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Development and validation of high performance liquid chromatographic method for the determination of cloxacillin sodium in bulk and pharmaceutical dosage form

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Abstract
A simple, sensitive, reliable and rapid reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the determination of cloxacillin sodium in bulk and pharmaceutical dosage form. The chromatographic system consisted of waters (784), 515 binary Pump, data Ace with UV-Visible detector. Separation was achieved on the thermo C18 (250 x 4.60), 5 µ particle size column in isocratic mode at room temperature. The sample was introduced through an injector valve with a 20 µl, sample loop. 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile 20:80 (%, v/v), was used as mobile phase with flow rate of 1 ml/min. UV detection was performed at 224 nm. A calibration graph was plotted which showed a linearity range between 5-25µg/ml with the correlation coefficient of 0.999. The LOD was 0.095µg/ ml, while the LOQ was 0.271 µg/ml. Validation studies revealed the method is specific, rapid, reliable and reproducible. To study the validity of the method, recovery studies and repeatability studies were carried out using the same optimum conditions. The system suitability studies were also calculated which includes column efficiency, resolution, capacity factor and peak asymmetrical factor. Therefore the proposed method is reliable, rapid, precise and selective so may be used for the quantitative analysis of cloxacillin.

Keywords: HPLC, cloxacillin, pharmaceutical dosage form, method validation

Introduction
Cloxacillin, chemically known as monosodium(2S,5R,6R)- 6-[o-(2-chlorophenyl)-5-methyl-4-isoxazole carboxamido]-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 b-lactamase [1, 2].The chemical structure of cloxacillin sodium is depicted in Figure 1. Unlike amoxicillin, this antibiotic is incompletely absorbed from the gastrointestinal tract, and absorption is reduced by the presence of food in the stomach. To produce a wider spectrum of activity, cloxacillin may be co-formulated with other antibacterials, in particular with amoxicillin (ratio 1:1, w/w) in capsules. The US Code of Federal Regulations [3] described two official methods for potency assay of cloxacillin: a microbiological method and iodimetric titration. The regulations state that the results obtained from the microbiological method shall be conclusive. The present official assay method of minimum requirements for antibiotic products of Japan [4] for the analysis of potency of cloxacillin in bulk drug substance and its preparations is a microbiological method. However, there has recently been a move to replace expensive microbiological assays by chemical assays, e.g., high-performance liquid chromatography (HPLC). Several HPLC methods for the determination of cloxacillin in biological fluids have been reported. Fewer methods have been reported for separating and identifying cloxacillin and the derivatives of penicillin and for the determination of cloxacillin in pharmaceutical samples [5, 6]. In the literature, the high performance liquid chromatography (HPLC) technique has been reviewed as a valuable tool for the analysis of antibiotics in formulated and unformulated samples [7]. As a result, this technique has been widely used for the determination of penicillins such as cloxacillin individually or simultaneous analysis of the two drugs in pharmaceuticals, veterinary combination, biological fluids and tissues [8-18]. Recently, we also developed a UV spectrophotometric method in drug combination [19, 20], stability-indicating [21, 22]. In the present work, we are therefore focused on to achieve the optimum chromatographic conditions for the

determination of cloxacillin in a capsule formulation. The developed method could be applied to quality control of the capsule dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines [23], which are mandatory also.

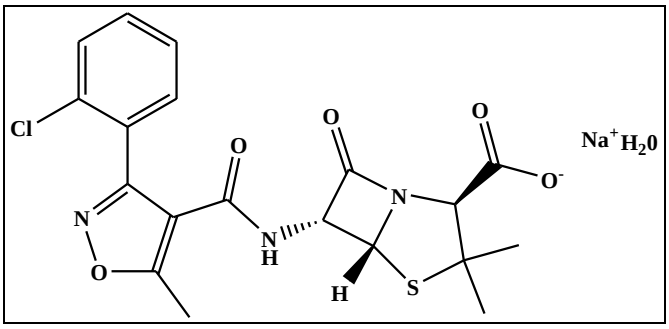


Fig 1: Chemical structure of cloxacillin sodium

Materials and Methods

Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Reagents and chemicals

Cloxacillin sodium was obtained as pure sample from Centuarian Laboratories, GIDC, Vadodara, India, as gift samples along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. Capsules Pitava 2, 2 mg Zydus Cadila Ahmedabad, India was purchased from local market. Capsules IP (500 mg) were obtained from Medipol Pharmaceutical India Pvt. Ltd.

Chromatographic conditions

The isocratic mobile phase consisted of 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile (20:80 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 224 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

Accurately weighed 10 mg of cloxacillin was transferred into 10 ml volumetric flask, dissolved in 5ml of ACN and volume was made up to 10ml with ACN to get concentration of solution 1000 µg/ml (Stock-A), 5ml of stock-A was taken and diluted up to 50ml to get concentration of 100µg/ml (Stock-B).

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 5-25 µg/ml for cloxacillin.

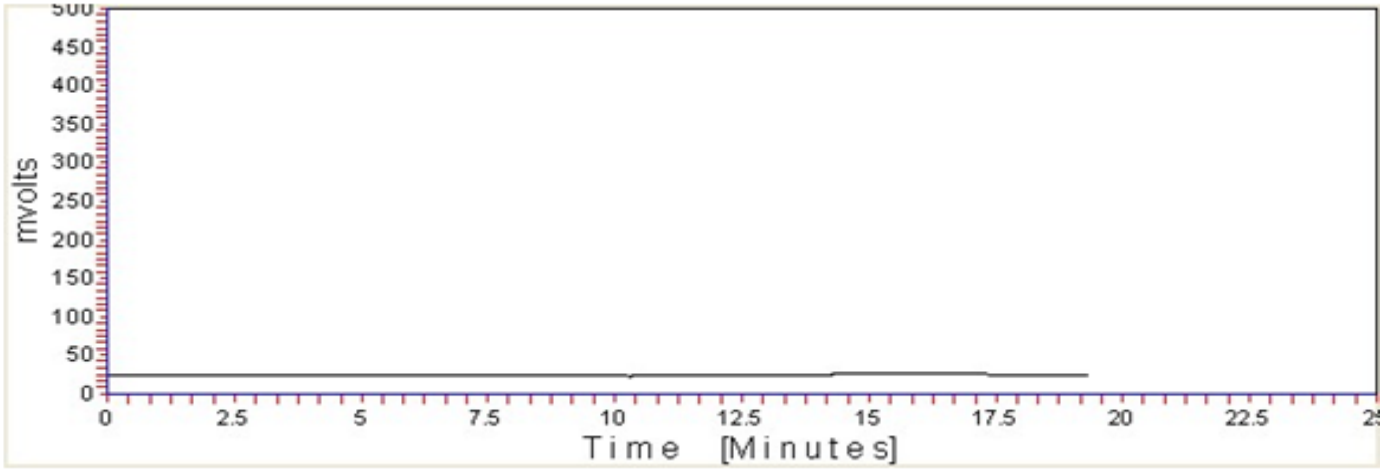
Sample preparation

Commercial formulations cloxacillin of Medipol 500mg was selected for analysis. Twenty capsules were taken and their average weight was determined. They are crushed to fine powder; amount equal to 10 mg of cloxacillin was taken in 100-ml volumetric flask. The volume is made up to the mark by mobile phase and filtered by whatmann filter paper (no.41) and Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analyzed.

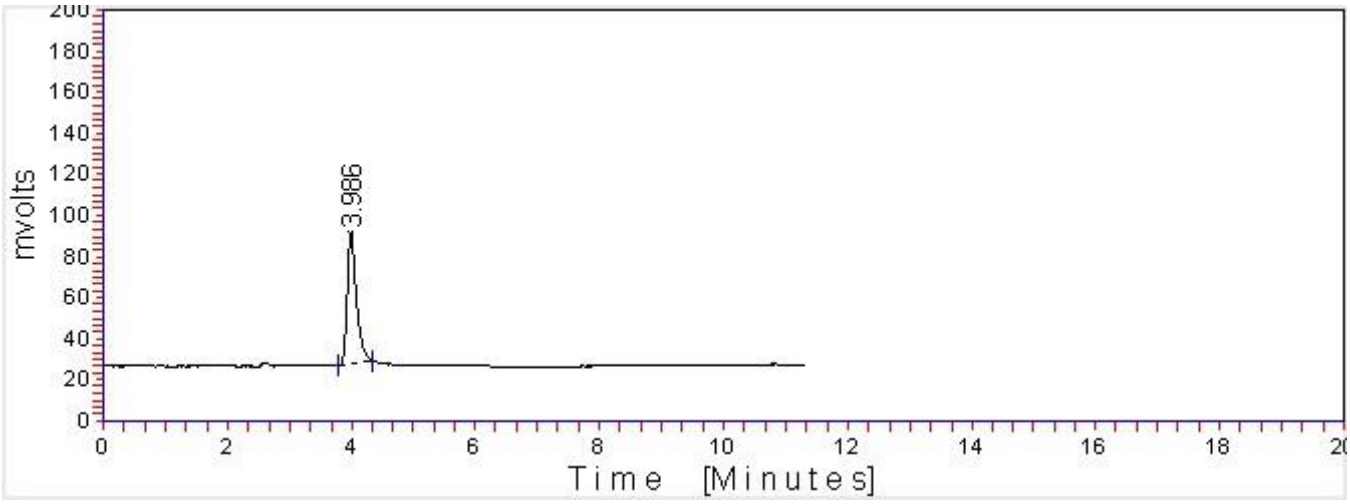
Results and Discussion

Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile (20:80 v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for cloxacillin was observed to be 3.989 ± 0.3 min. Total time of analysis was less than 6 min. The maximum absorption of cloxacillin was detected at 224nm, and this wavelength was chosen for the analysis Figure 2.



(A)



(B)

Fig 2: Chromatograms of (A) Blank mobile phase (B) Cloxacillin (10µg/ml) as reference substances

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for cloxacillin was 3553.33.

Table 1: Results of system suitability parameters

Parameters	Cloxacillin
AUC*	628.071
No. of Theoretical Plates	3553.33
Tailing Factor*	0.955
Retention time*	3.989
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25 µg/ml for cloxacillin. The linearity was represented by a linear regression equation as follows:

Y (cloxacillin) = 61.51 conc.+4.616 (r^2 = 0.997)

Accuracy

Method accuracy was performed by adding known amounts of cloxacillin to the preanalysed tablet solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80%, 100% and 120% of the nominal analytical concentration (10µg/ml for cloxacillin). Each level was made in triplicate table 2. The mean percentage recoveries obtained for cloxacillin was 99.78% and RSD was less than 1.

Table 2: Results of recovery study

Statistical data	Cloxacillin		
	80%	100%	120%
% Mean*	99.62	99.71	99.78
SD*	0.084	0.091	0.170
%R.S.D*.	0.084	0.092	0.170

*Mean of nine determinations (three replicates at three concentration level)

Precision

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations and results were found within acceptable limits (RSD < 2) as shown in table 3.

Table 3: Statistical data for precision

Statistical parameter	Cloxacillin		
	Mean*	S.D*	R.S.D*
Repeatability	99.74	0.028	0.028
Intermediate Precision (I) (A day to day)	99.27	0.041	0.383
(II) Analyst to Analyst	99.62	0.99	0.99
Robustness	99.45	0.065	0.546

*Mean of 15 determinations (three replicates at five concentration level)

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM KH_2PO_4 (pH-3.0 with OPA): Acetonitrile (20:80% v/v), to (15: 85% V/V) and method is found robust as RSD is again found < 2.0 table 3.

Specificity and selectivity

Commonly used excipients were spiked in to a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Figure 3. Where complete separation of cloxacillin in presence of capsule excipients.

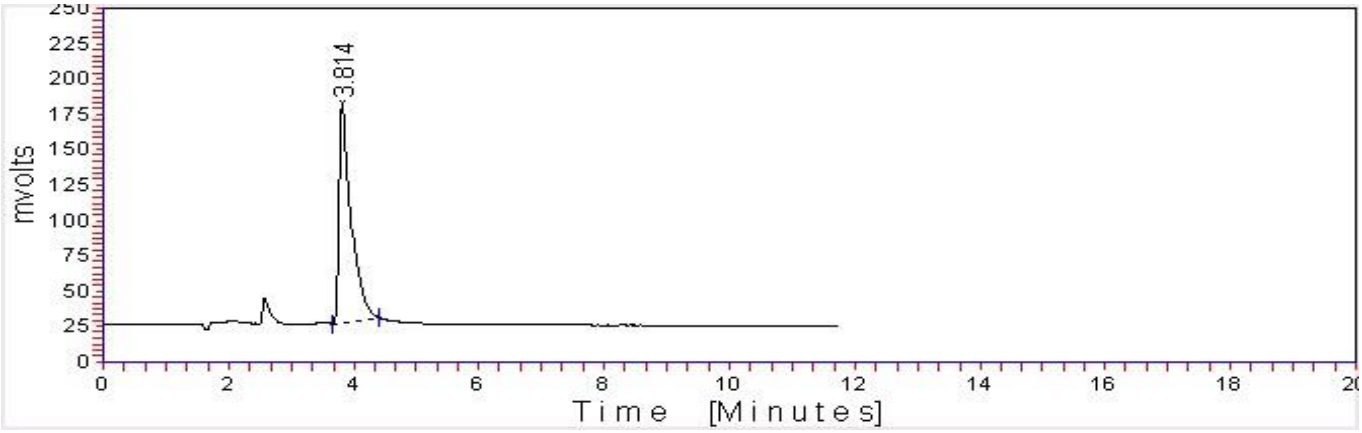


Fig: 3 Chromatograms of cloxacillin (25µg/ml) in a capsule formulation

Detection limit and quantitation limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve table 4.

Table: 4 LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Cloxacillin	0.095	0.271

Analysis of capsules

The concentration of cloxacillin in the capsules formulation was found to be 99.98%. The low values of % RSD indicate that the method is precise and accurate in table 5.

Table 5: Results of tablet analysis

S. No.	Parameter	Sample
		Cloxacillin
1	% Found	99.98
2	S.D.	0.125
3	% R.S.D.	0.125

* Mean of nine determinations

Conclusion

The proposed HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of cloxacillin by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of cloxacillin with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and the run time of less than six minutes allow its application for the routine determination of cloxacillin in the pharmaceutical dosage form.

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