Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

SPECTROPHTOMETRIC ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE AND CEFPODOXIME PROXETIL IN TABLET

TAMILARASI. G., DR. VETRICHELVAN.T., DR. VEKAPPAYYA.D., THARABAI. R., YUVARAJAN.K.,

[a)]Research scholar, Sastra university, Thanjavur-613402, India., [b)]Department of Pharmaceutical Chemisty, Madurai medical college, Madurai-625020, Tamilnadu, India.

Email: thamilarasi.mnc@gmail.com

Received: 11 Feb 2014 Revised and Accepted: 29 Feb 2014

ABSTRACT

Objective: The present study aims to develop simple, precise and accurate methods for the estimation of levofloxacin hemihydrate and cefpodoxime proxetil by simple UV method (Absorbance ratio method) in pure and tablet dosage form.

Methods: A simultaneous determination of levofloxacin hemihydrate and cefpodoxime proxetil by absorbance ratio spectrophotometric method has been developed in combined tablet dosage form. The method is based on the measurements of absorbance at isoabsorptive point. The absorbance measurements were made six times for the formulations at 266 nm, 295.4 nm. The amounts of levofloxacin hemihydrate and cefpodoxime proxetil were found by constructing Q-analysis equation method, from the Q-analysis equation to found the amount of sample.

Results: The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.87 ±0.2869 for Levofloxacin hemihydrate and 100.26 ± 0.1418 for Cefpodoxime Proxetil. The average %RSD values of Levofloxacin hemihydrate and Cefpodoxime Proxetil was found to be 0.2872 and 0.1414, respectively. The low % RSD value of drugs indicates that this method was accurate.

Conclusion: The wavelengths 266nm and 295.4nm were selected for the estimation of Levofloxacin hemihydrate and Cefpodoxime proxetil respectively. In this case 266 is the isobestic point for Levofloxacin hemihydrate and Cefpodoxime proxetil. The percentage label claim present in tablets was found to be 100.26 ± 0.9483 and 100.28 ± 1.9449 for Levofloxacin hemihydrate and Cefpodoxime proxetil, respectively. The percentage recovery was found to be in the range of 99.87 ± 0.2869 for Levofloxacin hemihydrate and 100.26 ± 0.1418 for Cefpodoxime proxetil.

INTRODUCTION

Levofloxacin hemihydrate chemically [5(s)-9 fluro 2,3- dihydro- 3-methyl-10-(4-methyl-1-piperazinyl 7-oxo 7H pyridol (1,2,3-de-)1,4-benzoxazin-6-carboxylic acid] which is an antibacterial mainly used for Urinary Tract Infection.

Cefpodoxime proxetil (LEVO) Chemically (6R-(6R- [6R-7α,7β(Z)]]-7-[(2-amino 4thiozoyl) (methoxy imino) acetyl]amino] 3-(methoxy – methyl)- 8- oxo 5-thia-1-azabicyclo [4;2;0]oct-2-ene -2-carboxylic acid 1-[(1-methyl ethoxy)carbonyl)oxy]ethyl ester [1-3]. Levo and CEFPO is a newer combination of tablet dosage form which is used for the treatment of severe bacterial infections.

Literature survey reveals that numerous methods are available for the determinate of LEVO and CEFPO alone and in combination with other drugs.

But there no methods were reported for the estimation of these drugs in combined dosage forms without earlier separation [4-11]. The present work deals with a development of simple rapid and reproducible method for the a concurrent estimation of LEVO and CEFPO in their combined dosage form using UV spectrophotometer.

MATERIALS AND METHODS

In the present method we have attempted to assay the LEVO and CEFPO combination in tablet using the concept of iso absorptive point. Iso absorptive point is defined as the wavelength at which both the drugs show same absorbance. In this method the absorbance were measured at two wavelengths one being the isoabsorptive point of two components and other being the wavelength of maximum absorption of one of the two components. The combination of drug substances can be estimated simultaneously using sample spectrophotometer. As a result such method if found suitable should be inexpensive fast and industry friendly.

Instrument

An UV/VIS spectrophotometer Shimadzu model 1800 was employed for all spectroscopic measurements with 1cm matched quartz cells. LEVO & CEFPO were obtained commercially.

Selection of solvent

The solubility of drugs were carried out using various polar to non polar solvents. The Solubility profiles of the drugs were determined as per the standards. The common solvent was found to be methanol. For the analysis of levofloxacin hemihydrate and cefpodoxime proxetil by the proposed method, methanol were chosen as solvent for Spectrophotometry, and it was selected on account of its ready availability, cost factor, solubility, and stability factor and cut off wavelength.

Preparation of standard stock solution of Levofloxacin hemihydrate

Pure raw material of levofloxacin hemihydrate 25 mg was accurately weighed and transferred to a 100 ml standard flask and dissolved in methanol and produce 100 ml with methanol. From this 10 mL of the solution was transferred in to the 100 ml volumetric flask and made up to required volume with methanol to get the concentration 25 g ml-1.It is used as a working standard.

Preparation of standard stock solution of Cefpodoxime proxetil

Pure raw material of cefpodoxime proxetil 20 mg was accurately weighed transferred to a 100 ml standard flask and dissolved in methanol and produce 100 ml with methanol. From this 10 ml of the solution was transferred in to the 100 ml volumetric flask and made up to required volume with methanol to get the concentration 20 g ml-1. It is used as a working standard.

Selection of wavelengths

The selection of wavelength for the estimation of levofloxacin hemihydrate and cefpodoxime proxetil, a suitable standard solution

to contain 10 gml-1 of levofloxacin hemihydrate and cefpodoxime proxetil were prepared individually and scanned in the entire range from 200-400 nm. From the overlaid spectra, the two wavelengths 295.4nm for levofloxacin hemihydrate and 260nm for cefpodoxime proxetil were selected for the construction simultaneous equation method. For Absorbance ratio method, the isobestic point was selected for both the drugs from their zero order spectra's, which is 266 nm, and other one is λ max of levofloxacin such as 295.4nm were chosen for the estimation of levofloxacin hemihydrate and cefpodoxime proxetil.

Linearity

The linearity studies for the Absorbance ratio method was performed at 266 nm, 295.40nm in the concentration range of 2.5-15 µg mL-1 for levofloxacin hemihydrate and 4-24 µg mL-1 for cefpodoxime proxetil.

Preparation of calibration curve

The calibration curves were constructed by plotting absorbance Vs concentration for absorbance ratio method. The optical characteristics and regression equation for the calibration curves are presented in Table 1.

Validation of developed method

Linearity and Range

From the calibration graphs plotted, levofloxacin hemihydrate and cefpodoxime proxetil shows the linearity between 2.5-15 µg mL-1, 4-24µg mL-1 for Absorbance ratio method.

Table 1: Optical characteristics and regression equation of proposed Method

S. No.	Parameters Values	LEVO	CEFPO
1.	λ- max(nm)	295.4	266
2.	Beer's law limit(µg/ml)	2.5-15µg/ml	4-24µg/ml
3.	Molar absorptivity	$1.397X10^4$	$1.657X10^4$
4.	Sandell's sensitivity (µg/cm^2/0.001AV)	77.8	0.0007748
5.	Regression equation(A Slope(b)	0.0778	0.19786
	Intercept(a)	0.00393	0.0025714
	Correlation coefficient(r)	0.9999	0.9999

A= a+bc (where **`c'** is the concentration in µg/ml).

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies.

Precision

The repeatability of the method was confirmed by the analysis of tablets repeated for 6 times with the same concentration. The amount of each drug present in the tablets were calculated. The percentage RSD was calculated. The amount of drugs was determined and the percentage RSD was calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using the average of slope and standard deviation of response.

Iso Absorptive method

For the present study two brands of commercial tablets designated as Tablet A and Tablet B each containing 250mg of LEVO and 200mg of CEFPO were procured from open market. Twenty tablets of each brand were weighed and crushed to fine powder. Next powder containing 25mg of LEVO and 20mg of CEFO was weighed and transferred and dissolved in methanol. The solution was filtered through whatmann filter paper No.41 and diluted to 100ml with methanol. The aliquots of solution was diluted to give final dilution of concentration 12.5mcg/ml of LEVO and 10mcg/ml CEFPO. The absorption of the solution was next measured at 295.4nm and 266nm. The concentration of LEVO and CEFPO in each tablet was calculated according to method of pernerwaski et al [12]. These such determination were carried out for each brand and the mean is calculated Table 2.

Table 2: Results of Commercial tablet formulation analysis

Tablet sample	Label Claim		Label Claim % of label claim		Standard Coefficient of (mg/tab) estimated* estimated		Deviation		Variance	
	LEVO	CEFPO	LEVO	CEFPO	LEVO	CEFPO	LEVO	CEFPO	LEVO	CEFPO
Tablet A	250mg	200mg	250.7	200.53	100.26	100.28	0.9483	1.9449	0.9457	1.9394
Tablet B	250mg	200mg	250.3	200.06	100.12	100.03	0.8076	0.7078	0.8059	0.707

*Average of ix Determiations.

The developed method was validated for Ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts, different sources of reagents and so on. In the present work it was confirmed by different analysts. The low % RSD value indicates that the developed method was more rugged. The results were shown in Table 3.

Table 3: Ruggedness studies of 50% pre analysed Formulation of proposed method

Drug	Percentage	Condition	% S.D	%R.S.D	S.E	Recovery
	80	Analyst 1	100.10	0.3579	0.3575	0.0397
		Analyst 2	100.31	0.4033	0.4027	0.0375
LEVO	100	Analyst 1	100.92	1.4648	1.4515	0.1627
		Analyst 2	101.25	0.4330	0.4276	0.0481
	120	Analyst 1	102.99	0.8653	0.8402	0.0961
		Analyst 2	99.79	0.2032	0.2019	0.0252
	80	Analyst 1	99.39	0.0346	0.0348	0.0038
		Analyst 2	99.41	0.6051	0.6038	0.0593
CEFPO	100	Analyst 1	98.97	0.4592	0.4640	0.0510
		Analyst 2	99.43	0.0866	0.0870	0.0096
	120	Analyst 1	99.52	0.1985	0.1995	0.0220
		Analyst 2	100.52	0.7032	0.7029	0.0221

Recovery studies

To study the accuracy reproducibility and precision of the proposed method, recovery studies were carried out by addition of standard drug solution to pre analyzed sample. Results of recovery studies were found to be satisfactory and are presented in Table 4.

Table 4: Recovery studies of pre analysed Formulation of proposed method

Tablet Sample	Concentration of added amount of drug in the final dilution in mcg/ml		Recovery in mcg/ml		%Recovery	
	LEVO	CEFPO	LEVO	CEFPO	LEVO	CEFPO
80	4	6.4	3.99	6.42	99.75	100.31
100	5	8	5.01	80.3	100.2	100.37
120	6	9.6	5.98	9.61	99.66	100.10

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of LEVO& CEFPO in combined dosage forms was found to be simple, accurate, economical and rapid.

The correlation coefficient for both the drugs was found to be around 0.9999.This indicates that both the drugs obey Beer's law and they were linear at the selected concentration range.

The concentration of solution containing 12.5g mL-1 of Levofloxacin hemihydrates and 10g mL-1 of Cefpodoxime Proxetil was prepared and the area of the solutions was measured at their respective wavelength ranges. The percentage purity of tablets was found to be 100.26±0.9483 and 100.28 ± 1.9449 for Levofloxacin hemihydrate and Cefpodoxime Proxetil, respectively. The amount present in tablets was in good concord with the label claim and the % RSD values were found to be 0.9457 and 1.9394 for Levofloxacin hemihydrate and Cefpodoxime Proxetil, respectively. The low % RSD value indicates that the method was highly precise.

The developed method was validated for Ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts, different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value 80%, 100%, 120% by analyst 1 and analyst 2 was found to be 0.3575, 1.4515, 0.8402 and 0.4027, 0.4276, 0.2019 for Levofloxacin hemihydrate and 0.0348, 0.4640, 0.1995 and 0.0.6038, 0.0870, 0.7029 for Cefpodoxime Proxetil, respectively. The low % RSD value indicates that the developed method was more rugged.

The accuracy of the method was performed by recovery studies. To the 50% pre analysed formulations, a known quantity of Levofloxacin hemihydrate and Cefpodoxime Proxetil raw material solutions were added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.87 ±0.2869 for Levofloxacin hemihydrate and 100.26 ± 0.1418 for Cefpodoxime Proxetil.

The average %RSD values of Levofloxacin hemihydrate and Cefpodoxime Proxetil was found to be 0.2872 and 0.1414, respectively. The low % RSD value of drugs indicates that this method was accurate.

The developed method was validated for Ruggedness. It refers to the specificity of one lab to multiple lab which may include different analysts, different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value 80%, 100%, 120% by analyst 1 and analyst 2 was found to be 0.3575, 1.4515, 0.8402 and 0.4027, 0.4276, 0.2019 for Levofloxacin hemihydrate and 0.0348, 0.4640, 0.1995 and 0.0.6038, 0.0870, 0.7029 for Cefpodoxime Proxetil, respectively. The low % RSD value indicates that the developed method was more rugged.

The accuracy of the method was performed by recovery studies. To the 50% pre analysed formulations, a known quantity of Levofloxacin hemihydrate and Cefpodoxime Proxetil raw material solutions were added at three different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.87 ±0.2869 for Levofloxacin hemihydrate and 100.26 ± 0.1418 for Cefpodoxime Proxetil.

The average %RSD values of Levofloxacin hemihydrate and Cefpodoxime Proxetil was found to be 0.2872 and 0.1414, respectively. The low % RSD value of drugs indicates that this method was accurate.

ACKNOWLDGEMENT

The authors thank SKAN Research lab, Pandicherry for the pure sample of levofloxacin hemihydrates and Biomarks Drugs India(p) ltd, Himachal Pradesh for the pure sample of Cefpodoxime Proxetil.

REFERENCES

1. British Pharmacopoeia HMSO Publication center London. I & II, 2009; 3933-3937, 5872-5877.
2. Merck Index, Merck Research Laboratories, Division of Merck and Co; Inc; NJ,1996; 322.
3. Sethi PD Quantitative analysis of Drugs in Pharmaceutical Preparations 3rd Edition. CBS Publications., 1997; 52-57.
4. Jagatap CA, Patil PB, Kane SR, Mohite SK, Magdum CS. Development and Validation of Simultaneous Spectrophotometric Estimation of Cefpodoxime Proxetil and Ofloxacin in Tablet Dosage Form, Journal of Pharmacy Research 2012;5(6):3181-3184
5. Patel Sanket A, Patel Satish A. Development and Validation of first order derivative Spectrophotometric method for simultaneous estimation of Ofloxacin and Cefpodoxime Proxetil in tablet dosage form. Journal of Pharmaceutical Science Bioscientific Research,2011; 1(2):108-112.
6. Shah MN, Dr.Patel HU, Patel CN, Spectrophotometric method for simultaneous determination of Cefpodoxime Proxetil and Ofloxacin in tablets, International Journal of Pharmaceutical Science & Research.2012; 3(2): 551-555.
7. .Malathi S, Dubey RN, Venkatnarayanan R. Simultaneous RP-HPLC estimation of cefpodoxime proxetil and clavulanic acid in tablets, International Journal of Pharmaceutical Science 2009;7(1):102-105.
8. Prathap B, Akalankadey, Srinivasa Rao G, Shahul Hussan, Simultaneous Estimation of Levofloxacin And Ambroxol Hydrochloride From Tablet Dosage form Using RP-HPLC. International Journal of Research in pharmaceutical science.2012; 3(3): 466-469.
9. .Suresh Kumar P. Navaneetha Krishnan S, Naveen Kumar V, Anil Kumar G, Kiran Kumar G, HPLC method development of levofloxacin by RP-HPLC method in its bulk dosage form. International Journal of Research in Ayurveda & Pharmacy.2011; 2(6):1790-1792.
10. Srividya B, Cardoza RM, Amir PD, Stability indicating HPTLC method of analysis of Ofloxacin. Indian drugs.2003;40(1):41-43.
11. Sethy & Savita yadav, Validated HPTLC method for Simultaneous quantitation of Cefixime and ofloxacin in Bulk drug and in Pharmaceutical formulation. International Journal of Comprehensive Pharmacy, 2011;4(05):1-5.
12. Becket AH, Stenlake TB, Practical Pharmaceutical Chemistry 4th edition CBS Publishers. II 1997:286-288.