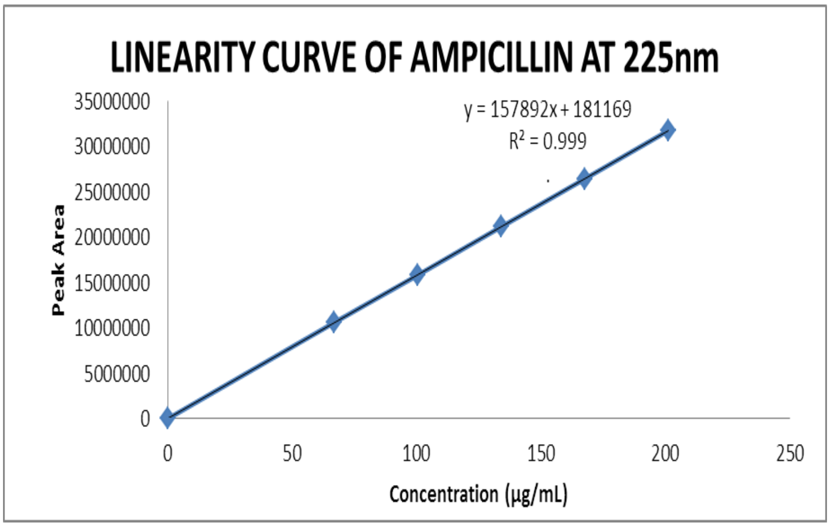


Figure 4: linearity graph of ampicillin.

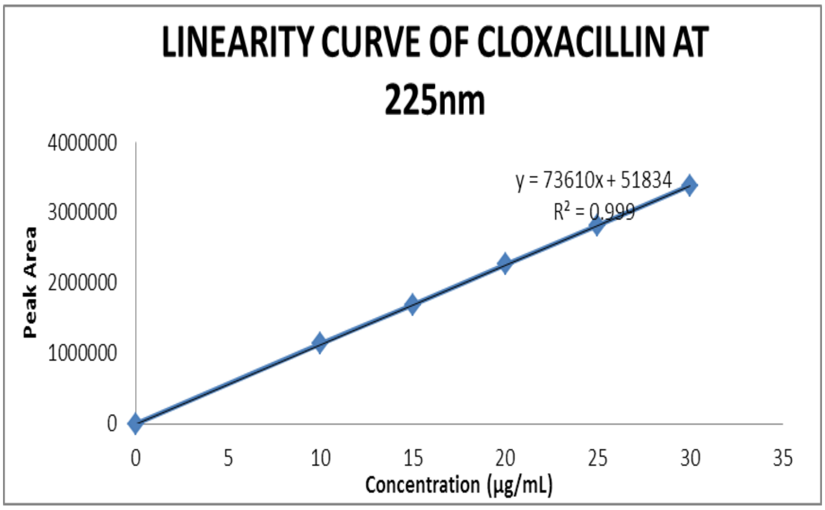


Figure 5: linearity graph of cloxacillin.

Table 3: linearity.

Concentration (%)	**Area Response**			**Degree Of Linearity**		
	AMP	**CLOX**				
80%	1419636	4093095		**R2**	**y-intercept**	**Slope**
90%	1570234	4588723	**AMP**	0.999	181169.40	1549785
100%	1746849	5090499	**CLOX**	0.999	73610.00	51834.80
110%	1876815	5595025				
120%	2041238	6181648				

Accuracy

Accuracy of the method was determined by recovery studies. The solutions were analyzed in triplicate each level by spiking the sample with 50%, 100% and 150% as per the proposed method. The % recovery and % RSD were calculated and results are shown in Table 4. Satisfactory recoveries were obtained by the proposed method which indicates that the proposed method is said to be accurate.

Table 4: accuracy for ampicillin and cloxacilin.

Concentration (%)	Added amount (mg)	(mg)	Removed	Amount (mg)	% of	Recovery
	Amp	Clox	Amp	Clox	Amp	Clox
80%	0.3626	0.3626	0.3867	0.3881	106.64	107.02
100%	0.45328	0.45328	0.4758	0.4827	104.98	106.48
120%	0.5439	0.5439	0.5560	0.5980	102.22	109.94

Robustness

The robustness study was performed by changing variations in flow rate and temperature. The drug was analyzed under these experimental conditions and observed that there is no significant change in tailing factor and theoretical plates as shown in Table 5.

Table: 5: Robustness.

S.No	Condition	%RSD of Ampicillin	%RSD of Cloxacillin
1	Flow rate (less) 1.0ml/min	0.0	0.1
2	Flow rate (actual) 1.2ml/min	0.2	0.1
3	Flow rate (more) 1.4ml/min	0.6	0.6
4	Temperature (less) 20°C	0.2	0.1
5	Temperature (actual) 25°C	0.8	0.8
6	Temperature (more) 30°C	0.3	0.3

Limit of detection and Limit of quantitation

Limit of detection and limit of quantitation was determined by signal to noise ratio method using dilute solution of standard and results were shown in Table 6.

Table 6: Limit of detection and Limit of quantitation.

Parameter	Ampicillin (µg/ml)	Cloxacillin (µg/ml)
LOD	0.018	0.05
LOQ	0.06	0.19

Degradation studies[8]

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. Further forced degradation studies were conducted for the simultaneous estimation of Ampicillin and Cloxacillin in acid/alkali hydrolysis, oxidative, thermal and photolytic stress indicating the stability of the method developed. Results were tabulated in table 7.

Table 7: Degradation results.

Stress conditions	% Assay of active substance	
	Amp	Clox
Acid hydrolysis(0.1 M)HCl)	92.5%	91.7%
Base hydrolysis(0.1 N NaOH)	91.6%	90.7%
Oxidation(3% H_2O_2)	84.6%	83.8%
Thermal degradation	88.6%	87.8%

CONCLUSION

The new stability indicating RP-HPLC method was developed and validated as per guidelines for the simultaneous determination of Ampicillin and cloxacillin in combined pharmaceutical dosage form. The proposed method was found to be accurate, precise, simple, economic, rapid and having good specificity. The developed method can be applied for the assay of commercial tablets containing Ampicillin and cloxacillin in routine quality control analysis.

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