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RP-HPLC Method for Simultaneous Estimation of Cefixime Trihydrate and Cloxacillin Sodium from Bulk and Tablet Dosage Form

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ABSTRACT

A simple and effective RP-HPLC method has been developed for the estimation of cefixime and cloxacillin in combination on C8 HiQsil column using phosphate buffer pH(3): Acetonitrile, (70:30 v/v) as mobile phase at flow rate of 1ml/min. Detection carried at 225 nm. Retention time found to be 2.120 for cefixime and 6.017 for cloxacillin. The linear dynamic ranges were 2-12 µg/ml (r^2>0.999) for Cefixime Trihydrate and 5-30µg/ml (r^2>0.995) for Cloxacillin sodium, respectively. The mean % recovery was found to be 100.314 % for Cefixime trihydrate and 100.830 % for Cloxacillin Sodium. The method was quantitatively evaluated in terms of linearity, precision, accuracy (recovery), selectivity and robustness in accordance with ICH guidelines. The obtained results show the proposed RP-HPLC method is simple, rapid, precise, accurate and cost-effective which is useful for the routine determination of Cefixime trihydrate and Cloxacillin sodium in bulk drug and in its tablet dosage form.

ABBREVATIONS: CFX-Cefixime, CLOXA-Cloxacillin, RSD- Relative Standard Deviation.

INTRODUCTION

Cefixime (CFX) is chemically 8-[[2-(2-Amino-1, 3-thiazol-4-yl)-2- (carboxymethoxyimino) acetyl] amino]-4-ethenyl-7-oxo-2-thia-6-azabicyclo [4.2.0] oct-4-ene-5-carboxylic acid. It is an oral third generation cephalosporin antibiotic which is used to treat a number of bacterial infections [1]. Cloxacillin(CLOXA) is chemically (2S,5R,6R)-6-[3-(2-chlorophenyl)5-methyl-1,2-oxazole-4-4-thia-1-azabicyclo[3.2.0]heptanes-2-carboxylic acid which act like β-lactamase resistant penicillin antibiotic with antibacterial activity[2]. The structures are presented in Figure 1.[3,4] Few methods have been reported for quantitative determination of drugs CFX and CLOXA in single or in combination with other drugs such as UV and RP-HPLC and HPTLC. [5-9] Few methods are reported for quantitative determination of CFX and CLOXA in combination such as UV spectrophotometry and RP-HPLC [10-14]. The reported method had long retention time, complex mobile phase composition and low linearity range. Therefore in the present study, an attempt was made to develop a simple, precise, accurate RP-HPLC for simultaneous estimation of drugs for the analysis of Cefixime and cloxacillin in pharmaceutical formulation.

a) b)

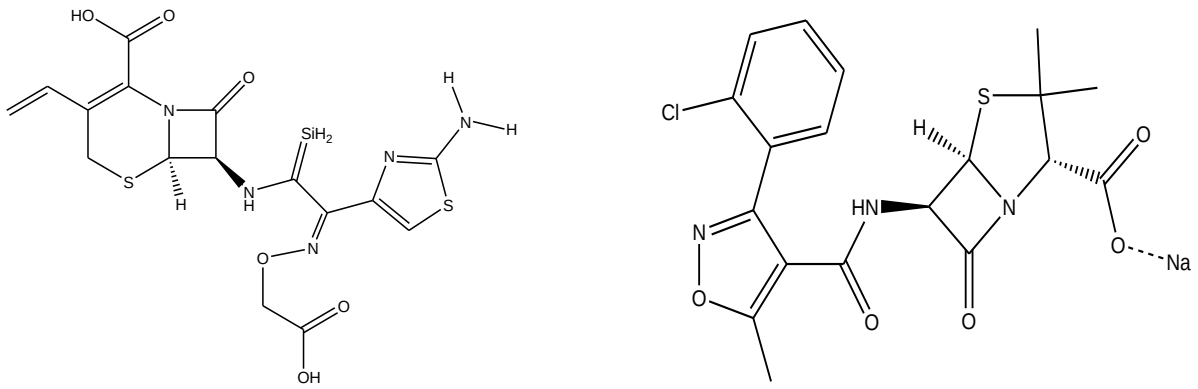

Figure 1: Structure of a) Cefixime and b) Cloxacillin

MATERIALS AND METHODS

INSTRUMENTATION

The RP-HPLC was carried on JASCO HPLC (PU 2080 Plus, Japan) equipped with Jasco UV detector (PU 2010 Plus, Japan). Samples were injected through Rheodyne sample injection port (50 µl), HiQSil C8 Column (250 x 4.5mm, i.d. 5 µm) was used. Data acquisition and integration were performed using Borwin software (version 1.5).

MATERIAL AND REAGENT

Pure drug samples (API) of cefixime and cloxacillin were obtained from Cadila Pharmaceuticals Ltd, Gujarat and KDL-Biotech Pharmaceutical Industries Ltd, Mumbai, respectively as gift sample. The drug samples were used without further purification. HPLC grade water was obtained from ELGA-Lab water purification system (PURELAB UHQ-II, United Kingdom). Acetonitrile used for HPLC was of HPLC grade (LOBA Chemie, Mumbai, MH, India). Cefolac-XL 200 tablets manufactured by MACLEODS pharmaceuticals Ltd. containing cefixime 200 mg and cloxacillin sodium 500 mg were procured from local pharmacy shop.

CHROMATOGRAPHIC CONDITIONS

The mobile phase was prepared by mixing Phosphate buffer pH(3): acetonitrile ratio (70:30 % v/v) and filtered through 0.45 µm membrane filter using vacuum pump and ultrasonicated for 10 min for degassing. The flow rate was 1 ml/min. 50 µl of the solution was injected and chromatograms were recorded. Quantization based on peak area was achieved using UV detector at 225 nm. All determinations were performed at ambient temperature. The retention time of CFX and CLOXA were 2.120 min and 6.017 min, respectively. The representative chromatogram is shown in Figure 2.

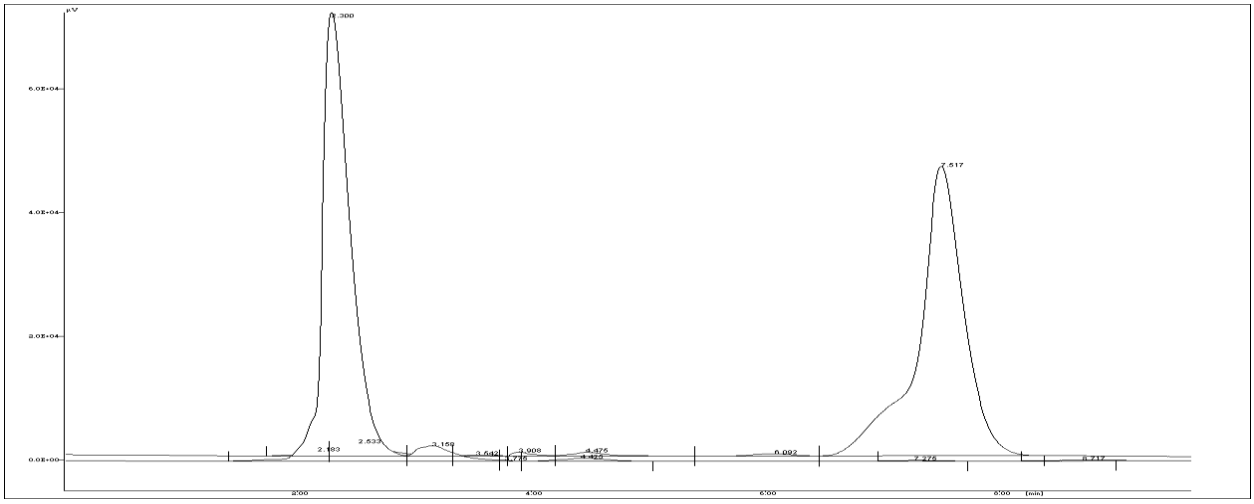

Figure 2: Chromatogram of standard Mixture CFX & CLOXA (10 µg/ml of each)

STANDARD STOCK SOLUTIONS

Stock solution (1000 µg/ml) of CFX and CLOXA were prepared by dissolving accurately weighed 10 mg of drug samples in 10 ml of methanol separately. From above solution, further 5 ml was pipette out and diluted to 50 ml to produce 100 µg/ml of CFX and CLOXA, each.

WORKING SOLUTIONS

Working standard solutions were prepared from the standard stock solution of 100 µg/ml by appropriate dilution to obtain final concentration of 2.0-12.0 µg/ml of CFX and 5.0-30 µg/ml of CLOXA for HPLC (dilution with mobile phase).

ANALYSIS OF DRUG IN MARKETED FORMULATION

Twenty Tablets, each containing 200 mg of Cefixime and 500 mg of Cloxacillin were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Cloxacillin was weighed and transferred to 50 ml volumetric flask. To this, methanol was added and sonicated for 10 min; the volume was made up to 10 ml with HPLC grade methanol to get solution of 1000 µg/ml. The solution was filtered using Whatman filter paper. From the filtrate, appropriate dilutions were made using mobile phase to obtain concentration 10.0 µg/ml for CLOXA (4.0 µg/ml for CFX). The sample solution was injected and chromatograph was obtained.

VALIDATION OF PROPOSED HPLC METHOD

For validation of the developed method, the ICH Q2 (R1) guidelines were followed. The requirement for drug assay follows these topics: linearity, precision, accuracy, specificity, robustness, LOD and LOQ. [15].

SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products, and matrix components. The specificity of the method was assessed by comparing the Chromatograms obtained from the drug standards with that obtained from the tablet solution.

LINEARITY

Linearity study of HPLC detector response for determination of CFX and CLOXA was evaluated by analyzing a series of standard solutions of six different concentrations of each compound. Calibration Curves were constructed by plotting average peak areas against respective concentrations. The results found to be linear over the concentration range of 2.0-12.0 µg/ml and 5.0-30.0 µg/ml for CFX and CLOXA, respectively. Regression analysis has been carried out with coefficient of determination (r^2) 0.999 and 0.995 for CFX and CLOXA, respectively.

Calibration curve for both drugs is shown in Figure 3 and Figure 4.

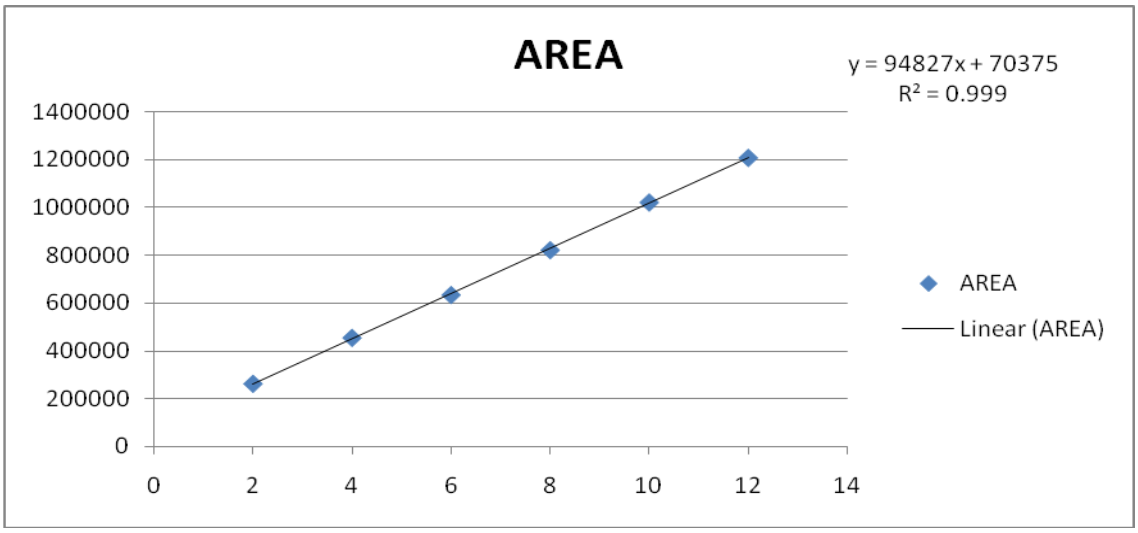

Figure 3: Calibration Curve of CFX

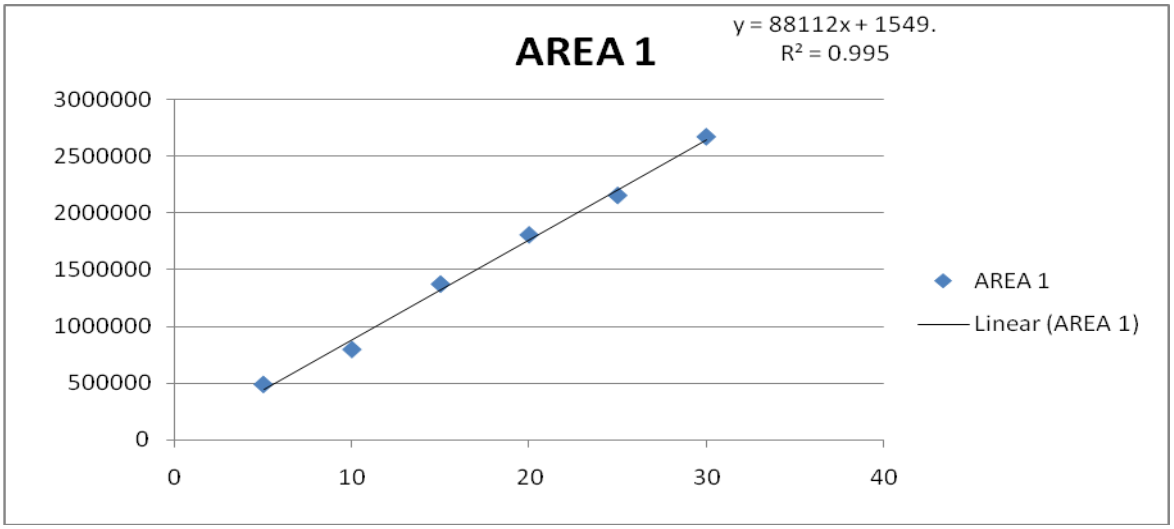

Figure 4: Calibration Curve of CLOXA

PRECISION

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The intraday and inter-day precision study of the analytical method was carried out by analyzing three replicates of three concentrations in linear range and percentage amount of Cefixime and Cloxacillin in the tablet formulation was calculated. The mean % assay value, standard deviation and percent relative standard deviation was calculated. The results obtained for intra-day and inter-day variations are shown in Table 1 and Table 2, respectively.

Table 1: Intra-day Precision of CFX and CLOXA

Intra-day Precision					
CFX			CLOXA		
Conc.(µg/ml)	Avg. area	% RSD	Conc.(µg/ml)	Avg. area	% RSD
6	641150.064	0.64	15	906023.083	0.959
8	842679.417	0.292	20	1333446.778	0.742
10	1037362.496	1.405	25	1834195.428	0.897

Table 2: Inter-day Precision of CFX and CLOXA

Inter-day Precision					
CFX			CLOXA		
Conc.(µg/ml)	Avg. area	% RSD	Conc.(µg/ml)	Avg. area	% RSD
6	644656.075	0.294	15	907363.279	0.284
8	837257.064	1.009	20	1340087.865	0.127
10	1049139.411	0.533	25	1841555.339	0.982

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

From the linearity data, the LOD and LOQ were calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y-intercept of linearity equations and S = slope of the calibration curve of the analyte. The LODs for CFX and CLOXA were found to be 0.016 and 0.145 respectively, and the LOQs for CFX and CLOXA were 0.049 and 0.439 ug/ml, respectively.

ASSAY:

Cefolac XL-200 tablet formulation analysis was carried out as mentioned under section preparation of sample solution. The procedure was repeated for six times. The sample solution was injected and the area was recorded. Concentration and % recovery was determined from the linear equation. The results obtained are shown in Table 3.

Table 3: Assay of Marketed Formulation

Sr No	Amount Present (µg/ml)		Area Found (µg/ml)		% Assay	
	CFX	CLOXA	CFX	CLOXA	CFX	CLOXA
1	4	10	457820.123	912124.626	101.898	101.222
2	4	10	459919.111	904110.643	102.445	100.338
3	4	10	450232.908	906019.135	99.919	100.548
4	4	10	449071.111	904265.116	99.616	100.355
5	4	10	456991.76	908824.602	101.682	100.858
6	4	10	450069.093	901124.626	99.876	100.009
Mean			454017.351	906078.125	100.906	100.555
SD			4743.732	3893.634	1.237	0.429
% RSD			1.044	0.429	1.226	0.429

ACCURACY

The accuracy of the method was evaluated by standard addition method in which a known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery of CFX and CLOXA was calculated at three concentration levels of 50%, 100%, and 150%. The solutions were analyzed in triplicate at each level. The percent recovery and % RSD at each level was calculated. The results obtained are shown in Table 4.

Table 4: Accuracy of CFX and CLOXA

Level of % Recovery	Mean (% Recovery)		± SD		% RSD	
	CFX	CLOXA	CFX	CLOXA	CFX	CLOXA
50	100.770	102.166	0.984	1.299	0.977	1.271
100	100.314	100.830	0.870	2.049	0.868	2.032
150	101.112	102.745	0.566	0.100	0.560	0.097

ROBUSTNESS

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, detection wavelength, flow rate were altered and the effect on the area were noted. The method found to be robust.

CONCLUSION

The method represents a fast analytical procedure for the simultaneous quantization of Cefixime and Cloxacillin. The sample preparation is simple, the analysis time is short and the elution is isocratic.

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