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Development and Validation of new RP-HPLC method for the estimation of Oxacillin sodium in injectable dosage forms

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ABSTRACT:

A rapid and simple RP-HPLC chromatographic method has been developed for the estimation of Oxacillin in API and Injectable dosage form. Chromatographic separation was achieved on Phenomenex C18 column (250× 4.6 mm; 5 µm) using 0.1% *Ortho* – phosphoric acid buffer (pH 6) and acetonitrile in the ratio 50:50 v/v as mobile phase at a flow rate of 1ml/min. The detection wavelength was set as 240 nm. The retention time of the drug was found to be 2.837 min. The method was applied to injectable dosage form, without any interference from excipients. The calibration curve was linear over the range of 10-150 µg/ml. The performance of the method was validated according to ICH guidelines and it was found suitable for the analysis of oxacillin in routine analysis.

Keywords: oxacillin , RP-HPLC, Method development, Validation, ICH guidelines.

INTRODUCTION

Oxacillin (Fig. 1) is a Anti-biotic binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall. Oxacillin inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Oxacillin interferes with an autolysin inhibitor.

Literature survey revealed that several analytical methods have been reported for the quantitative estimation of Oxacillin in tablet dosage forms by HPLC and UV spectrophotometric techniques [1-4]. We tried to develop and validate RP-HPLC method with short retention and run times in bulk and injectable dosage forms. Conformation of the applicability of the developed method was validated according to ICH guidelines.

Fig 1: Structure of Oxacillin

MATERIALS AND METHODS

EXPERIMENTAL SECTION

Chemicals, Solvents and Drugs used:

Ortho - phosphoric acid, triethylamine GR grade and acetonitrile HPLC grade was purchased from Merck Chemicals Limited. HPLC grade water was prepared using Millipore Milli-Q system. Oxacillin working standard was obtained from Aurobindo Pharma Ltd. (Hyderabad, India) as gift sample.

Equipment and chromatographic conditions:

The chromatographic system consisted of Shimadzu HPLC fitted with Prominence LC 20 AD Series pump and SPD 20A UV detector using LC Solutions software as data handling system. Phenomenex C18 (250 x 4.6 mm, 5 μ m) was used for this method. All chromatographic runs were carried out in isocratic mode with a flow rate of 1.0 mL/min. *Ortho* – phosphoric acid buffer was prepared by dissolving 0.3mL of *ortho* – phosphoric acid in 300mL of triple distilled water. pH was adjusted to 6 using 10% v/v triethylamine. It was filtered through 0.45 μ filter and sonicated. The mobile phase consisted of buffer and acetonitrile (HPLC grade) in the ratio of 50:50%v/v. The detector wavelength was set at 240 nm. The injection volume was 20 μ L.

Table 1: Optimized chromatographic conditions

Mobile phase	Buffer and acetonitrile in the ratio of 50:50 v/v
Diluent	Buffer
Stationary phase	Phenomenex C18 250x4.6 mm, 5 μ column
Flow rate	1ml/min
Column temperature	22°C
Volume of injection	20 μ l
Detection wavelength ($\lambda_{\max}$)	240nm
Run time (min)	10

Preparation of Diluents:

Mobile Phase was used as diluent.

Preparation of buffer: 0.3ml of orthophosphoric acid was dissolved in 300 ml of triple distilled water and the pH was adjusted to 6 with 10% of triethyl amine.

Preparation of standard drug solutions:

Stock solution of Oxacillin sodium was prepared by dissolving 100mg of Oxacillin sodium in a 100ml volumetric flask with small quantity of diluent and the

mixture was sonicated for about 15min and then made upto volume with diluent. From the stock solution, 100 μ g/ml of Oxacillin sodium solution was prepared by pipetting 1ml into a 10ml volumetric flask and diluted upto the mark with the diluent. All the stock solutions are stored at 20°C.

Recommended procedure for standard graph:

The dilutions containing concentrations in the range of 10-125 μ g/ml of Oxacillin sodium was prepared with the diluent. The solutions were filtered through a 0.22 μ Millipore filter and prior to use. These solutions were injected into HPLC system for six times each. The run time was 10 min and peak areas were measured. Calibration curve data was subjected to plot between concentration (μ g/mL) and mean area of injections of the drug solution.

Estimation of the drug from Injectable dosage forms:

A vial was reconstituted with 10 ml of water and shaken vigorously. Contents were transferred into a clean and dry 100ml volumetric flask. The vial was washed with diluent thrice and the washings were collected into the 100ml volumetric flask. The volume was made upto the mark using diluent and mixed thoroughly. 10 ml of the above solution was transferred into a 100ml volumetric flask and made upto mark with diluent and mixed. This solution was filtered through 0.45 μ m membrane filter (discarding first few ml), and used as stock solution. The above solution was then chromatographed six times. The mean peak area of the drug was calculated and the drug content in the formulation was calculated by the regression equation of the method.

RESULTS AND DISCUSSION

During initial method optimization studies C18 columns with different column lengths were tried. Finally below mentioned chromatographic conditions were finalized after evaluating column efficiency parameters like theoretical plates and tailing. Wavelength was selected by scanning standard solution of the drug in diluent, over 200nm to 400nm. Using mobile phase, base line separation for the oxacillin peak was achieved. Under these conditions the retention time of oxacillin was found to be 2.837 min.

Specificity

A good analytical method should be able to measure the analytes accurately in the presence of suspected interferences such as blank, excipients, and degradation products. Fig. 2 shows chromatographic base line separation of oxacillin. Fig.3 demonstrates that no interferences were found at the retention time of oxacillin in its dosage form due to excipients.

Fig. 2: Representative chromatogram obtained from the analysis of oxacillin from working standard solution

Fig .3: Chromatogram of oxacillin separation in dosage form from its excipients

Linearity

The regression of the plot was computed by least square regression method and is shown in the Fig 4. The calibration curve (n=3) constructed for the drug was linear over the concentration range of 10-150 µg/ml. the correlation coefficient is greater than 0.99 and the % RSD for each concentration studied was less than 2. The linearity data is shown in the Table. 2 and Fig. 4 demonstrates the linearity plot for oxacillin concentrations.

Table 2: Linearity data

S. No	Concentration (µg/ml)	Mean Peak Area (n=3)
1	10	293351
2	20	711693
3	50	1663605
4	75	2432705
5	100	3243606
6	125	4054508
7	150	4959823

deviation of the percentage recovery were calculated and represented in Table 3. The high percentage of recovery indicates that the proposed method is highly accurate. The precision of the method was demonstrated by inter-day and intra- day variation studies. Six replicate injections of sample solutions were made and the percentage RSD was calculated and represented in Table 4. From the data obtained the developed RP-HPLC method was found to be precise.

Robustness study

The robustness of the method was determined as per USP guidelines under a variety of conditions including change in flow rate and pH of buffer, detection wavelength. The results obtained by deliberately variation in method parameters and data are summarized in Table 5.

System suitability parameters:

System suitability parameters were studied with six replicates of standard sample solution and the parameters are presents in Table 6.

Table 3: Accuracy data for the proposed method

Analyte	Amount of the analyte taken (µg/ml)	Mean recovery (µg/ml) ± SD	% mean recovery ± SD
Oxacillin	80%	79.62251±0.34	99.5281±0.42
	100%	99.06541±0.954	99.0654±0.95
	120%	119.089±1.28	99.24080±1.06

Table 4: Precision data of the proposed method

	Intra-day precision	Inter-day precision
Mean peak area	3234009.667	3241424.833
SD	14986.45	16037.89
% RSD	0.46	0.49

Table 5: Robustness study

		Retention time	Peak areas	Theoretical plates	USP tailing	
Oxacillin	Flow rate	0.9 ml/min	3.273	3703542	7128.855	1.35
		1.1ml/min	2.628	2767231	5916.178	1.42
	Detection wavelength	239 nm	2.866	3162778	6194.068	1.4
		241nm	2.866	2843350	6191.42	1.42
	Mobile phase	51:49	2.823	3059402	6130.452	1.38
		49:51	2.917	3082927	6150.157	1.37

Table 6: System Suitability Parameters

Parameter	Value
Retention time(min)	2.84 min
Asymmetry	1.380
Theoretical plates	6140.210
HETP	24.429

Fig. 4: Linearity plot for Oxacillin

CONCLUSION

The proposed RP-HPLC method is sensitive, precise, accurate and robust and can be used for the routine quality control analysis for the determination of oxacillin in its injectable dosage form.

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