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RESEARCH ARTICLE

Method Development and Validation RP-HPLC Method for the Simultaneous Estimation of Levofloxacin Hemihydrate and Cefpodoxime Proxetil in Pharmaceutical Dosage Form

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Abstract: A new simple, sensitive, accurate and precise method has been developed for the simultaneous estimation of Levofloxacin hemihydrate and Cefpodoxime proxetil in pharmaceutical dosage form by RP-HPLC. After optimization good chromatographic separation was achieved by isocratic mode with a mixture of K_2HPO_4 and methanol pH 8 in the ratio of 40:60 v/v as the mobile phase with YMC C_{18} (250 mm × 4.6 mm i.d × 5 µm) column as stationary phase at flow rate of 1mL/min and detection wavelength of 275 nm. The retention time of Levofloxacin hemihydrate and Cefpodoxime proxetil were found to be 2.8 and 3.9 min respectively. The linearity of this method was found in the concentration range of 250 µg/mL to 750 µg/mL for Levofloxacin hemihydrate and 200 µg/mL to 600 µg/mL for Cefpodoxime proxetil. The method was extensively validated according to ICH guidelines Q2B for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: High performance liquid chromatography, Levofloxacin hemihydrates, Cefpodoxime proxetil

Introduction

Chemically, Levofloxacin Hemihydrate is [(s)-9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-piperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxazine-6-carboxylic acid Hemihydrate][1] as show in Figure 1.

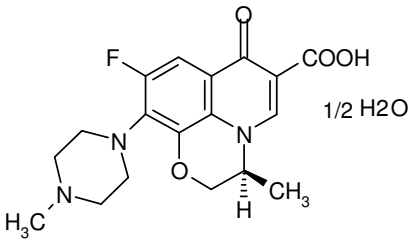

Figure 1. Structure of Levofloxacin Hemihydrate

Levofloxacin inhibits bacterial type II topoisomerases, topoisomerase IV and DNA gyrase. Levofloxacin, like other fluoroquinolones, inhibits the subunits of DNA gyrase, two subunits encoded by the gyrA gene. This results in strand breakage on a bacterial chromosome, supercoiling and resealing; DNA replication and transcription are inhibited[2].

Chemically, Cefpodoxime Proxetilis [1(isopropoxycarbonyloxy) ethyl (6R, 7R)-7-[2-(2-amino-4-thiazolyl)-(*Z*)-2(methoxyimino)acetamido]-3-methoxymethyl-3-cephem-4-carboxylate][3] as show in Figure 2.

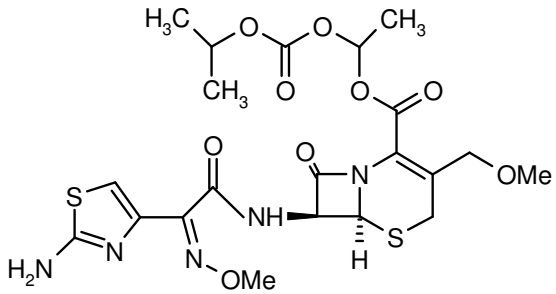

Figure 2.Structure of Cefpodoxime Proxetil

Cefpodoxime is active against a wide spectrum of Gram-positive and Gram-negative bacteria. Cefpodoxime is stable in the presence of beta-lactamase enzymes. As a result, many organisms resistant to penicillins and cephalosporins, due to their production of beta-lactamase, may be susceptible to Cefpodoxime. Cefpodoxime is inactivated by certain extended spectrum beta-lactamases. The bactericidal activity of Cefpodoxime results from its inhibition of cell wall synthesis. The active metabolite of cefpodoxime binds preferentially to penicillin binding protein 3, which inhibits production of peptidoglycan, the primary constituent of bacterial cell walls[4].

In the literature survey it was found that Levofloxacin and Cefpodoxime were estimated in combination drugs by Spectrophotometric[5,6] and HPLC methods,[7-9] Levofloxacin Hemihydrate and Cefpodoxime Proxetil alone in tablet dosage form[10-13]. The plan of work is to develop a simple, sensitive, accurate and precise method for its analysis in combination drug formulation. After a detailed study a novel RP-HPLC method was decided to be developed and validated.

Experimental

Pharmaceutical grade Levofloxacin Hemihydrate, Cefpodoxime Proxetil, methanol and water were supplied by Rainbow Parma training Lab., Hyderabad, India. Gudcef-L tablet was purchased from market for analysis.

The analysis of the drug was carried out on a Waters HPLC system 2695 series consisting pump, auto sampler, photodiode array detector, thermostat column running on empower-2 software.Chromatographic separation was achieved on YMC C18 (250 mm×4.6 mm, 5 µ) column using mobile phase composition of 0.1 M Dipotassium hydrogen ortho phosphate buffer: Methanol (40:60 v/v) pH 8. Flow rate was maintained at 1 mL/min with detection 275 nm. The retention time of Levofloxacin Hemihydrate and Cefpodoxime Proxetil were found to be 2.8 and 3.9 min respectively as show in Figure 3.

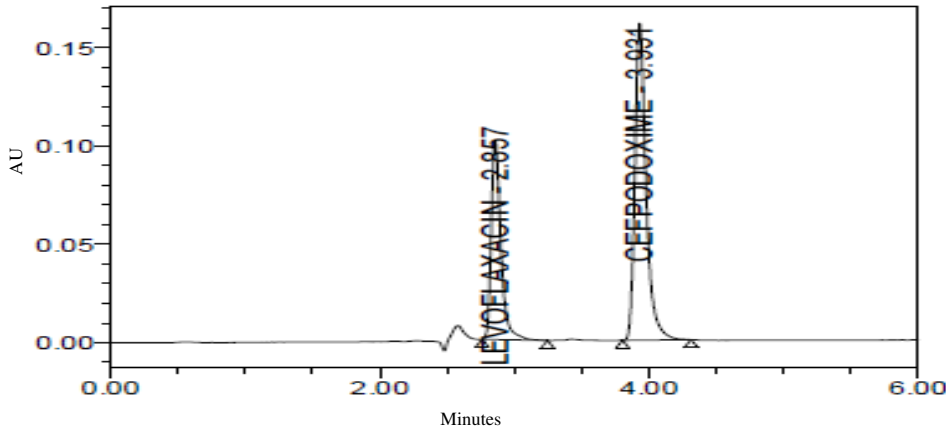

Figure 3. Chromatogram of Levofloxacin Hemihydrate and Cefpodoxime Proxetil

Preparation of mobile phase

The mobile phase was pumped by mixing 0.1 M Dipotassium hydrogen orthophosphate: methanol in ratio of 40:60 v/v.

Preparation of standard solution

Standard combined solution was prepared by transferring accurately weighed 62.5 mg of Levofloxacin Hemihydrate and 50 mg Cefpodoxime Proxetil into a 25 mL of clean dry volumetric flask. Thereafter 10 mL of methanol was added. The result solution was sonicated to dissolve the drug and volume was made up to the mark with HPLC grade water. From the above standard solution, 5 mLwas pipette into a 25 mL volumetric flask and diluted up to the mark with HPLC grade waterwhich is concentration 500 µg/mL and 400 µg/ mL Levofloxacin Hemihydrate and CefpodoximeProxetilrespectively.

Preparation of working standard solution

Accurately weighed 20 tablets and transferred tablet powder equivalent to 250 mg of Levofloxacin Hemihydrateand 200 mg of Cefpodoxime Proxetil taken into a 100 mL clean dry volumetric flask and added about 20 mL of methanol. It was sonicated for 30 min to dissolve completely and volume up to the mark with the same HPLC grade water. This stock solution contains 2500 µg/mL of Levofloxacin and 2000 µg/mL of Cefpodoxime. From the above test stock solution, 5 mL of solution was pipette into a 25 mL volumetric flask and make up to the mark with HPLC grade waterwhich is of concentration 250 µg/mL and 200 µg/mL of Levofloxacin Hemihydrate and Cefpodoxime Proxetil respectively.

Optimization method

To optimize the HPLC parameters, several mobile phase composition with different columns with different columns were tried. The optimized method had been concluded by theoretical plates, tailing and resolution achieved within limits. For the optimization condition in Figure 3 and Table 1.

Table 1.HPLC optimization condition

HPLC System	Water HPLC System 2695 Series
Detector	PDA
Column	YMC Column (250 x 4.6 mm, 5 µm)
Mobile Phase	0.1 M K_2HPO_4, Methanol (40: 60)
Flow Rate	1 mL/ min
Sample Volume	10 µL
Temperature	30 ^oC
Wavelength	275 nm
Run time	6 min
Retention time	LVF -2.867 min and CEP-3.930 min
Theoretical plates	8390
Tailing	1.41
Resolution	7.70
%RSD	<1

Results and Discussion

Method Validation: Linearity

The calibration curves were obtained within the concentrations of the standard solutions of Levo 250-750 µg/mL, Cefpo 200-600 µg/mL. From the linearity data in Table 2**,** the correlation coefficient for linear curve obtained between concentration *vs*. area for standard preparations of Levofloxacin Hemihydrate and Cefpodoxime Proxetil was 0.999, 0.999 respectively. The relationship between the concentration of Levofloxacin Hemihydrate and Cefpodoxime Proxetilarea of both drugs is linear in the range examined since all points lie in a straight line as shown in Figure 4 and 5 respectively and the correlation coefficient is well within limits.

Table 2. Linearity of Levofloxacin Hemihydrate and CefpodoximeProxetil

S.No.	Parameters	Results	
		Levofloxacin	Cefpodoxime
1	Linearity range	250-750 µg/mL	200-600 µg/mL
2	Correlation coefficient	0.999	0.999
3	Slope	992.6	2424
4	Intercept	411.4	24760

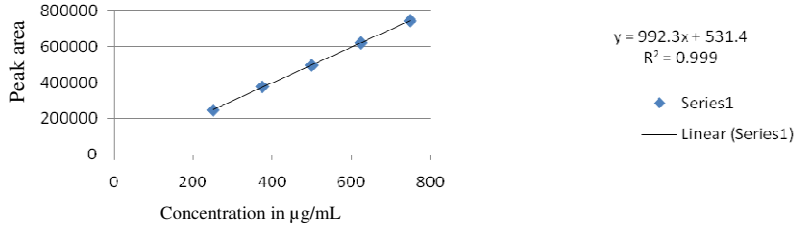

Figure 4. Linearity plot of Levofloxacin Hemihydrate

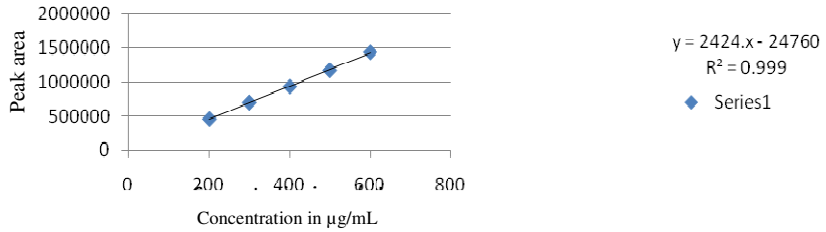

Figure 5. Linearity plot of Cefpodoxime Proxetil

System suitable parameters

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. System suitability was evaluated by injecting the standard drugs of Levofloxacin Hemihydrate and Cefpodoxime Proxetil six times. The theoretical plates, tailing factor, resolution and %RSD was found within limits (Table 1).

Assay of tablet dosage form

The sample peaks found to be without any interference. % Assay was calculated and listed in Table 3 the values were found to be within limits (98-102%).

Table 3. Assay results of Levofloxacin and Cefpodoxime in tablet dosage form

Drug	Label claim	Amount found	% Assay
Levofloxacin	250 mg	245.83	98%
Cefpodoxime	200 mg	198.66	99%

Accuracy

The accuracy of the test method was demonstrated by preparing recovery samples of blend mixture at the level of 50%, 100% and 150% of target concentration. The recovery samples were prepared triplicate at each level. The samples at different level were injected and the percentage recovery for the amount added was estimated. From the data of Tables 4 and 5 the % mean recovery for Levofloxacin Hemihydrate is 100 and for Cefpodoxime Proxetil is100.

Table 4. Recovery studies of Levofloxacin Hemihydrate

S.No	Spiked Level, %	µg/mL added	µg/mL found	% Recovery
1	50	245	242.27	99
2	100	490	490.97	100
3	150	735	740.73	101

Table 5. Recovery studies of Cefpodoxime Proxetil

S.No	Spiked Level, %	µg/mL added	µg/mL found	% Recovery
1	50	198	198.08	100
2	100	396	396.75	101
3	150	594	594	100

Precision

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the relative standard deviation (%RSD) of the replicate measurements.The relative standard

deviation (%RSD) of 6 determinations of peak areas for Levofloxacin Hemihydrate and CefpodoximeProxetilfor precision was found to be within the acceptance criteria of less than 2.0% (Table 6).

Table 6. Precision studies for Levofloxacin and Cefpodoxime

Average mean area	Levofloxacin	Cefpodoxime
	495467	935431
SD	3716	3453
%RSD	0.7	0.3

LOD and LOQ

For this method, the LOD value was found to be 2.358 µg/mL for Levofloxacin Hemihydrate, 1.183 µg/mL for CefpodoximeProxetil. For this method, the LOQ value was found to be 7.862 µg/mL for Levofloxacin Hemihydrate, 3.944 µg/mL for CefpodoximeProxetil.

Robustness

As part of the Robustness, deliberate change in the temperature and flow rate composition was made to evaluate the impact on the method. From the results of robustness by variations in temperature and flow rate (Table 7), it was observed that not much variation in resolution, tailing factor and plate count was observed with deliberate changes in temperature and flow rate. The resolution, tailing factor and plate count was found to be within the limits for Levofloxacin Hemihydrate and Cefpodoxime Proxetil.

Table 7. Results of Robustness by variation in temperature and flow rate

Parameters	Value	Levofloxacin		Cefpodoxime	
		RT	Area	RT	Area
Temperature	25 ^{0}C	3.1	544072	4.2	1032295
	30 ^{0}C	2.8	503783	3.9	943568
	35 ^{0}C	2.6	455398	3.6	872582
Flow rate	0.8 mL/min	3.1	542209	4.2	1030353
	1 mL/min	2.9	513783	3.9	94356
	1.2 mL/min	2.6	455596	3.6	865303

Conclusion

A new simple, sensitive, accurate and precise method was developed by RP-HPLC for the simultaneous estimation of Levofloxacin Hemihydrate and Cefpodoxime Proxetilin combined tablet dosage form. After observing all the satisfactory results in optimized chromatographic conditions and validation parameters, the % recovery was found 100%. It indicates the accuracy of the method. The %RSD was found less than 1% it indicates the method is precise. It was concluded that this method is specific and selective for Levofloxacin Hemihydrate and Cefpodoxime Proxetil as there were no excipient peaks in Chromatogram. From the results of robustness it was found that a little variation in methods does not affect the intended use.

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