



DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN HEMIHYDRATES AND CEFIXIME TRIHYDRATE IN MARKETED FORMULATION

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ABSTRACT

A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of Levofloxacin hemihydrates and Cefixime trihydrate from tablet dosage forms using C18 column (Grace Smart (250mm x 4.6mm, 5µm). The sample was analyzed using 0.08M Potassium dihydrogen phosphate: Acetonitrile in the ratio of 60:40 (%v/v) pH adjusted to 6.5 with triethylamine, as a mobile phase at a flow rate of 1.0ml/min and detection at 254nm. The retention time for Cefixime trihydrate and Levofloxacin hemihydrates was found to be 2.19min and 3.60min respectively. The method can be used for estimation of combination of these drugs in tablets. The method was validated as per ICH guidelines. The linearity of developed method was achieved in the range of 20-120µg/mL ($r^2=0.9995$) for Cefixime trihydrate and 20-120µg/mL ($r^2=0.9995$) for Levofloxacin hemihydrates and assay of tablets were between 98.0-102.0%. Due to these attributes, the proposed method could be used for routine quality control analysis of these drugs in combined dosage forms.

Keywords: RP-HPLC Method, LEVO and CEF, Levofloxacin hemihydrates and Cefixime trihydrate.

INTRODUCTION

Cefixime trihydrate (CEF) is official in Indian pharmacopoeia. Chemically, it is (6R,7R)-7- {[2-(2-amino-1,3-thiazol-4-yl)-2 (carboxymethoxyimino) acetyl]amino}-3-ethenyl 8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid, clinically used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary tract infection. The antibacterial effect of cefixime results from inhibition of mucopeptide synthesis in the bacterial cell wall. Levofloxacin hemihydrates are official in Indian pharmacopoeia. Chemically, it is (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo [7.3.1.0^{5,13}] trideca 5(13),6,8,11-tetraene-11-carboxylic acid. It is used to treat the Pneumonia and

exacerbations of chronic bronchitis, sinusitis, enteric fevers, Pyelonephritis and Skin/Soft tissue infections. Literature survey revealed that a number of analytical methods which include HPLC⁶, HPTLC⁷, UV^{8,9}, were reported for the estimation of Cefixime trihydrate and Levofloxacin hemihydrates individually and in combination with other drugs. But there is not any reported HPLC method for this combination. The aim of the present study was to develop accurate, precise and selective HPLC methods for the analysis of Cefixime trihydrate and Levofloxacin hemihydrate¹⁻⁵. This paper describes RP-HPLC method for the simultaneous estimation of LEVO and CEF in combination in Tablet dosage form. Structures of LEVO and CEF are shown in Figure 1.

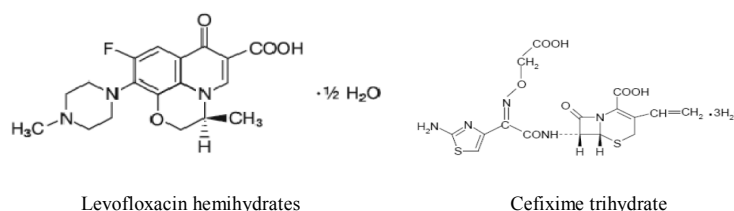


Figure: 1 Structure of LEVO and CEF

MATERIALS AND METHODS

Materials

Working standards of pharmaceutical grade Levofloxacin and Cefixime were obtained as generous gifts from Cadila pharmaceuticals. It was used without further purification. Fixed dose combination Tablet Cefi-L- (manufactured by Akum Drugs & Pharma) containing 500mg Levofloxacin and 400mg Cefixime were procured from local market. All chemicals and reagents of analytical grade were purchased from s d Fine-chem. Ltd.

Preparations of mobile phase and stock solution

600ml 0.08M potassium dihydrogen phosphate was prepared in double distilled water. This solution was mixed with 400ml of Acetonitrile, finally pH was adjusted to 6.5 with triethylamine (mobile phase). The solution was sonicated for 10minutes and filtered using Whatman filter paper (No.1). LEVO and CEF were weighed (25mg each) and transferred to two separate 25ml volumetric flasks and dissolved in mobile phase and volume was adjusted to mark with mobile phase, which gives 1000µg/ml of LEVO and CEF, respectively. For LEVO 25.0ml was further diluted to 50ml with mobile phase to obtain the final concentration of 500µg/ml from this working solution of LEVO

(20,40,60,80,100,120µg/ml) were prepared. For CEF 25.0ml was further diluted to 50ml with mobile phase to obtain the final concentration of 500µg/ml from this working solution of LEVO (20,40,60,80,100,120µg/ml) were prepared.

Chromatographic conditions

A reverse phase C18 column equilibrated with mobile phase 0.08M KH₂PO₄: Acetonitril (60:40) (adjusted to pH 6.5 with the triethylamine) was used. Mobile phase flow rate was maintained at 1.0ml/min and effluents were monitored at 254nm. The sample was injected using a 20µL fixed loop, and the total run time was 8min. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for LEVO and CEF.

Determination of LEVO and CEF in their combined dosage forms

Twenty tablets were taken and weighed and powdered. Powder equivalent to LEVO 500.0mg and 400.0mg CEF was accurately weighed and transferred to a 100ml volumetric flask, 20ml of mobile phase was added and sonicated for 5min then volume was adjusted up to the mark with mobile phase. The above solution was filtered using Whatman filter paper No.1. 1ml volume of the aliquot was transferred to a 10ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 500µg/ml of LEVO and 400µg/ml of CEF. Further 1 volume of the aliquot was transferred to a 10ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 50µg/ml of LEVO and 40µg/ml of CEF. The solution was injected at above chromatographic conditions (n=6) and peak areas were

measured. The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

Accuracy

The accuracy of the method was determined by calculating recoveries of LEVO and CEF by method of standard additions. Known amount of LEVO (40, 50, 60µg/ml) and CEF (32, 40, 48µg/ml) were added to a pre-quantified sample solution and the amount of LEVO and CEF were estimated¹¹.

Precision

The intraday and inter day precision study of LEVO and CEF was carried out by estimating the corresponding responses 3times on the same day and on 3 different days for 3 different concentrations of LEVO (40,60,80µg/ml) and CEF (40,60,80µg/ml) and the results were reported in terms of relative standard deviation (RSD), [Table 2]. The Repeatability studies were carried out by estimating response of one test concentration concentrations (50+40µg/ml) for 6times and results are reported in terms of relative standard deviation (RSD)¹¹.

Detection limit and Quantitation limit

A calibration curves (n=5) were prepared using concentrations in the range of 20-120µg/ml for LEVO and 20-120µg/ml for CEF. The standard deviation of y-intercepts of regression lines were determined and kept in following equation for the determination of detection limit and quantitation limit.

Detection limit = $3.3\sigma/s$; quantitation limit = $10\sigma/s$;
where σ is the standard deviation of y- intercepts of regression lines and s is the slope of the calibration curve¹¹.

Table 1: Regression Analysis of the Calibration Curves for the Proposed Method

Parameters	CEF	LEVO
Linearity Range (µg/ml)	20 - 120	20 - 120
Slope	18.38	14.89
Standard deviation of Slope	0.005	0.005
Intercept	-7.43	69.48
Standard deviation of Intercept	0.314	0.908
Correlation co-efficient	0.9995	0.9995

Table 2: Summary of Validation Parameters

Parameters	CEF	LEVO
Limit of detection(µg/ml)	0.056	0.201
Limit of quantification(µg/ml)	0.171	0.609
Accuracy (%)	98.02-100.68	98.38-101.42
Precision (RSD%)		
Intraday(n=3*3)	0.602 – 1.641	0.439 – 1.497
Interday (n=3*3)	0.708 – 1.680	0.554 – 2.001
Robustness	1.43	1.56
Repeatability(RSD %)(n=6)	0.97	0.57

Table 3: System Suitability Test Parameters for CEF and LEVO by the Proposed Method

System suitability Parameters	CEF	LEVO
Retention time (min)	2.19	3.60
Resolution		3.80
Theoretical plate	8900	6000
Tailing Factor (asymmetric factor)	1.35	1.47

Table 4: Assay Results of Combined Dosage Form Using Proposed Method

Formulation	Labelled mg/tablet		Obtained % of label claim ± S.D*	
	CEF	LEVO	CEF	LEVO
Cefi-L (Akum Drugs & Pharma	400	500	101.4 ± 0.86 %	101.56 ± 1.02%

CEF = Cefixime Trihydrate
LEVO = Levofloxacin Hemihydrates

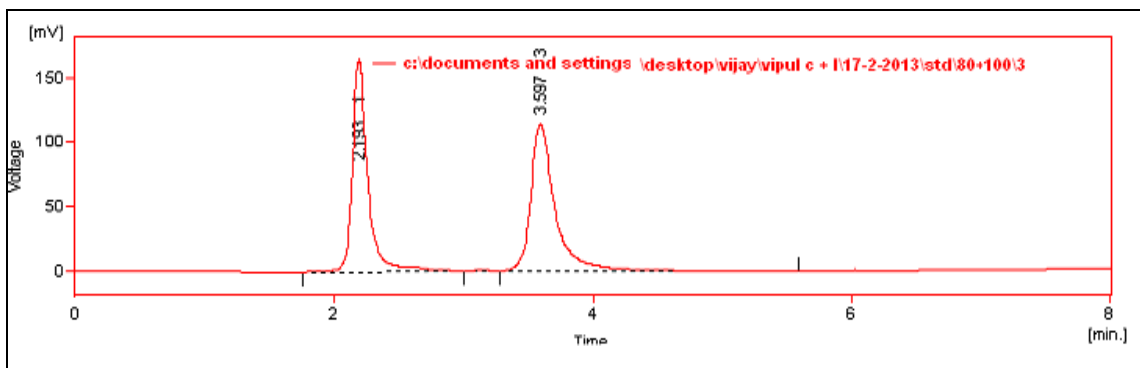


Figure 2: Chromatogram of CEF and LEVO Mixture

Robustness

Robustness of the method was studied by changing the composition of organic phase by $\pm 5.0\%$ and the pH by ± 0.2 and also by observing the stability of the drugs for 24h at 35° temperature in the mobile phase.

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both LEVO and CEF. The mobile phase 0.08M potassium dihydrogen Phosphate: Acetonitrile (60:40), adjusted to pH 6.5 with triethylamine was found to be satisfactory and gave two symmetric and well-resolved peaks for LEVO and CEF. The resolution between LEVO and CEF was found to be 3.8, which indicates good separation of both the compounds. The retention time for CEF and LEVO were 2.19min and 3.60min, respectively [Figure 2]. The asymmetric factors for LEVO and CEF were 1.47 and 1.35 respectively. The calibration curve for LEVO was obtained by plotting the peak area of LEVO versus the concentration of LEVO over the range of 20-120 $\mu\text{g/ml}$, and it was found to be linear with $r^2=0.9995$. Similarly, the calibration curve for CEF was obtained over the range of 20-120 $\mu\text{g/ml}$ and was found to be linear with $r^2=0.9995$. The data of regression analysis of the calibration curves are shown in [Table 1]. The detection limits for LEVO and CEF were 0.20 $\mu\text{g/ml}$ and 0.05 $\mu\text{g/ml}$, respectively. The quantitation limits for LEVO and CEF were 0.60 $\mu\text{g/ml}$ and 0.17 $\mu\text{g/ml}$, respectively. The validation parameters are summarized in [Table 2]. The recoveries of LEVO and CEF were found to be in the range of 98.02-100.68% and 98.38-101.42%, respectively. The system suitability test parameters are shown in [Table 3]. The liquid chromatographic method was applied to the determination of LEVO and CEF in their combined dosage forms. The results for LEVO and CEF were comparable with the corresponding labeled amounts [Table 4]. Proposed study describes a new RP-HPLC method for the estimation of LEVO and CEF combination in mixture using simple mobile phase. The method gives good resolution between both the compounds with a short analysis time ($<8\text{min}$). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation.

Therefore, the proposed method can be used for routine analysis of LEVO and CEF in their combined dosage form.

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