INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Novel Validated RP-HPLC Method for the Simultaneous Estimation of Levofloxacin and Cefpodoxime Proxetil in Bulk and Tablet Dosage Form

Rama Prasad Padhy[1], K. Srinivas*[2], Subhash Chandra Dinda[3].

[1]Jeypore College of Pharmacy, Rondapalli, Jeypore, Koraput, Odisha., India – 764002

[2]Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, A.P., India - 534 101.

[3]C.H.S. Mekelle University, Mekelle, Ethiopia.

ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of levofloxacin and cefpodoxime proxetil in tablets. A column having 250 x 4.6 mm, 5µ i.d. in isocratic mode with mobile phase containing Orthophosphoric acid:Methanol (70:30) was used. The flow rate was 1.0 ml/min and effluent was monitored at 230 nm. The retention time (min) and linearity range (ppm) for levofloxacin and cefpodoxime proxetil were (3.7, 4.8) and (25-75, 20-60), respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of levofloxacin and cefpodoxime proxetil in tablets.

Keywords: Levofloxacin, cefpodoxime proxetil, RP-HPLC, simultaneous determination, tablets.

INTRODUCTION

Levofloxacin, (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, is an orally active anti-bacterial agent belongs to the class of fluoroquinolones, which inhibits the nucleic acid synthesis in bacteria[1-4]. Cefpodoxime proxetil is an orally administered antibacterial drug belongs to the class of cephalosporins, widely used in antibacterial pharmaceutical formulations, alone or in combination with other drugs, which acts by interfering with bacterial cell wall synthesis and division by binding to the cell wall of the bacteria, causes cell to die. It is chemically (6R,7R) -7- [(2z) -2- (2-amino - 1, 3 - thiazol-4-yl) -2- (methoxyimino) acetamido] -3- (methoxymethyl) -8- oxo -5- thia -1- azabicyclo [4.2.0] oct -2- ene -2- carboxylic acid, and was successfully used as one content in association with other drugs in the treatment of bacterial infections[1-4]. There are very few methods appearing in the literature for the simultaneous determination of levofloxacin and cefpodoxime proxetil in tablets. Since these methods were based on UV-derivative spectrophotometry[5-9], HPLC[10-15] and HPTLC[16] the procedure was inconvenient for determination and the run times were rather long. The aim of this study was to develop a simple, precise and accurate reverse-phase high performance liquid chromatographic method to estimate levofloxacin and cefpodoxime proxetil in tablets. This method was simple and rapid and provides accurate and precise results, as compared to other methods which have been reported. Criteria employed for assessing the suitability of said solvent system were cost-effectiveness in terms of time required for analysis, solvent noise and preparatory steps involved in the extraction of the drug from the formulation excipients for the estimation of drug contents. The retention times for levofloxacin and cefpodoxime proxetil were 3.7 and 4.8 min, respectively.

EXPERIMENTAL

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Waters symmetry shield C_{18} column (250 mmx4.6mm; 5µm), a 2695 LC20AT binary pump, a 20 µl injection loop and a 2998 PDA detector and running on Waters Empower-2 software. The UV spectrum of the drugs was taken using an Elico SL-159 UV-Visible spectrophotometer.

Chemicals and solvents

The reference sample of levofloxacin and cefpodoxime proxetil was supplied by Lara drugs Pvt Ltd., Hyderabad. HPLC grade water and methanol were purchased from E. Merck (India) Ltd., Mumbai.

Procedure

A mixture of ortho phosphoric acid and methanol in the ratio of 70:30 v/v was found to be the most suitable mobile phase for ideal separation of levofloxacin and cefpodoxime proxetil. The solvent mixture was filtered through a 0.45µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 230 nm. The run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drugs levofloxacin and cefpodoxime proxetil was 3.7 min and 4.8 min. A typical chromatogram showing the separation of the drug is given in Fig. 1.

Calibration plot

Buffer preparation:

Transferred 1ml of orthophosphoric acid into 500 ml volumetric flask and made upto volume with HPLC grade water and degassed.

Preparation of stock solution:

Reference solution: Weigh accurately about 25mg of levofloxacin and 20mg of cefpodoxime proxetil. Transferred into a 100ml volumetric flask, dissolved in 30ml of diluent, which contains methanol and water in the ratio 80:20,with the help of sonication. And the solution is cooled to room temperature and made upto volume with the diluent.

Preparation of working standard solution:

From the above stock solution, 5ml was taken into a 50ml volumetric flask and dissolved using diluent and made up to the mark. It is sonicated and filtered through 0.45µ membrane.

Preparation of sample drug solution for pharmaceutical formulations:

Twenty tablets were weighed accurately and crushed to get a powdered blend. From this a quantity of powdered blend equivalent to about 25mg of levofloxacin and 20mg of cefpodoxime proxetil is weighed and taken into a 100 ml volumetric flask. To this add 60 ml of diluent, sonicate for 30min with intermediate shaking [ensure that no lumps are formed], and made upto the volume with diluent and mixed well. Filtered through 0.45µm membrane filter by discarding the first 5 ml and from the above stock solution, 5ml was taken into a 50ml volumetric flask and dissolved using diluent and made up to the mark. It is sonicated and filtered through 0.45µ membrane.

Procedure for calibration curve:

The contents of the mobile phase were filtered before use through 0.45micron membrane and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The chromatographic separation was achieved using a mobile phase consisting of Orthophosphoric acid : Methanol at 70:30V/V the eluent was monitored using PDA detector at a wavelength of 230nm.The column was maintained at ambient temperature (27^0c) and an injection volume of 20µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time, peak areas of drug was recorded graph was plotted by taking concentration of the drug on x-axis and peak area on y-axis.

Calculation:

The amount of drugs present in each pharmaceutical formulation was calculated by using the standard calibration curves (concentration in ppm was taken on x-axis and peak area on y-axis).The results of linearity are furnished in Table 1&2.

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of Levofloxacin and Cefpodoxime proxetil. Solution containing 50µg/ml for levofloxacin and 40µg/ml for cefpodoxime proxetil was subjected to the proposed HPLC

analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-3&4. The accuracy of the HPLC method was assessed by analyzing solutions of levofloxacin and cefixime at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Table-5&6. The system suitability parameters are given in Table-7.The results of assay of the drugs was furnished in Table 8&9.

RESULTS AND DISCUSSION

In the proposed method, the retention time of levofloxacin and cefpodoxime proxetil was found to be 3.7 min and 4.8 min. Quantification was linear in the concentration range of 25-75ppm for levofloxacin and 20-60ppm for cefpodoxime proxetil. The regression equation of the linearity plot of concentration of levofloxacin and cefpodoxime proxetil over its peak area was found to be Y=5719+16616X (r^2=0.99) for levofloxacin and Y=5382+19288X (r^2=0.99) for cefpodoxime proxetil, where X is the concentration of levofloxacin and cefpodoxime proxetil (ppm) and Y is the corresponding peak area. The number of theoretical plates calculated was 10362 for levofloxacin and 8134 for cefpodoxime proxetil, which indicates efficient performance of the column. The limit of detection and limit of quantification for levofloxacin were found to be 0.002 µg and 0.008 µg and for cefpodoxime proxetil were found to be 0.006 µg and 0.02 µg respectively, which indicates the sensitivity of the method. The use of orthophosphoric acid and methanol in the ratio of 70:30 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of levofloxacin and cefpodoxime proxetil and can be reliably adopted for routine quality control analysis of levofloxacin and cefpodoxime proxetil in its tablet dosage forms. The proposed method is cost effective, using isocratic mode and the retention time of the drugs is very less when compared with the existing methods and analysis of the samples can be done in less time.

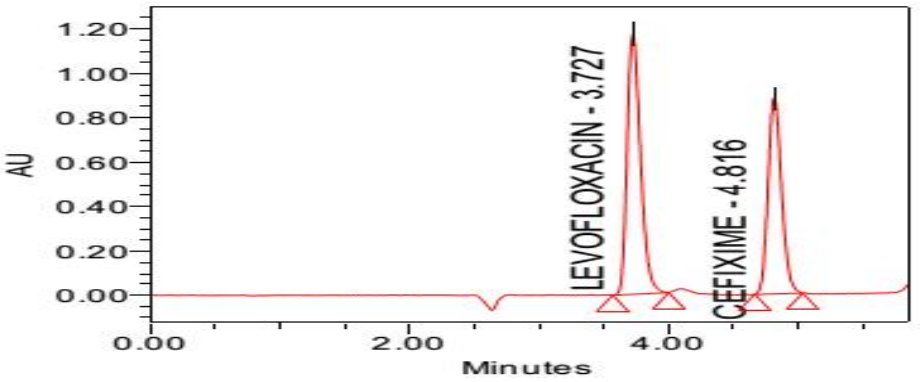

Fig 1
Typical chromatogram of levofloxacin and cefpodoxime proxetil

Table 1
Calibration data of the method (levofloxacin)

Concentration («g/ml)	Mean peak area (n=5)
0	0
25	2333535
37.5	3505494
50	4673695
62.5	5841370
75	7015486

Table 2
Calibration data of the method (cefpodoxime proxetil)

Concentration («g/ml)	Mean peak area (n=5)
0	0
20	1906610
30	2859061
40	3810769
50	4763750
60	5716217

Table 3
Precision of the proposed HPLC method (levofloxacin)

Concentration of levofloxacin(50«g/ml)	Peak area	
	Intra day	Inter day
Injection-1	4678289	4675865
Injection-2	4677549	4679586
Injection-3	4676508	4678921
Injection-4	4675202	4668954
Injection-5	4677862	4675462
Injection-6	4678224	4672168
Average	4677272	4675159
Standard Deviation	1202.8	4044.4
%RSD	0.02	0.08

Table 4
Precision of the proposed HPLC method (cefpodoxime proxetil)

Concentration of cefpodoxime proxetil(40«g/ml)	Peak area	
	Intra day	Inter day
Injection-1	3818769	3825649
Injection-2	3812585	3824892
Injection-3	3812077	3827965
Injection-4	3812886	3826413
Injection-5	3817130	3824673
Injection-6	3811304	3829641
Average	3814125	3826539
Standard Deviation	3054.6	1931.7
%RSD	0.08	0.05

Table 5
Accuracy studies (levofloxacin)

Concentration	Amount added (ppm)	Amount found (ppm)	% Recovery	% Mean recovery
50%	24.7	24.96	101.05	100.68%
100%	49.71	49.93	100.44	
150%	74.52	74.94	100.56	

Table 6
Accuracy studies (cefpodoxime proxetil)

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	19.80	19.96	100.80%	100.56%
100%	39.60	39.86	100.65%	
150%	59.49	59.63	100.23%	

Table 7
System suitability parameters

Parameter	Result (Levofloxacin)	Result (Cefixime)
Linearity (µg/ml)	25-75	20-60
Correlation coefficient	0.99	0.99
Theoretical plates (N)	10362	8134
Tailing factor	1.19	1.26
LOD (µg/ml)	0.002	0.006
LOQ (µg/ml)	0.008	0.02

Table 8
Assay (Levofloxacin)

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	50	50.09	100.18
Brand 2	50	50.13	100.26

Table 9
Assay (Cefixime)

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	40	12.45	101.12
Brand 2	40	12.59	101.47

REFERENCES

1. Satoshkar RS, Bhandarkhar SD and Ainapure SS. Pharmacology and pharmacotherapeutics, 17th edition, Popular Prakashan, Mumbai, India, 2001.
2. Skoog DA, West DM and Holler FJ (1996). *Fundamentals of Analytical Chemistry*. 7th ed. Philadephia: Saunders College Publishing. p. 1-3.
3. Snyder LR, Kirkland JJ and Glajch JL (1997). *Practical HPLC Method Development.* 2nd ed. New York: Wiley. p. 1-20.
4. Levofloxacin 219.1 (15283-V),Cefixime 166.2 (12006-g),Martindale,3rd edition.
5. Development And Validation Of Analytical Methods For Simultaneous Estimation Of Cefixime And Levofloxacin In Pharmaceutical Dosage Form, American Journal Of Pharmatech Research, 2013; 2249-3387.
6. Payal Patel, Helly Shah, Kushbu Patel, Dr. Mandev Patel; Development And Validation Of Spectrophotometric Methods Simultaneous Estimation Of Cefixime Trihydrate And Levofloxacin Hemihydrate In Their Combined Tablet Dosage Form; International Journal Of Pharmaceutical Research And Bio-Science; 2012, 1(5): 502-515.
7. Makale Künye Bilgisi; Development and Validation Of Spectrophotometric Methods For Simultaneous Estimation Of Cefixime Trihydrate And Levofloxacin Hemihydrate In Their Combined Tablet Dosage Form; International Journal Of Pharmacy And Pharmaceutical Research 2012, 1, 110-113.
8. Rajeshree C.Kavar, Birju M. Savaliya, Arvind J. Lakkad, Smita V. Soriya, Kaushik G.Kapuriya, Shital D. Faldu; Q-Absorbance Ratio Spectrophotometric Method For The

Simultaneous Estimation Of Cefpodoxime Proxetil And Levofloxacin Hemihydrate In Their Combined Dosage Form; International Bulletin Of Drug Research, 2012, 2(3); 22-30.

9. Sunil Singh, Nitin Dubey, Dinesh Kumar Jain, Lalit Kumar; Spectrophotometric and rp-hplc methods For Simultaneous Estimation Of Cefpodoxime Proxetil And Clavulanate Potassium In Combined Tablet Dosage Form; American-Eurasian Journal Of Scientific Research; 2010, 5(2); 88-93.
10. Sejal K. Patel, Sumeet I. Chhabra. Development And Validation Of RP-HPLC Method For Simultaneous Determination Of Cefixime Trihydrate And Levofloxacin Hemihydrates In Tablet Formulation, Pharmatutor, Pharmacy Infopedia, 1873.
11. Rakesh Radadiya, Nirav Patel, Chetana Ribadia, Nimish Talaviya, Jenisha Rokad; Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Cefixime Trihydrate And Levofloxacin Hemihydrate In Bulk Drug And In Combined Dosage Form; Inventi Journals; Inventi Rapid: Pharmaceutical Analysis & Quality Assurance, 2013.
12. Mahesh H Khodifad, Kuldip R Marwada, Pratik M Oza; Simultaneous Estimation Of Levofloxacin Hemihydrate And Cefpodoxime Proxetil In Pharmaceutical Dosage Form By Rphplc Method; Inventi Rapid: Pharmaceutical Analysis And Quality Assurance; 2012.150-159.
13. Darshan Shah, Smita Talaviya, Mandev Patel; Simultaneous Estimation Of Cefpodoxime Proxetil And Ofloxacin In Pharmaceutical Dosage Form By Rp-Hplc ; International Journal Of Pharmacy And Pharmaceutical Sciences; 2012, Volume 4, 32-35.
14. Ajit R. Wankhede, Prashant Y. Mali, Vikram Karne, Anubha R. Khale; Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Cefixime And Cloxacillin In Tablet Dosage Form; International Journal Of Pharmaceutical And Bio Archieves; 2010, 1(2), 317-320.
15. Ceema Mathew, Tiwari Priya, Godavariya Vijay; Development And Validation Of Rp-Hplc Method For The Simultaneous Estimation Of Levofloxacin Hemihydrates And Cefixime Trihydrate In Marketed Formulation; International Research Journal Of Pharmacy; 2013, 4(6),68-71.
16. Pritam Jain, Amar Chaudhari, Anup Bang And Sanjay Surana; Validated Stability-Indicating High-Performance Thin-Layer Chromatographic Method For Estimation Of Cefpodoxime Proxetil In Bulk And In Pharmaceutical Formulation According To International Conference On Harmonization Guidelines; Journal Of Pharmacy And Bio-Allied Sciences; 2012, 4(2), 101-106.