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Method Development and Validation for The Simultaneous Estimation of Levofloxacin and Cefpodoxime Proxetil by Using RP-HPLC in Combined Tablet Dosage Form

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ABSTRACT

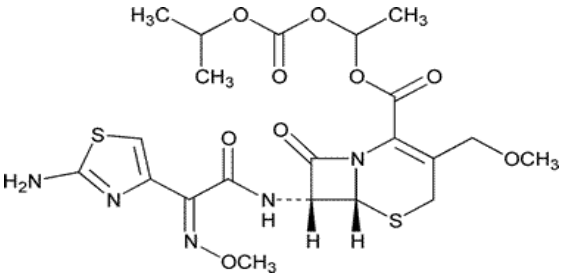
An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Levofloxacin and Cefpodoxime proxetil in combined-dosage form. Alliance -Waters System with Agilant Zorbax Eclipse XBD-C8, (150mm×4.6; 5µm) column with mobile phase containing water with Ortho phosphoric acid: Methanol in the ratio of (80: 20, v/v) was used. The flow rate was 0.5 ml/min, column temperature was 40°C and effluents were monitored at 270 nm. The retention times of Levofloxacin and Cefpodoxime proxetil were 3.096min and 4.559min, respectively. The correlation co-efficient for Levofloxacin and Cefpodoxime proxetil was found to be 1.0 and 1.0, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Levofloxacin and Cefpodoxime proxetil in formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied for the estimation of Levofloxacin and Cefpodoxime proxetil in combined dosage form.

Key words: RP-HPLC, Levofloxacin and Cefpodoxime proxetil.

INTRODUCTION

Cefpodoxime proxetil

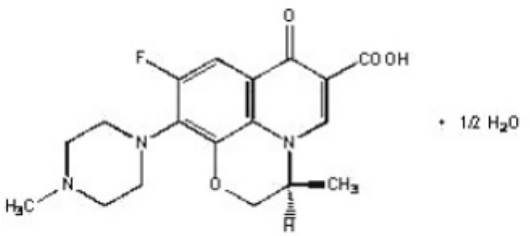
Cefpodoxime proxetil, (6R,7R)-7-{[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino acetyl]amino} -3(methoxy methyl)-8-oxo-5-thia-1 azabicyclo[4.2.0]oct-2-ene-2carboxylic acid is a broad spectrum antibiotic implicated in the treatment of upper respiratory tract and urinary tract infections. The drug is official in Indian Pharmacopoeia and United States Pharmacopeia .The recommended dose of cefpodoxime proxetil is 200 to 400mg per day. The molecular weight of Cefpodoxime Proxetil is 557.6



Structure of Cefpodoxime proxetil

Levofloxacin

Levofloxacin hemi hydrate is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is used to treat severe life-threatening bacterial infection or bacterial infection that has failed to respond to other antibiotic classes. IUPAC



name is (S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylicacid.

Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software with Agilant Zorbax Eclipse XBD-C8, (150mm×4.6;5µm) column.

Chemicals and Reagents

Cefpodoxime proxetil and Levofloxacin was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Ortho phosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and mille Q water.

HPLC Conditions

The mobile phase consisting of water (pH adjusted with Ortho phosphoric acid : Methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 80:20v/v was pumped into the column at a flow rate of 0.5ml/min. The column temperature was 40°C. The detection was monitored at 270nm and the run time was 6min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

Preparation of standard solution

Weigh a quantity of 50mg of Cefpodoxime Proxitel and 40mg of Levofloxacin and transfer it into 100ml clean and dry volumetric flask. Then add mobile phase and sonicate for 30mins and make up the volume with mobile phase and filter through the 0.45µm filter paper. Transfer 5ml of above solution 5ml into 25ml volumetric flask and make up the volume with mobile phase.

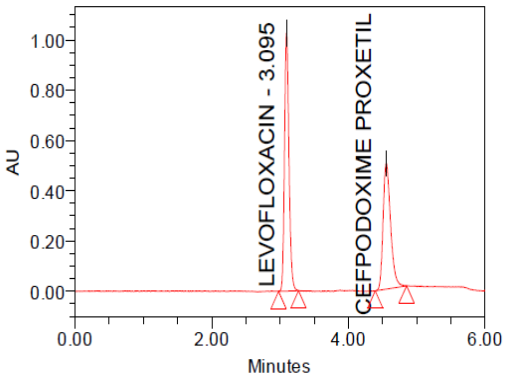


Fig. 1: Standard chromatogram for levofloxacin and cefpodoxime proxetil

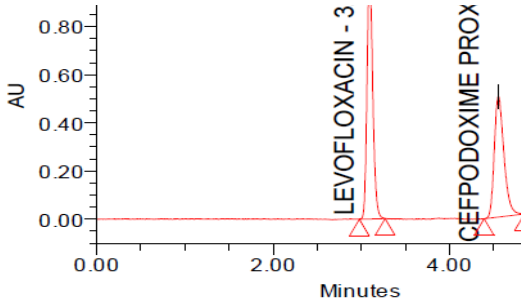


Fig. 2: Formulation chromatogram for Levofloxacin and Cefpodoxime proxetil

Preparation of sample solution

Accurately weighed 1037.10mg of sample

Transfer the sample powder into 100ml of volumetric flask added 25ml of mobile phaseand sonicate for 30mins. Then make up the volume with mobile phase and filter through the 0.45µm

Table1: System Suitability Parameters

Parameters	Levofloxacin	Cefpodoxime proxetil
Correlation Coefficient	1	1
Regression Equation	y = 46746x	y = 38111x
LOD	6.1344	5.474
LOQ	20.4479	18.246
Theoretical plates	11627	8801
Tailing	1.202	1.285

filter paper. Transfer 5ml of above solution 25 ml volumetric flask and make up the volume with mobile phase.

Method validation

System Suitability Studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table1).The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during rountine performance ofthemethod.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may expect to be present. Typically these might include impurities, degradants, matrix, etc

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times. The percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate (Table-3&4). The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated.

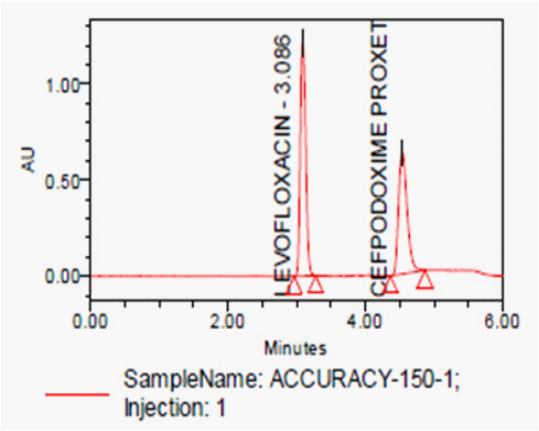


Fig. 3: Accuracy Chromatograms-50% of Levofloxacin and Cefpodoxime proxetil

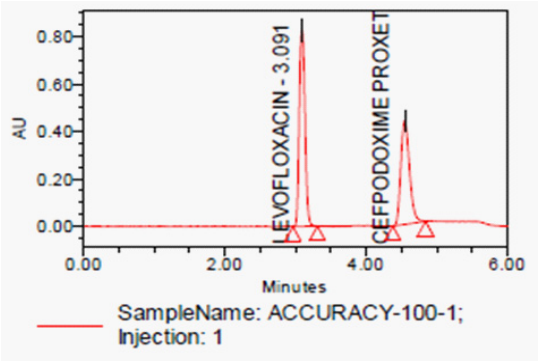


Fig. 4: Accuracy Chromatograms-100%of Levofloxacin and Cefpodoxime proxetil

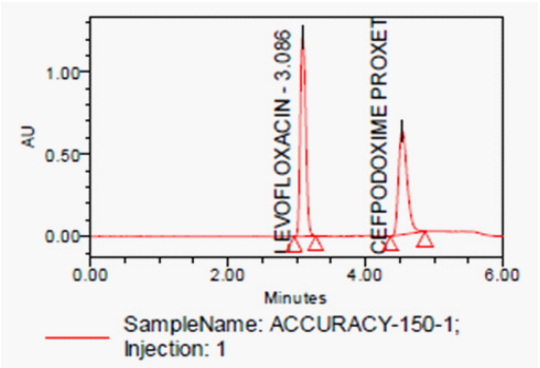


Fig. 5: Accuracy Chromatograms-150% of Levofloxacin and Cefpodoxime proxetil

The chromatograms of three different levels shown in Fig 3, 4 &5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2)

Linearity and range

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was Y=46746X(R^2=1) for Levofloxacinand Y=38111X (R^2=1) for Cefpodoxime proxetil. The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated

S No.	Sample Wt(mg)	Area (Levo)	Area (Cepo)	%Assya (Levo)	(%Assya (Cepo)
1	1037.1	4678289	3818769	100	100
2	1037.1	4677549	3812585	100	100
3	1037.1	4676508	3812077	100	100
4	1037.1	4675202	3812886	100	100
5	1037.1	4677862	3817130	100	100
6	1037.1	4678822	3811304	100	100

Table 3: Accuracy for Levofloxacin

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	518.55	2336174	247.500	249.57	101	101
50%	518.55	2339139	247.500	249.89	101	
50%	518.55	2339510	247.500	249.93	101	
50%	518.55	2338848	247.500	249.86	101	
50%	518.55	2332161	247.500	249.14	101	
50%	518.55	2335555	247.500	249.51	101	
100%	1037.10	4671180	495.000	499.02	101	101
100%	1037.10	4670407	495.000	498.94	101	
100%	1037.10	4679997	495.000	499.96	101	
150%	1555.70	7018686	742.524	749.81	101	101
150%	1555.70	7012947	742.524	749.19	101	
150%	1555.70	7016342	742.524	749.55	101	
150%	1555.70	7015939	742.524	749.51	101	
150%	1555.70	7012013	742.524	749.09	101	
150%	1555.70	7010094	742.524	748.89	101	

Table 4: Accuracy for Cefpodoxime proxetil

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	518.55	1903284	198.000	199.57	101	101
50%	518.55	1900439	198.000	199.27	101	
50%	518.55	1909331	198.000	200.20	101	
50%	518.55	1905283	198.000	199.78	101	
50%	518.55	1901541	198.000	199.38	101	
50%	518.55	1906397	198.000	199.89	101	
100%	1037.10	3818410	396.000	400.37	101	101
100%	1037.10	3811113	396.000	399.61	101	
100%	1037.10	3810929	396.000	399.59	101	
150%	1555.70	5710221	594.019	598.74	101	101
150%	1555.70	5717081	594.019	599.46	101	
150%	1555.70	5716768	594.019	599.42	101	
150%	1555.70	5711949	594.019	598.92	101	
150%	1555.70	5714713	594.019	599.21	101	
150%	1555.70	5718874	594.019	599.65	101	

Table 5: Robustness for Levofloxacin

	Sample name	INJ	Name	RT	Area	USP Tailing	USPPlatecount
1	TEMP-1	1	Levofloxacin	3.858	5842122	1.222	7582
2	TEMP-2	1	Levofloxacin	3.091	4657862	1.158	9234
3	FLOW-1	1	Levofloxacin	3.092	4618822	1.097	8403
4	FLOW-2	1	Levofloxacin	3.858	5842122	1.222	7582

Table 6: Robustness for Cefpodoxime proxetil

	Sample name	INJ	Name	RT	Area	USP Tailing	USPPlatecount
1	TEMP-1	1	Cefpodoxime proxetil	5.691	4400459	1.294	7274
2	TEMP-2	1	Cefpodoxime proxetil	4.541	3707130	1.225	7899
3	FLOW-1	1	Cefpodoxime proxetil	4.538	3721304	1.245	7448
4	FLOW-2	1	Cefpodoxime proxetil	5.691	4400459	1.294	7274

Table 7: LOD and LOQ For Levofloxacin and Cefpodoxime proxetil

S.No	Sampel Name	inj	Name	RT	Area
1	LOD	1	LEVO	3.074	73571
2	LOQ	1	LEVO	3.089	212865
1	LOD	1	Cefpo	4.564	13320
2	LOQ	1	Cefpo	4.578	122890

above. The overlay chromatograms of Linearity for Levofloxacin and Cefpodoxime proxetil shows in Fig 6 and the results for calibration curves are given in Fig 7&8.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which

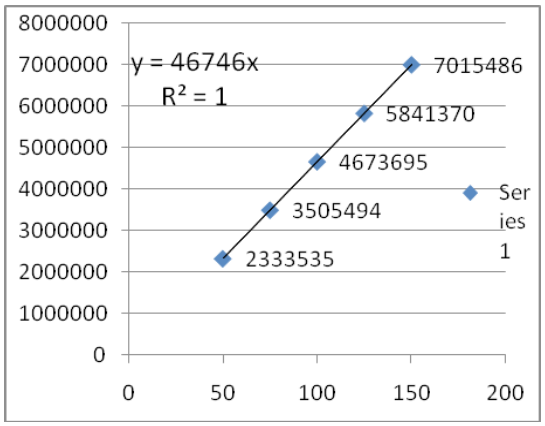


Fig. 7: Linearity Curve for Levofloxacin

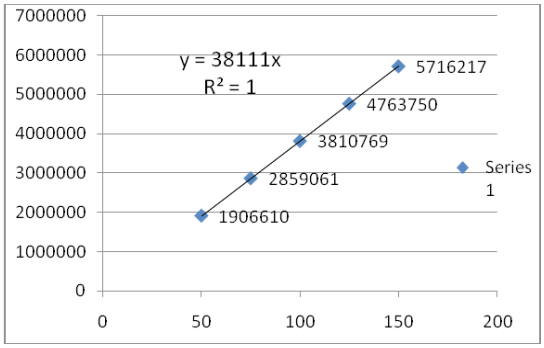


Fig. 8: Linearity Curve for Cefpodoxime proxetil

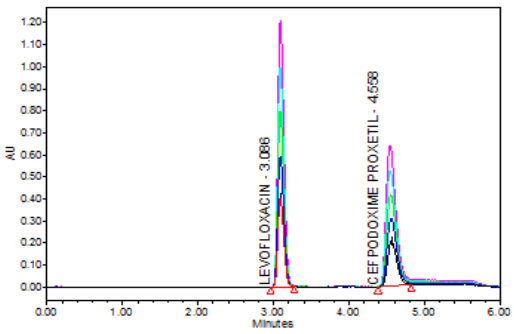
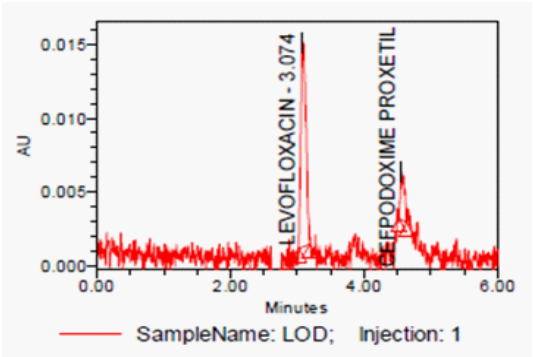


Fig. 9: Overlay chromatograms of Linearity for Cefpodoxime proxetil and Levofloxacin



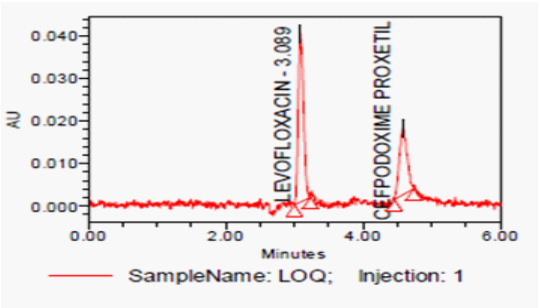
Where
σ = residual standard deviation of response
S = slope of the calibration

RESULTS AND DISCUSSION

System suitability results were given by table1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1. So we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Levofloxacin and Cefpodoxime proxetil standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Levofloxacin and Cefpodoxime proxetil studies. The method accuracy was evaluated by recovery studies. Levofloxacin and Cefpodoxime proxetil recovery was founded 100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given below fig: 7&8 and plot the graph three different concentrations versus areas to construct the demonstrated that the RP HPLC method developed, are robust (Table-5&6).

LOD&LOQ

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Levofloxacin and Cefpodoxime proxetil. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precisionwas established at these predicted levels.
(a) LOQ = 10 σ / S
(b) LOD = 3.3 σ / S



linear regression equation and to calculate the value of correlation co-efficient.Linear correlation was found to be Y=46746 for Levofloxacin and y = 38111 for Cefpodoxime proxetil Method robustness results were given by table 5&6, LOQ and LOD Results were given by table 7.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Levofloxacin and Cefpodoxime proxetil pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for rountine quality control analysis of Levofloxacin and Cefpodoxime proxetil pure and its pharmaceutical dosage forms.

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REFERENCES

1. USP 22/NF 17. General chapters<1649>, NF Monographs. Rockville MD. United states Pharmacopoeia convention. 1710-12 (2006).
2. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization,IFPMA, Geneva, 2003 Indian Pharmacopoeia-2007. General chapters<p.No.78> Monographs. United states Pharmacopoeia convention. (2006).
3. MonikaBakshi,SaranjitSingh., Development of validated stability indicating assay methods-critical review, *Journal of Pharmaceutical and Biomedical Analysis.,* **28**(6); 1011-1040 (2002).
4. Reynolds, D.W., Facchine, K.L., Mullaney, J.F., Alsante, K.M., Hatajik, T.D., Motto, M.G., Available guidance and best practices For conducting forced degradation studies, *Pharm Tech.,* 48-56 (2002).
5. FDA Guidance for Industry, Analytical Procedures and Methods Validation (draft guidance), (2000).
6. T. Kumar, A. Chitra, V. Amrithraj, and N. Kumar, "New RP-HPLC method development and validation for estimation of levofloxacin in tablet dosage form," *Journal of Global Trends in Pharmaceutical Sciences*, **2**(3): 264–276, (2011).
7. K. Kothekar, J. Balasundaram, A. Khandhar, and R. Mishra, "Quantitative determination of levofloxacin and ambroxol hydrochloride in pharmaceutical dosage form by reversed-phase high performance liquid chromatography," *Eurasian Journal of Analytical Chemistry,* **2**(1): (2007).
8. T. Santhoshi, K. Kumar, V. Rao, and A. Ravipati, "Development and validation of RP-HPLC method for simultaneous estimation of levofloxacin and ornidazole in pharmaceutical dosage form," *Journal of Pharmacy Research*, **4**(11): 3864 (2011).
9. N. S. Lakka and N. Goswami, "A novel isocratic RP-HPLC method development and validation for estimation of 5HMF in Levofloxacin Hemihydrate intravenous infusion," *International Journal of Research in Pharmaceutical Sciences,* **2**(1): 45-51 (2011).
10. U. Neckel, C. Joukhadar, M. Frossard, W. Jäger, M. Müller, and B. X. Mayer, "Simultaneous determination of levofloxacin and ciprofloxacin in microdialysates and plasma by high-performance liquid chromatography," *Analytica Chimica Acta,* **463**(2): 199-206 (2002).
11. J. Mehta, Y. Pancholi, V. Patel, N. Kshatri, and N. Vyas, "Development and validation of a sensitive stability indicating method for quantification of levofloxacin related substances and degradation products in pharmaceutical dosage form," *International Journal of PharmTech Research,* **2**(3): 1932–1942 (2010).
12. M. Green, a Practical Guide to Analyticalmethod validation; Analytical chemistry news and features, P. 309A (1996).
13. Chen, X, Bates, Morris, K.R. *Journal of Pharmaceutical biomedical analysis* 26-63 (2001).
14. Gombas A., Antal, Szabo, Marton. S., Eros.I, *International Journal of pharmaceutical analysis* 256,25 (2003).
15. Kamau F.N, Naugi. J.K, Roets. E. and Chepkwony, H.K. "Isocratic liquid chromatographic method for the analysis ofCefpodoxime proxetil in Bulk sample". *Journal of Chromatographic science.* **24**(4): 529-533 (2002).
16. Biljana Nigovic and Branimir Simunic." Voltammetric assay of Cefpodoxime proxetil in pharmaceutical dosage forms". *Journal of pharm Biomed Anal*. **27**(6):115-120 (2003)