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RP-HPLC METHOD FOR THE 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, (70:30v/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 6.107 for cloxacillin. The linear dynamic ranges were 2-12µg/ml (r^2>0.999) for cefixime trihydrate and 5-30µg/ml (r^2>0.995) for cloxacillin sodium, respectively. The mean percent 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.

KEYWORDS: Cefixime trihydrate and Cloxacillin, RP-HPLC, Method Validation.

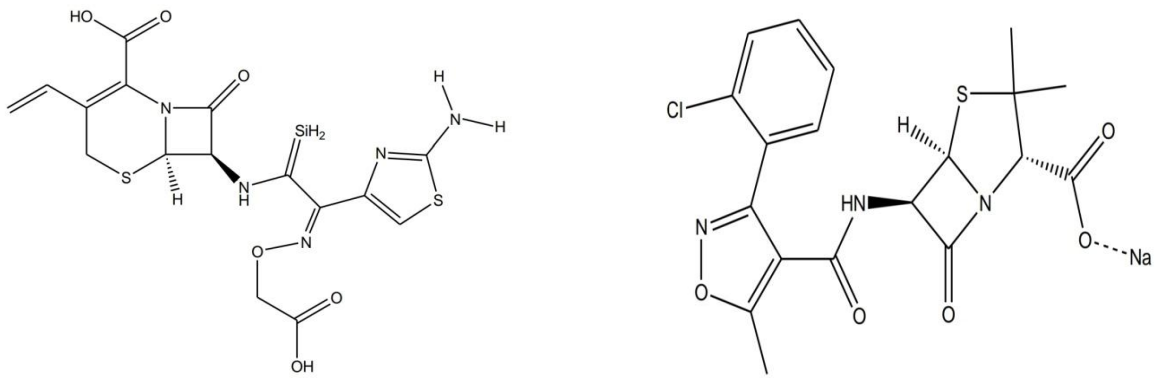
Abbrevations: CFX – Cefixime, CLOXA – Cloxacillin, RSD- Relative Standard Deviation.

INTRODUCTION

Cefixime (CFX) is chemically [2-(2-amino-1,3-thiozol-4-yl)-2-(carboxymethoxyimino) amino]-4-ethenyl-7 -oxo-2-thia-6-azabicylo [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. Cloxacilin(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 acts like β-lactamase resistant.

Penicillin antibiotic with antibacterial activity. The structures are presented in figure 1. 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. Few methods are reported for quantitative determination of CFX and CLOXA in combination such as UV spectrophotometry and RP-HPLC. 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 formulations.



a) **b)**

Figure 1: Structure of a) Cefixime 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 (50µl), HiQsil C8 column (250×4.5mm, i.d µm) was used. Data acquisition and integration were performed using Borwin software (version 1.5).

MATERIAL AND REAGENT

Pure drug samples (API) 0f cefixime and cloxacillin were obtained from cadila pharmaceuticals Ltd, Gujrat and KDL-Biotech pharmaceutical Industries ltd, Mumbai, respectively as gift samples. 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 pharmaceutical ltd. Containing cefixime 200mg and cloxacillin sodium 500mg were procured from local pharmacy shop.

Chroatographic conditions

The mobile phase was prepared by mixing phosphate buffer pH 3: acetonitrile ratio (70:30%v/v) and filtered through 0.45µm membrane filter using vaccum 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 225nm. All determinations were performed at ambient temperature. The retention time of CFX and CLOXA were 2.210 min anf 6.017 min, respectively. The representative chromatogram is shown in figure 2.

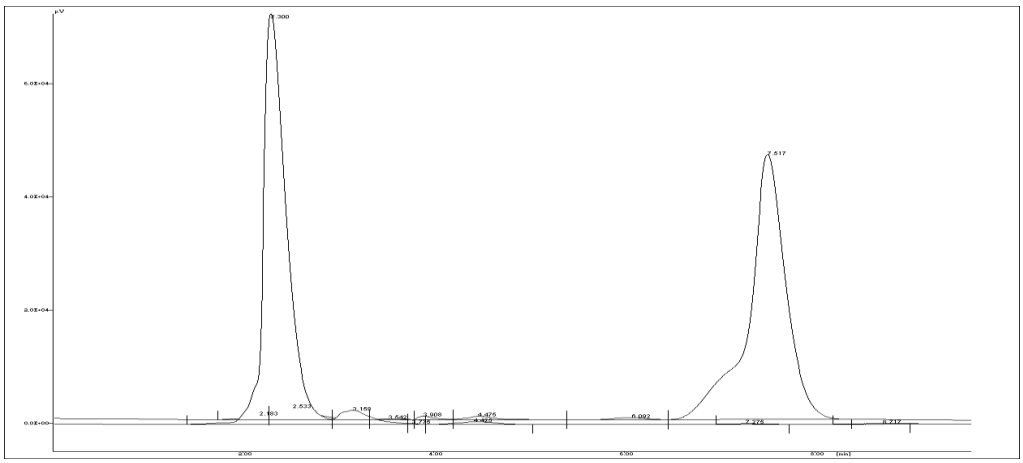


Figure 2: Chromatogram of standard mixture CFX & CLOXA.

Standard stock solution

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 5ml was pipette out and diluted to 50 ml to produce 100µg/ml of CFX and CLOXA, each.

Working solutions

Working standard solution were prepared from the standard stock solution of 100 µ/ml by appropriate dilution to obtain final concentration of 2.0-12.0 µg/ml of CFX and 5.0-3.0 µg/ml of CLOXA for HPLC (dilution with mobile phase).

Analysis of drug in marketed formulation

20 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 clocacillin was weighed and transferred to 50 ml of volumetric flask. To this methanol was added and sonicated for 10 min; the volume was made upto 10 ml with HPLC grade methanol to get solution of 1000µg/ml. The solution was filtered using whatmann filtered paper. From the filtrate, appropriate dilutions were made using mobile phase to obtain concentration 10.0µg/ml for CLOXA (4.0µg/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.

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 drugs standards with that obtained from the tablet solution.

Linearity

Linearity study of HPLC detectors 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 by 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.

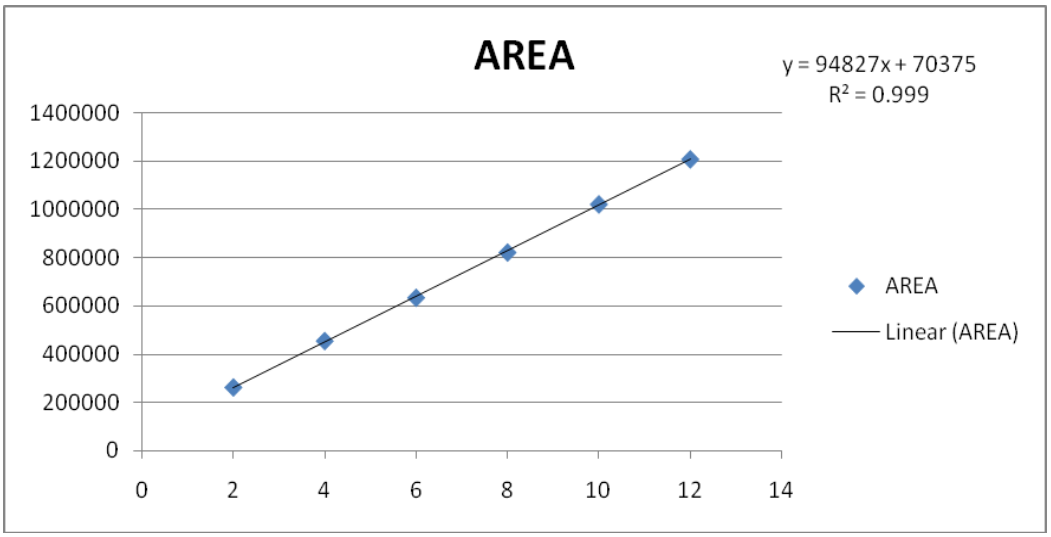


Figure 3: Calibration curve for CFX.

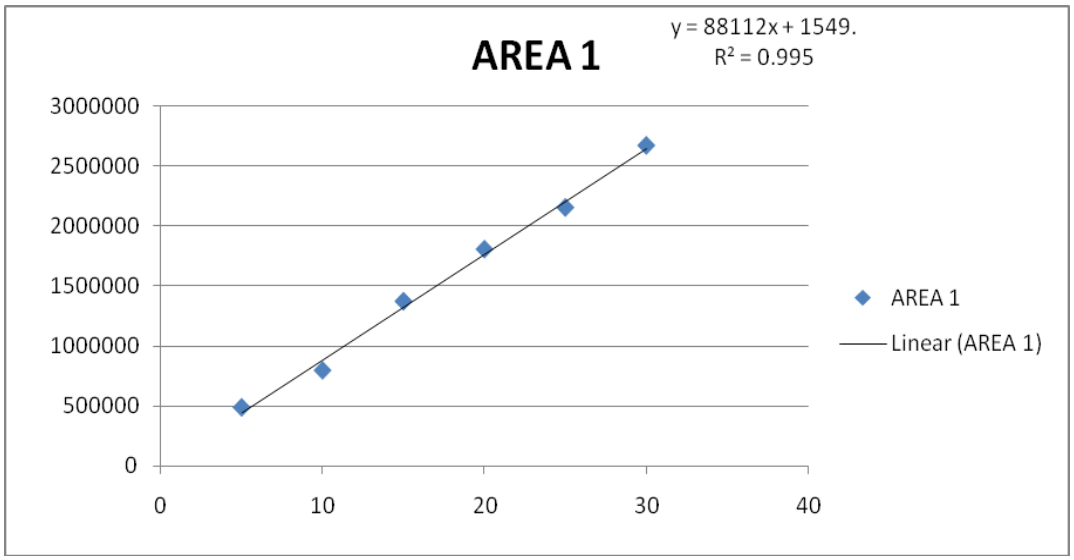


Figure 4: Calibration curve for CLOXA.

Precision

The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The intra-day and inter day precision study of the analytical method was carried out by amazing three replicates of three concentrations in linear range and the percentage amount of cefixime and cloxacillin in the tablet formulation was calculated. The results obtained by 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		
Sample	**Avg. area**	**%RSD**	**Sample**	**Avg. area**	**% RSD**
Sample1	641150.064	0.64	Sample 1	906023.083	0.959
Sample2	842679.417	0.292	Sample 2	1333446.778	0.742
Sample3	1037362.496	1.405	Sample 2	1834195.428	0.897

Table 2: Inter day precision of CFX and CLOXA.

Inter-day precision					
CFX			CLOXA		
sample	**Avg.area**	**%RSD**	**Sample**	**Avg.area**	**%RSD**
Sample1	644656.075	0.294	Sample 1	907363.279	0.284
Sample2	837257.064	1.009	Sample 2	1340087.865	0.127
Sample3	1049139.411	0.533	Sample 2	1841555.539	0.982

Limlt of detection (lod) and Limit of quantification (Lod)

From the linearity data, the LOD and LOQ were calculated, using the formula LOD = 3.3 σ/S and LOQ= 10σ/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 a and CLOXA were 0.049 and 0.439 µg/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 formulations.

Sr. no	Sample		Area found (µg/ml)		% Assay	
	CFX	CLOXA	CFX	CLOXA	CFX	CLOXA
1	Sample1	Sample1	457820.123	912124.626	101.898	101.222
2	Sample2	Sample2	459919.111	904110.643	102.445	100.338
3	Sample3	Sample3	450232.908	906019.135	99.919	100.548
4	Sample4	Sample4	449071.11	904265.116	99.616	100.355
5	Sample5	Sample5	456991.76	908824.602	101.682	100.858
6	Sample6	Sample6	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 50%, 100%, 150%. The solutions were analyzed in triplicate at each level. The percent recovery and the percent 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
0	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	0.560	0.560	0.097

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile face compositions, detection wave length, 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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