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A Validated RP – HPLC Method for Simultaneous Estimation of Cefixime and Cloxacillin in Tablets

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Abstract: This paper presents a RP-HPLC method for the simultaneous estimation of cefixime and cloxacillin in tablets. The process was carried out on C₁₈ column (5 µm, 25 cm x 4.6 mm, i.d) using phosphate buffer (pH 5.0), acetonitrile and methanol in the ratio 80:17: 3 respectively as a mobile phase at a flow rate of 2mL/min. Wavelength was fixed at 225 nm. The retention time of cefixime and cloxacillin was found to be 5.657 and 6.200 min, respectively. The developed method is rapid and sensitive and it can be used for estimation of combination of these drugs in tablets.

Keywords: Cefixime, Cloxacillin, RP-HPLC Estimation.

Introduction

Cefixime, chemically designated as (6R, 7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-(Z)-[o-(carboxymethyl)-oxime] trihydrate is an antibiotic, and an oral third-generation cephalosporin. It is highly stable in the presence of *beta*-lactamase enzymes. Cloxacillin, chemically 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 semisynthetic antibiotic in the same class as penicillin. It used against staphylococci that produce *beta*-lactamase. From literature survey it was found that various methods have been reported for both the drugs individually but no HPLC methods were reported for such a combination in any type of pharmaceutical dosage form so far. The present work describes a simple, precise, and accurate reverse phase HPLC method for simultaneous estimation of Cefixime and Cloxacillin in combined dosage form.

Experimental

Acetonitrile and methanol used were of HPLC grade and obtained from Merck Chemicals. All other chemicals used were of AR grade and obtained from Sd Fine Chemicals, Mumbai. Reference standards of cefixime and cloxacillin were obtained from Concept Pharmaceuticals Ltd., Aurangabad, India.

Instrumentation

Quantitative HPLC was performed on a isocratic HPLC of SHIMADZU prominence consisting of LC – 20AT liquid pump, manual with 20 μ L sample injection loop and SPD 20A UV-visible absorbance detector. The output – signal was monitored and integrated by Shimadzu spin chrome software.

Chromatographic conditions³⁻⁵

The process was carried out on C₁₈ column (5 μ m, 25 cm x 4.6 mm, *i.d*) using the mobile phase consisting of phosphate buffer (pH 5.0), acetonitrile and methanol^{1,2} in the ratio (80:17: 3 v/v) respectively at a flow rate of 2mL/minutes. Wavelength was fixed at 225 nm. The mobile phase was filtered through 0.2 μ membrane filter and degassed.

Preparation of solutions

Standard solution of the pure drug was prepared by dissolving 100 mg of cefixime trihydrate and 250 mg of cloxacillin sodium in a 100 mL volumetric flask using 25 mL of methanol. Then the volume made up to the mark with the same solvent. Appropriate volume from this solution was further diluted to get appropriate concentration levels according to the requirement.

Twenty tablets were weighed the average weight was determined and these were powdered. Sample solution was then prepared by dissolving the powdered tablets equivalent to 100 mg of cefixime and 250 mg of cloxacillin in a 100 mL of volumetric flask. Then the drugs were dissolved by using 25 mL methanol and the volume was made up to the mark with methanol. 5 mL of this solution was further diluted to 25 mL with the same solvent.

20 μ L of solution was injected into HPLC system to obtain chromatogram for standard drug solution and sample solution. Concentrations of cefixime and cloxacillin in the formulation were calculated by comparing AUC of the sample with that of the standard.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of cefixime and cloxacillin was found to be 4.657 and 6.200 min respectively. This procedure was repeated for the sample solution obtained from the formulation (Table 1) and recovery studies (Table 2).

Table 1. Analysis of formulation

S.No	Brands/ Manufactures	Label amount, mg	Amount recovered*, mg	
			Cefixime	Cloxacillin
1.	Formulation-1	Cefixime 200	198.57 \pm 0.8203	499.94 \pm 0.7637
2.	Formulation-2	Cloxacillin 500	199.70 \pm 0.7071	505.72 \pm 0.0495
3.	Formulation-3		198.35 \pm 0.1626	502.40 \pm 0.5515

*Each value is average of three determinations \pm SD

Method validation^{6,7}

Linearity and range of method was determined on standard solution by analyzing 80 to 120 % of test concentration, and the calibration curve was plotted using AUC versus concentration of standard solution. Accuracy of method was ascertained by recovery study by adding a known amount of standard drug ($\pm 20\%$ of test concentration) to pre-analyzed sample and reanalyzing the samples by the proposed method. Precision was studied by analyzing five replicates of standard solution. Specificity was carried out by injecting placebo solution. Robustness of method was evaluated by performing the assay with variations in wavelength, pH and flow rate. The chromatographic parameters were also validated by system suitability studies (Table 3), which were carried out on freshly prepared standard stock solution.

Results and Discussion

The typical chromatogram obtained from the formulation is presented in Figure 1. The retention time for cefixime and cloxacillin was found to be 4.657 and 6.200 minutes respectively. Peaks were well resolved with resolution of 6.986 between the two drugs and were symmetrical in shape with asymmetry factor less than 2.00. Linearity was observed in the concentration range of 160 -240 $\mu\text{g/mL}$ for cefixime and 400 – 600 $\mu\text{g/mL}$ cloxacillin, with the correlation coefficient of 0.9998 for cefixime and 0.9999 for cloxacillin. Accuracy of the method was ascertained by recovery study ($n=3$). The concentration of standard spiked to the sample was 80% - 120% of the assay level. Recovery data form the study is reported in Table 2. The method was found to be accurate with percent recoveries between 99.99% and 102.24%. There was good repeatability of proposed method with percentage RSD 0.69 for cefixime and 0.77 for cloxacillin. The results of specificity studies indicated no interference from excipients and mobile phase; the peak response was due to individual components only.

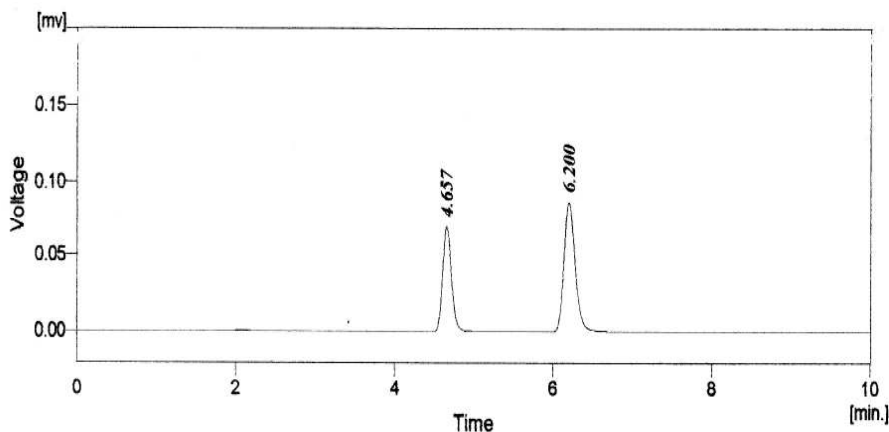


Figure 1. Typical chromatogram of the sample solution

Table 2. Recovery Studies

Sample ID	Statistical analysis Amount recovered*, mg	
	Cefixime	Cloxacillin
S1 - 80 %	Mean* = 98.99 \pm 0.1552	Mean* = 102.24 \pm 0.1950
S2 - 100 %	Mean* = 99.45 \pm 0.1450	Mean* = 101.37 \pm 0.1007
S3 - 120 %	Mean* = 100.21 \pm 0.2739	Mean* = 101.49 \pm 0.1916

*Each value is average of three determinations \pm SD

Table 3. System suitability parameters

Parameter	Cefixime	Cloxacillin
Calibration range, µg/mL	160 - 240	400 - 600
Theoretical plates	8826	10366
Resolution	6.986	
Tailing factor	1.269	1.242

Conclusion

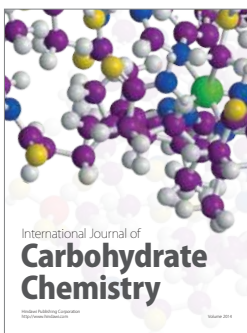
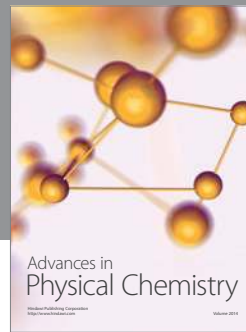
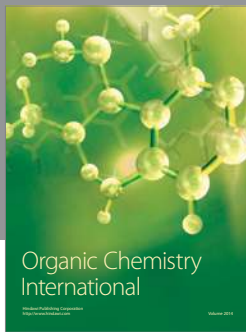
The proposed method was found to be simple, precise, accurate and rapid for determination of cefixime and cloxacillin from tablets. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims. Hence, it can be easily and conveniently adopted for routine analysis of cefixime and cloxacillin in tablets.

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