Research Article

SPECTROPHOTOMETRIC ESTIMATION OF AMPICILLIN AND CLOXACILLIN IN PURE AND CAPSULE DOSAGE FORM BY USING DIFFERENT METHODS

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ABSTRACT

Ampicillin and cloxacillin are the β-lactum antibiotic drug. Two sensitive, precise, accurate and simple UV spectrophotometric methods have been developed for simultaneous Estimation of Ampicillin and cloxacillin in capsule dosage form. Method A employs formation and Solving of simultaneous equation using 216 nm and 248 nm as two analytical wavelengths for both drugs in Distilled water, Whereas Method B involved formation of Q-absorbance equation at isobestic point (195 nm). Ampicillin and cloxacillin shows Linearity in a concentration range of 5-30μg /ml and 5-30μg/ml. Recovery studies for Ampicillin 97.83 % and 99.22% for cloxacillin in case of simultaneous equation method confirming the accuracy of the proposed method. The Proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.

Keywords: Ampicillin, cloxacillin, Simultaneous equation method, Q-Absorbance ratio method.

INTRODUCTION

Ampicillin and Cloxacillin sodium are available in capsule dosage form in the ratio of 1:1. Ampicillin is (6R)-6-(α-phenyl-D-glycylamino) penicillanic acid. Ampicillin is official in Indian Pharmacopoeia and U.S.P. Cloxacillin Sodium is sodium (6R)-6-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido] penicillanate monohydrate.[4] Some HPLC and spectrophotometric method have been reported in literature for its estimation. Many methods have been reported in literature for determination of Ampicillin with other drugs individually and in combination. However there is no UV spectrophotometric method for study of Ampicillin and Cloxacillin in capsule dosage form in pharmaceutical preparations has been found in literature survey. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Ampicillin and cloxacillin in capsule dosage form. This communication forms the first report of simple, sensitive and reproducible methods for the simultaneous estimation of Ampicillin and Cloxacillin from combined dosage form.

Ampicillin[1]   Cloxacillin[1]
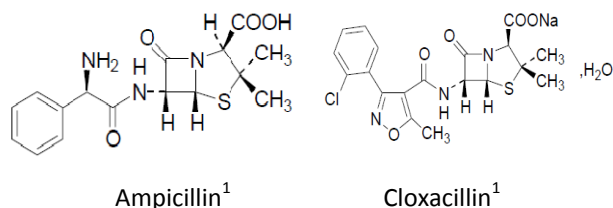
MATERIALS AND METHODS

A Jasco V-630 UV- Visible double beam spectrophotometer with 1 cm matched Quartz cells were used for spectral measurement. Schimadzu AX 200 Analytical balance was used for weighing purposes. The Reference Standard of Ampicillin and Cloxacillin Sodium was taken and capsule was procured from market (AMPOXIN-500) were utilized for the study. All chemicals and reagent used were of analytical grade.

Selection of common solvent

Main criteria for media selection was solubility and stability, i.e. Ampicillin and Cloxacillin should be soluble as well as stable for sufficient time in selected media. The media used in reported method was distilled water. Hence Distill water selected as analytical media for present work.

Preparation of standard drug solution

Standard stock solutions containing Ampicillin (AMPI) and cloxacillin (CLOXA) were prepared individually by dissolving 10 mg of Ampicillin and 10 mg of Cloxacillin separately in 50 ml of distilled water. It was then sonicated for 10 minutes and the final volume of both the solutions were made up to 100 ml with distilled water to get stock solutions containing 100mcg/ mL each of AMPI and CLOXA in two different 100 ml volumetric flasks.

Determination of absorption maxima

By appropriate dilution of two standard drug solutions with distilled water, solutions containing 10 μg/ml of AMPI and 10 μg/ml of CLOXA were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. AMPI and CLOXA showed absorbance maxima at 216 nm (λ1) and 248 nm (λ2) respectively.

Method A: Simultaneous Equation Method

Two wavelengths selected for the method are 216 nm and 248 nm that are absorption maxima of AMPI and CLOXA in Distilled water respectively. The stock solutions of both the drugs were further diluted separately with Distilled water to get a series of standard solutions of 5-30μg /ml concentrations of AMPI and 5-30μg /mL concentrations of CLOXA. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of six independent determinations. Concentrations in the sample were obtained by using following equations:[8]

$$Cx = \frac{A1\ ay2 - A2\ ay1}{ax1ay2 - ax2ay1} \dots\dots\dots Eq.\ (i)$$

$$Cy = \frac{A1\ ax2 - A2\ ax1}{ay1ax2 - ay2a\ x1} \dots\dots\dots Eq.\ (ii)$$

Method B: Absorption Ratio Method (Q Method)

The solutions of AMPI and CLOXA (10 mcg/ml) were scanned in the range of 100 to 400 nm against distilled water as blank. For Q method, 195 nm (isobestic point) and 248 nm (λmax of Cloxacillin) were selected as wavelengths of measurements. Concentrations of AMPI and CLOXA were determined using following equations.[8]

$Cx = (Qm-Qy). A1 / (Qx-Qy). ax1$

$Cy = (Qm-Qx). A1 / (Qy-Qx). ay1$

Where

$Qm = A2/ A1$

$Qx = ax2/ ax1$

$Qy = ay2/ ay1$

Where, A1 and A2 are absorbance's of mixture at 216 nm and 248 nm respectively ax1 and ax2 are absorptivities of AMPI at λ1 and λ2 respectively and ay1 and ay2 are absorptivities of CLOXA at λ1 and λ2 respectively. Cx and Cy are concentrations of AMPI and CLOXA respectively.

Application of the Proposed Methods for the Determination of ampicillin and cloxacillin in capsule

Marketed Capsule formulation containing ampicillin 250 mg and cloxacillin sodium equiv. to 250 mg of cloxacillin was analyzed using this method. From the 20 capsules, an amount equivalent to 250 mg of ampicillin and 250 mg of cloxacillin was weighed and from that the amount equivalent to 10mg of ampicillin and 10 mg of cloxacillin was weighed and dissolved in 50 ml of distill water and sonicated for 10 minutes. Then the volume made up to 100ml with distilled water to get a stock solution containing 100mcg/ml of ampicillin and 100mcg/ml of

cloxacillin. Then the solution was filtered through Whatman filter paper no. 41. Appropriate aliquots of ampicillin and cloxacillin within the Beer's law limit were taken. The absorbances of resulting solutions were measured at 216 nm and 248 nm. The concentration of Ampicillin and Cloxacillin present in the sample solution was calculated by using the equation generated from calibration curve of respective drugs. In this method the concentration of both AMPI and CLOXA were determined by measuring the absorbance of the sample at 216 nm and 248 nm. Values were substituted in the respective formula to obtain concentrations.[6, 7]

Validation

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.[2, 3, 5]

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%). Percent recovery for AMPI and CLOXA, by this method, was found in the range of 99.56-100.14%

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of AMPI and CLOXA (figures 1 and 2). For simultaneous equation method the Beer- Lambert's concentration range was found to be for 5-30 μg/ml for AMPI and 5-30 μg/ml CLOXA.
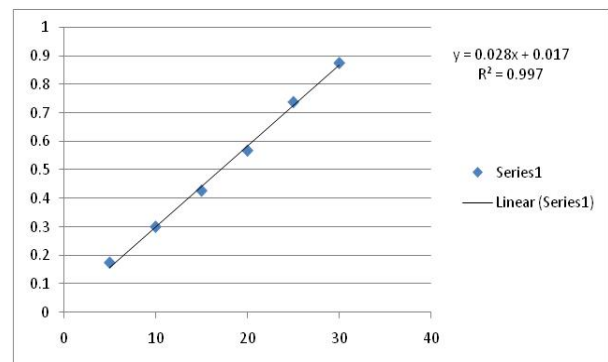
$y = 0.028x + 0.017$
$R^2 = 0.997$

Figure 1: Linearity curve for ampicillin

$y = 0.009x + 0.011$
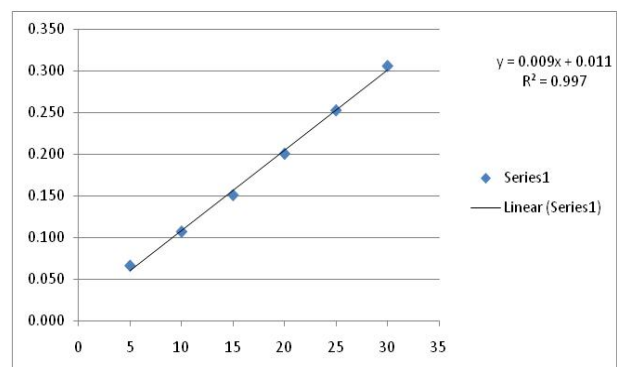$R^2 = 0.997$

Figure 2: Linearity curve for cloxacillin

Table 1: Analysis of Capsule formulation

Drug	Label claim (Mg/ tab.)	Amount found (mg)		% Drug found ±SD		Standard Error	
		Method A	Method B	Method A	Method B	Method A	Method B
AMPI	250	248.29	248.25	99.31 ± 0.35	99.3± 0.31	0.14	0.11
CLOXA	250	249.39	249.25	99.75 ± 0.20	99.7± 0.18	0.085	0.079

Values expressed mean± SD (n=6)

Table 2: Recovery study of AMPI AND CLOXA

Drug	Level of addition (%)	Amount added (µg/ml)	Amount recovered (µg/ml)		% Recovery ± SD	
			Method A	Method B	Method A	Method B
AMPI	80	4	3.87	3.86	96.75± 0.055	96.5 ± 0.045
	100	5	4.97	4.95	99.4 ± 0.045	99.00 ± 0.043
	120	6	5.87	5.90	97.83 ± 0.062	98.33 ± 0.059
CLOX	80	4	3.97	3.98	99.25 ± 0.081	99.5 ± 0.078
	100	5	4.98	4.92	99.6 ± 0.045	98.4 ± 0.041
	120	6	5.93	5.94	98.83 ± 0.041	99.00 ± 0.039

Values expressed mean± SD (n=3)

Table 3: Optical characteristics data and validation parameters

Parameters	Values for AMPI	Values for CLOXA
Absorption maxima (λ max)	216nm	248nm
Beer's law limit (µg/ml)	5-30	5-30
Regression equation	y = 0.028x + 0.017	y = 0.009x + 0.011
Correlation coefficient (R2)	0.997	0.997
Molar absorptivity	1.04×10^4	5.09×10^3
A(1%,1cm)	300	107
Accuracy (%Recovery ± SD)	97.83±1.33	99.22±0.38
Precision		
Intraday*(Analyst 1)	99.31±0.35	99.75±0.2
Interday*(Analyst 2)	100.86±0.86	98.66±0.81
LOD (µg/ml)	0.2582	0.1428
LOQ (µg/ml)	0.7823	0.4327

*Average of six determinations ± SD

Limit of Detection (LOD) and Limit of Quantization (LOQ)

The LOD and LOQ of ampicillin and cloxacillin by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in table 3.

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, precision and recovery studies were performed. The drugs obey beer's law in the concentration range 5-30 µg/ml for AMPI and 5-30 µg/ml for CLOXA for all the methods with good correlation coefficient 0.997. The results of commercial formulation analysis are presented in table 1. Results of recovery studies are shown in table 2. The accuracy and reproducibility is evident from the data as results are close to 100 % and low standard deviation. The proposed methods are simple, economical, rapid, precise and accurate. Hence these can be used for routine analysis of AMPI and CLOXA in capsule formulation.

CONCLUSION

The most striking feature of this method is its simplicity, economy and rapidity, non- requiring consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. These are new and novel methods and can be employed for routine analysis in quality control analysis. The described methods give accurate and precise results for determination of Ampicillin and Cloxacillin mixture in marketed formulation.

REFERENCES

1. Indian pharmacopoeia Vol II, New Delhi, The controller of Publications. Govt of India, 2010. p822,823,1123,1124.

2. Validation of Analytical Procedures: Methodology (in Q2(R1)) http://www.ich.org/cache/compo/276-254-1.html

3. ICH Q2A, Text on validation of analytical procedures, International Conference on Harmonization tripartite guidelines, adapted 27 Oct 1994.

4. Block JH and Beale JM (Jr.) Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th edition, 2004, 311, 312.

5. International Conference on Harmonization (ICH), Q2B, Validation of Analytical Procedures: Definitions and Terminology, Vol.60, US FDA Federal Register, 1995.

6. Abuirjeie MA, Abdel-Hamid ME., Simultaneous high-pressure liquid chromatographic Analysis of ampicillin and cloxacillin in serum and urine. J Clin Pharm Ther. 13(2):1988 Apr; 101-8.

7. BURNS, DT, O'CALLAGHAN, M, Smyth, Franklin and AYLING, CJ. high performance Liquid-chromatographic analysis of ampicillin and cloxacillin and its application to an intramammary veterinary preparation. Fresenius journal of analytical chemistry, 340 (1), 1991, 53-56.

8. Stenlake J. B. and Backett A. H.; Practical pharmaceutical Chemistry; C. B. S. Publishers and Distributors; New Delhi; 4th Ed., Part II, 1997, 281-306.

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