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Research article

NEW ANALYTICAL METHOD FOR THE QUANTITATIVE ESTIMATION OF DICLOXACILLIN SODIUM AS AN ACTIVE PHARMACEUTICAL INGRIDIENT AND IN CAPSULE DOSAGE FORM

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ABSTRACT

Dicloxacillin Sodium is a Beta-lactum antibiotic. The aim of the present study is to develop a new analytical method for the estimation of Dicloxacillin Sodium in bulk and in tablet dosage form. Spectroscopic method has been developed for the quantification of Dicloxallin Sodium in bulk and in the formulation. This method is simple, cost effective, accurate and precise. In this method, Dicloxacillin Sodium showed maximum absorbance at 274 nm in double distilled water, which is selected as solvent for our analysis based on its stability. Beer's law obeyed in the concentration range of 5-25 mcg/ ml. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.516682 and 1.565703 mcg/ml respectively. This method is validated as per ICH guidelines. The method was found to be simple, accurate, Precise and rapid.

Key Words:- UV-spectroscopic method, Dicloxacillin Sodium, precise.

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INTRODUCTION

Dicloxacillin Sodium is chemically (2*S*, 5R, 6R)-6-{[3-(2, 6-dichlorophenyl)-5-methyl-oxazole-4-carbonyl] amino}-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid. Its Molecular formula is $C_{19}H_{17}Cl_2N_3O_5S$ and possessing a molecular weight of 510.32. It is Soluble in aqueous solvents and insoluble in organic solvents. Dicloxacillin Sodium is used as an Beta-lactum antibiotic. The structural formula of Dicloxacillin Sodium is shown fig 1.

Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development in the recent era. Standard analytical procedure for newer drugs or formulation may not be available in pharmacopoeia. Hence, it is essential to develop newer analytical methods, which are accurate, precise, and specific, linear, simple and rapid. From the extensive literature survey, it was revealed that there were a very few methods reported for the estimation of Dicloxacillin Sodium from plasma and for Pharmaceuticals dosage forms. Therefore, here an attempt was made to develop simple, cost, effective, and accurate for the estimation of Dicloxacillin Sodium in bulk and in capsule dosage forms (Anonymous 1-3).

MATERIALS AND METHOD

Materials

Drug Sample (Raw material)

Dicloxacillin sodium was obtained as a gift samples from Assure Pharma, 105-Advait complex Ahmedabad, India.

Formulation used

Klox-D* (Hetero Healthcare Ltd., hyderbad) - containing 50 mg of Dicloxacillin sodium. The formulation was procured from the local market.

Chemicals and solvents used

Double distilled water was purchased from Aravind labs, Tirupathi.

Instruments used

Different instruments used to carry out the present work,

- Wensar Digital balance
- Analytical Spectroplus 2060
- ELICO pH meter (Model LI-120)
- Micropipette
- Melting point apparatus

METHOD

UV Spectrophotometric Method

Selection of Solvent

The solubility of Dicloxacillin sodium was determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility test for Dicloxacillin sodium was carried out in different polar and non-polar solvents. From the solubility studies double distilled water was selected as suitable solvent for proposed method (Boveley *et al*., 2008).

Preparation of Standard Stock Solution

50 mg of Dicloxacillin sodium raw material was accurately weighed and transferred into the 100 ml volumetric flask and dissolved in minimum quantity of water and made up to 100 ml with water. The solution was observed to contain 500 µg/ml.

Selection of λ_{max}

The standard stock solution was further diluted with double distilled water to get 10 µg/ml concentration. The solution was scanned between the ranges of 200-400 nm using double distilled water as blank. From UV spectra, 274 nm was selected as λ_{max} for analysis of Dicloxacillin sodium. Stability was studied by measuring the same solution in different time intervals. It was observed that Dicloxacillin sodium in double distilled water was stable for 1 hour.

Calibration graph

In this method, the aliquots of stock solution of Dicloxacillin sodium (1 – 5 ml of 500 µg/ ml) were transferred into 100 ml volumetric flask and made up to the mark with double distilled water. The absorbance of different concentration solutions was measured at 274 nm against double distilled water as blank. The samples were found to be linear with the concentration range of 5 – 25 µg/ ml. The calibration curve was plotted using concentration against absorbance. The curve obtained was linear with the concentration range of 5 - 25 µg/ml.

Quantification of Raw material

2 ml of standard stock solution (500 µg/ ml) was taken into six 100 ml volumetric flasks individually and the volume made up to mark with double distilled water. The absorbance of solutions was measured at 274 nm. The amount of Dicloxacillin sodium present in bulk was determined by using slope and intercept values from calibration graph.

Quantification of formulation

Twenty capsules of formulation (Klox-D) containing 50 mg of was accurately weighed to find out the average weight and powdered. Powdered capsule equivalent to 50 mg of Dicloxacillin sodium was transferred into a 100 ml volumetric flask, added water to dissolve and made up to the volume. Then the solution was sonicated for 10 minutes. After sonication the solution was centrifuged at 100 rpm for 10 minutes. The solution was filtered through Whatmann filter paper No.41. From the clear solution, 2 ml of the solution was transferred into a 100 ml standard flask and made up to the mark with double distilled water to produce 10 µg/ ml concentration. The absorbance measurements were made six times for the formulation at 274 nm. The amount of Dicloxacillin sodium present in formulation was determined by using slope and intercept values from calibration graph.

Recovery studies

The recovery experiment was done by adding known concentrations of Dicloxacillin sodium raw material to the pre analyzed formulation. 50 mg equivalent of Dicloxacillin sodium formulation was taken into a series of three 50 ml standard flasks. To that 25 mg, 50 mg and 75 mg of raw material were added in to series 1, 2 and 3 respectively. Dissolved with water and made up to volume with water .The solutions were sonicated for 10 minutes. After sonication, the solution was centrifuged at 100 rpm for 10 minutes. The solutions were filtered through Whatmann filter paper No. 41. From each standard flask, 2 ml of the clear filtrate was transferred into a series of six 100 ml standard flasks and made up to volume with double distilled water. The absorbances of the resulting solutions were measured at 274 nm against blank and the amount of drug recovered from the formulation was calculated by using slope and intercept values. The procedure was repeated for three times for each concentration (Walsh *et al*., 2007).

Validation of developed method

Linearity

A calibration curve was plotted between concentration and absorbance. Dicloxacillin sodium was linear with the concentration range of 5 - 25 µg/ml at 274 nm.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. To the preanalyzed formulation, a known quantity of raw material of Dicloxacillin sodium was added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration.

Precision

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration. The amount of each drug present in the capsule formulation was calculated. The percentage RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs were determined, percentage RSD also calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of intercept (Zamith *et al*., 2004; Rosenberg *et al*., 2005; Krystal *et al*., 2003).

RESULTS AND DISCUSSION

One simple, precise and accurate method was developed for the estimation of Dicloxacillin sodium in pure form and in its capsule dosage form. The method employed for analysis of Dicloxacillin sodium was,

UV Spectrophotometric Method

Dicloxacillin sodium was identified by IR spectrum and as shown in Figure 1. The solubility of Dicloxacillin sodium was determined as per Indian Pharmacopoeia. The numbers of polar and non-polar solvents tried were double distilled water, 0.1M HCl, 0.1M NaOH, Methanol, Ethanol, Acetone, Isopropyl alcohol, N-Butanol, Carbon tetrachloride, Petroleum ether, Dichloromethane, Ethyl acetate, Toluene, Diethyl amine and Acetonitrile. From the solubility data Double distilled water was selected as a solvent for simple UV method because of its solubility and easy availability. Dicloxacillin sodium was dissolved in double distilled water and further diluted with double distilled water to get a concentration of 10 µg/ml. The solution was scanned in UV region in the wavelength range from 200 to 400 nm against double distilled water as blank. The spectrum of Dicloxacillin sodium in double distilled water was recorded as shown in Figure 2. From the spectrum of Dicloxacillin sodium the wavelength maxima was found to be 274 nm. The absorbance of the solution was measured at the selected wavelength in different time interval. It was observed that Dicloxacillin sodium in double distilled water was stable for 1 hour. Hence this wave length was selected for analysis.

Different aliquots of Dicloxacillin sodium in double distilled water was prepared in the concentration range of 5 - 25µg/ ml. The absorbance of solutions was measured at 274 nm. The calibration curve was plotted using concentration against absorbance. The procedure was repeated for six times. The optical parameters like correlation co-efficient, slope, intercept, Sandell's sensitivity, molar absorptivity, LOD and LOQ was calculated. These are shown in Table 1. The correlation co-efficient value for the calibration graph was found to be 0.99569 it indicates that the concentration of Dicloxacillin sodium has good linearity. The calibration graph is shown in Figure 3.

Standard Dicloxacillin sodium solution was prepared (10 µg/ml) in double distilled water for six times. The absorbance of these solutions was measured at 274 nm and the amount of each solution was calculated by slope and intercept values from calibration graph. The amount of Dicloxacillin sodium was found to be 100.45% ± 1.1431. This indicates that the proposed method can be applied for the analysis of formulation. The results of raw material analysis are shown in Table 2. Klox-D* capsules (Hetero Health care Hyderabad) containing 500 mg of Dicloxacillin sodium was selected for analysis. Twenty capsules were weighed accurately and the average weight was found. The nominal concentration of Dicloxacillin sodium (10 µg/ml) was prepared from the calibration graph for six times. The absorbance of the solutions was measured at 274 nm and the amount of each test solution was calculated. The percentage label claim of Dicloxacillin sodium present in capsule formulation was found to be 99.995% ± 0.5914. The amount found was in good agreement with the label claim and the percentage relative standard deviation (% RSD) value was found to be 0.5914 indicates that the method has good precision. The results of formulation analysis are shown in Table 3.

Further the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The percentage RSD value was found to be 0.1209 and 0.3264 for intraday and inter day analysis of Dicloxacillin sodium, respectively. The reports of analysis are shown in Table 4. Hence the precision was confirmed.

The accuracy of the method was performed by recovery studies. To the pre analyzed formulation, a known quantity of Dicloxacillin sodium raw material solution was added at different levels viz., 50%, 100%, and 150%. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 97.24– 99.62%. The amount of the drug recovered from the formulation was very close to the expected value and the % RSD value also very low (1.2504). This indicates

that this method is very accurate. The recovery data is shown in Table 5.

All the above parameters combined with the simplicity and ease of operation ensures that the application of proposed method in the assay of Dicloxacillin sodium in bulk and capsule form. Hence the UV spectroscopy method can be applied for the estimation of Dicloxacillin sodium in bulk and in capsule dosage form.

Table 1. Optical Characteristics of Dicloxacillin Sodium by UV Method

Parameter	Value
λ_{max}(nm)	274
Beers law limit (µg/ml)	5 – 25
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.2062284
Molar absorptivity (L mol^{-1} cm^{-1})	2265.8467
Correlation coefficient (r)	0.99569
Regressionequation (y=mx+c)	Y= 0.01685667x – 0.002639255
Slope(m)	0.01685667
Intercept(c)	0.002639255
LOD (µg/ml)	0.516682
LOQ (µg/ml)	1.565703
Standard error of mean of Regression line	0.000535

Table 2. Quantification of Raw Material by UV Method

S.No	Expected amount (µg/ml)	Amount found (µg/ml)	Percentage obtained	Average (%)	S.D.	% RSD	S.E
1.	10	10.022	100.22				
2.	10	10.162	101.62				
3.	10	10.162	101.62	100.45	1.1431	1.1379	0.4667
4.	10	9.882	98.82				
5.	10	9.952	99.52				
6.	10	10.092	100.92				

Table 3. Quantification of Formulation – KLOX-D* by UV Method

S.No	Labeled amount (mg/Cap)	Amount found (mg)*	Percentage obtained*	Average* (%)	S.D.	%RSD	S.E
1	500	499.28	99.85				
2	500	501.43	100.28				
3	500	496.04	99.20				
4	500	504.67	100.93				
5	500	498.20	99.64			0.5914	0.2414
6	500	500.35	100.07	99.995	0.5914		

*Mean of six observations

Table 4. Intra Day and Inter Day Analysis of Formulation - KLOX-D* by UV Method

S.No	Labeled Amount (mg/tab)	Amount Obtained *		S.D*		% RSD*	
		Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
1.	500	501.43	501.43	0.1212	0.3262	0.1209	0.3264
2.	500	500.35	498.20				
3.	500	501.43	499.28				
	Mean	**501.07**	**499.64**				

* Mean of Three observations.

Table 5. Recovery Analysis of Formulation - KLOX-D* by UV Method

S.No	Amount Present (µg/ ml)	Amount Added (µg/ ml)	Amount Found* (µg/ ml)	Amount Recovered* (µg/ ml)	Percentage Recovery*	S.D	% RSD	S.E
1.	10	5	14.981	4.981	99.62	1.2277	1.2504	0.7088
2.	10	10	19.769	9.769	97.69			
3.	10	15	24.586	14.586	97.24			
				Mean	**98.183**			

* Mean of Three Observations.

Figure 1. Chemical structure of Dicloxacillin Sodium

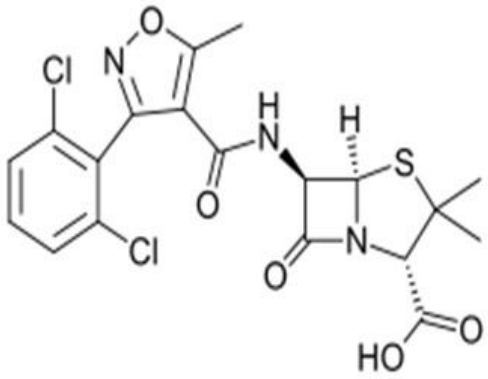
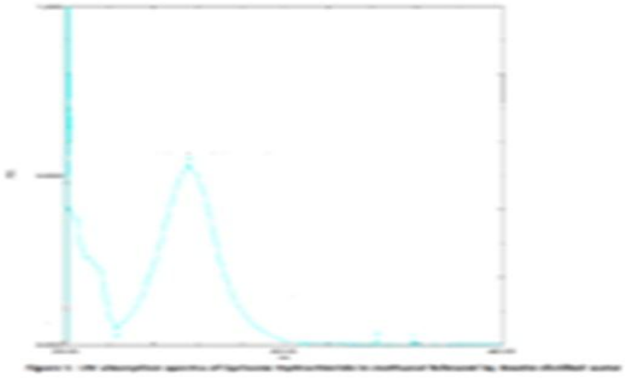


Figure 2. UV spectrum of Dicloxacillin sodium



CONCLUSION

One simple, precise, rapid and accurate method was developed for the estimation of Dicloxacillin sodium in bulk and in capsule formulation. Estimation of Dicloxacillin sodium was achieved by UV method. After considering the solubility and stability, double distilled water were selected as solvent. Dicloxacillin sodium 10 µg/ml solution was prepared and scanned in the UV region. From the spectra 274 nm was selected as an analyzing wavelength. Calibration curve was plotted by using concentration versus absorbance. From the calibration curve it was found that Dicloxacillin sodium obeys Beer's law in the range of 5 - 25 µg/ml, Correlation coefficient: 0.99569, Slope: 0.016856667, Intercept: 0.002639255, LOD: 0.516682 µg/ml and LOQ: 1.565703µg/ml were calculated. Quantification of raw material was performed by the proposed method. Percentage amount of Dicloxacillin sodium raw material was found to be 100.45% ± 1.1431. Twenty capsules were weighed and capsule powder equivalent to 50 mg of Dicloxacillin sodium was weighed, dissolved and diluted to get 10 µg/ml using solvent. The amount of Dicloxacillin sodium in formulation (Klox-D*) was found to be 99.995% ± 0.5914. The precision of the method was studied by making repeated analysis. The recovery studies were also carried out to ensure the accuracy of the method by adding known concentration of pure drug to a pre analyzed formulation. The average percentage recovery for formulation (Klox-D*) was found to be in the range of 97.24 - 99.62%. The method was further studied by intraday and inter day analysis.

One simple, rapid and accurate analytical method was developed for the determination of Dicloxacillin sodium in bulk and in capsule formulation by using UV spectrophotometry. The method showed excellent sensitivity, reproducibility, accuracy and repeatability, which is evidenced by low percentage relative standard deviation. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. Hence it is suggested that the proposed UV spectrophotometric method can be effectively applied for the routine analysis of Dicloxacillin sodium in bulk and in tablet formulation and the obtained results will be presented elsewhere.

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