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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND LEVOFLOXACIN HEMIHYDRATE IN THEIR COMBINED TABLET DOSAGE FORM

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Abstract

Two simple, accurate, precise and economical spectrophotometric methods have been developed for the estimation of Cefixime trihydrate (CEF) and Levofloxacin hemihydrates (LEVO) simultaneously in tablet dosage form. Simultaneous method (Method 1) and Absorbance Ratio (Q-Absorbance) method (Method 2) were used. The wavelength ranges 240.27 nm and 296.28 nm in Simultaneous method were selected to determine Cefixime trihydrate (CEF) and Levofloxacin hemihydrate (LEVO), respectively and wavelength ranges 289.39 nm (iso-absorptive point) and 240.27 nm (λmax of Cefixime trihydrate) were selected for Absorbance ratio (Q-Absorbance) method. Beer's law is obeyed in the concentration ranges of 3-15 µg/ml and 3-15 µg/ml for Cefixime trihydrate(CEF) and Levofloxacin hemihydrate(LEVO) for Simultaneous method as well as Absorbance ratio method. The % assay for commercial formulation was found to be in the range 99.92% – 100.07% for Simultaneous method and 99.85–100.04 % for Absorbance Ratio by the proposed methods. Recovery was found in the range of 98.33-101.25% for Cefixime trihydrate and 98.66-101 % for Levofloxacin hemihydrate by Simultaneous spectroscopic method and 98.33-100.75 % for Cefixime trihydrate and 98.66 –99% for Levofloxacin hemihydrate by Absorbance ratio method for both the Formulations.

The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out according to ICH guidelines.

INTRODUCTION

Cefixime trihydrate (CEF) is official in British 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 gonorrhea, 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 hemihydrate(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 HPLC, UV, were reported for the estimation of Cefixime trihydrate and Levofloxacin hemihydrate individually and in combination with other drugs. The aim of the present study was to develop accurate, precise and selective uv methods for the analysis of Cefixime trihydrate and Levofloxacin hemihydrate.
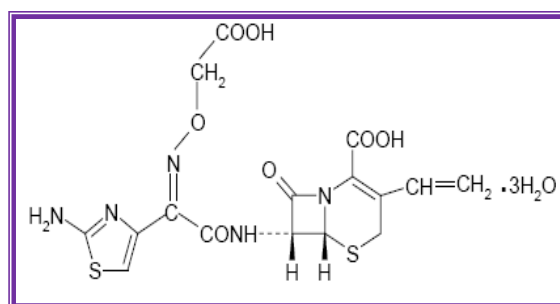
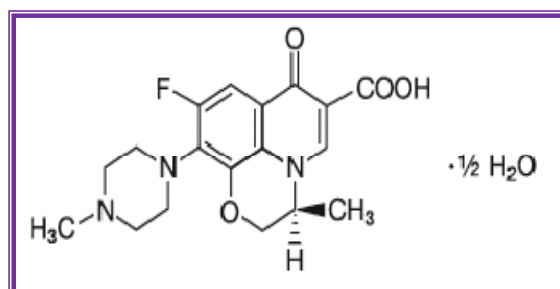**STRUCTURE:**

Figure1 Chemical structure of Cefixime Trihydrate

Figure2 Chemical structure of Levofloxacin hemihydrate

MATERIALS & METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (SHIMADZU 1800) with 10 mm matched quartz cells was used. Al weighing were done on electronic balance (Model Shimadzu AUW-220D), Ultrasonicator model 5.5L150H were used.

Material used:

Api of Cefixime trihydrate (gift sample from West-Coast Pharmaceutical Works LTD, Ahmedabad,Gujarat,India)

Api of Levofloxacin hemihydrate (gift sample from Zydus Cadila Pharmaceuticals Ltd., Ankleshwar,Gujarat,India)

The pharmaceutical dosage form used in study was Cefi-L (label claim CEF 400 mg, LEVO 500 mg) manufactured in India by Abbott Pharma.

Reagent used

Methanol: AR grade (Finar Chemicals Pvt. Ltd, Ahmedabad, India)

METHODS

Preparation of Standard Solutions

A 10 mg of standard CEF and LEVO were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100μg/ml each of CEF and LEVO.

METHOD

1. SIMULTANEOUS EQUATION METHOD

In simultaneous equation method (vierodt's method) two wavelengths were selected i.e. 240.27 nm and 296.28 nm absorbance maxima of Cefixime trihydrate and Levofloxacin hemihydrate respectively. For calibration curves, stock solutions of Cefixime and Moxifloxacin in the concentration of range of 3-15μg/ml and 3-15 μg/ml respectively. The absorbance of Cefixime trihydrate and Levofloxacin hemihydrate were measured at 240.27 and 296.28 nm, calibration curves were plotted. The absoptivities of both the drugs at both the wavelengths were determined. The content of both ingredient in the sample were obtained by using following equations:

$$Cx = A2ay1 – A1ay2/ax2ay1 – ax1\ ay2$$
$$Cy = A1ax2 – A2ax1/\ ax2ay1 – ax1\ ay2$$

Where,

A1 = Absorbance of the diluted sample at 240.27 nm

A2 = Absorbance of the diluted sample at 296.28 nm

ax1 = Absorptivity of cefixime trihydrate at 240.27 nm

ax2= Absorptivity of cefixime trihydrate at 296.28 nm

ay1 = Absorptivity of Levofloxacin hemihydrate at 240.27nm

ay2= Absorptivity Levofloxacin hemihydrate of at 296.28 nm

Cx = Concentration of cefixime trihydrate

Cy= Concentration of Levofloxacin hemihydrate

2. Q- ABSORPTION METHOD

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that CEF and LEVO show an isoabsorptive point at 289.39nm. The second wavelength used is 240.27nm, which is the λ-max of CEF.Five working standard solutions having concentration 3,6, 9,12,15, μg/ml for CEF and 3,6,9,12,15

μg/ml for LEVO were prepared in methanol and the absorbances at 289.39 nm (isoabsorptive point) and 240.27 nm (λ-max of CEFI) were measured and absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance/ Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

Concentration of Cefixime trihydrate

CX = [(QM – QY) / (QX -QY)] × A1/ax1

Concentration of Levofloxacin hemihydrate

CY = [(QM – QX) / (QY -QX)] × A1/ay1

Where,

A1 and A2 = Absorbance of sample solution at 289.39nm and 240.27nm

QM = A2 / A1

QX = ax2 / ax1

QY = ay2 / ay1

SPECTROPHOTOMETRIC CONDITION

VALIDATION OF THE METHOD

These methods were validated with respect to linearity, accuracy, intraday and interday

Precision, limit of detection (LOD) and limit of quantitation (LOQ), in accordance with ICH guideline.

Linearty

For both drugs, appropriate dilutions of standard stock solutions were analyzed as per the developed method. Calibration curve was plotted in the concentration range of 3-15 µg/ml for Cefixime trihydrate and 3-15 µg/ml for Levofloxacin hemihydrate.

Precision

Precision of the method was confirmed by interday and intraday analysis i.e. the analysis of

formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and %RSD also calculated.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by Calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Accuracy (Recovery study)

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder, a known quantity of standard Cefixime trihydrate and Levofloxacin hemihydrate added at 50%, 100% and 150% level and the contents were re-analysed by the proposed method. The %recovery and %RSD were calculated.

Assay

It was tested by analysis of commercially available marketed formulation.Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 400 mg of Cefixime trihydrate was transferred to 50 ml volumetric flask containing 40 ml of methanol, gentle shaking was carried out for 5 min and ultrasonicated for 5 min. The volume was made up to the mark with methanol.The tablet sample solution was filtered through Whatman filter paper no. 41. 5 ml of filtrate

was further diluted to 25 ml of methanol to get 100 µg/ml concentrations.From the 100 µg/ml of sample stock solution take 0.9 ml of solution and diluted up to the mark in 10 ml volumetric flask. So the final solution was made which contains 9 µg/ml Cefixime trihydrate and 9 µg/ml Levofloxacin hemihydrate both.

RESULTS AND DISCUSSION

SIMULTANEOUS EQUATION METHOD

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of CEF and LEVO. In simultaneous equation method, wavelengths selected for analysis were 240.27 nm for CEF and 296.28 nm for LEVO. Linearity was observed in the concentration rage of 3-15 µg/ml for CEF and 3-15µg/ml for LEVO correlation coefficient was found to be 0.999 and 0.999 at 240.27nm and 296.28 nm respectively. The proposed method was applied for pharmaceutical formulation and % label claim for CEF and LEVO was found to be 100.07 and 99.92, respectively. The method is accurate and precise and can be used for routine pharmaceutical analysis

ABSORBANCE RATIO METHOD

UV-spectrophotometric method by using absorbance ratio method was developed. Absorbances selected were 289.39 nm (isoabsorptive point) and 240.27 nm (λ max of CEF) Linearity was observed in the concentration range of 3-15 µg/ml and 3-15µg/ml. correlation coefficient was found to be 0.997 and 0.999 respectively The proposed method was applied for pharmaceutical formulation; % label claim for CEF and LEVO was found to be 99.85 and 100.04, respectively.

For parameters like linearity, precision, accuracy, LOD, LOQ. the data for which are presented in the Table 1, 2. Analytical recovery experiments were carried out by standard addition method to check the accuracy of the developed methods and to study the interference of formulation additives (Table1.3 and2.3). The validated method was successfully applied for the determination of in tablets mixture of CEF and LEVO the results are given in Table1.4 and2.4 indicate that the amount of drug in tablet samples met with requirements.

CONCLUSION

The proposed simultaneous equation method and Q absorption method provides

simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of CEF and LEVO in combined tablet dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness and reproucibility. The method can be used for routine analysis of CEF and LEVO in combined dosage form.
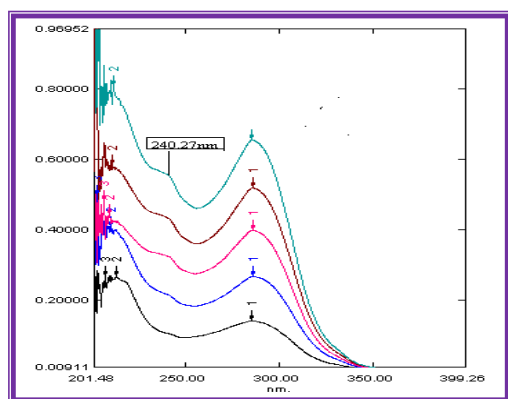
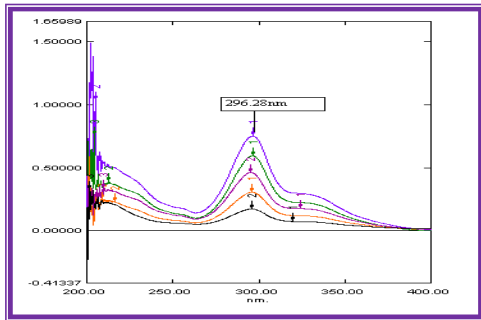**Figure3: Overlay spectra of CEF at 240.27nm**

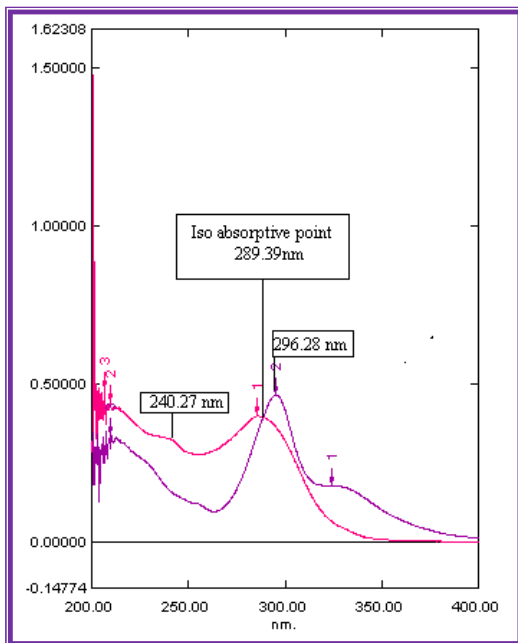**Figure4: Overlay spectra of LEVO at 296.28nm**

Figure 5: Overlay spectra of CEF (9 µg/ml) and LEVO (9 µg/ml)

Table 1.1

Summary of Validation Parameters of Simultaneous Method

Parameters	CEF		LEVO	
	240.27nm	**296.28nm**	**240.27**	**296.28**
Recovery %	98.33-101.25		98.66-101	
Precision				
Intra-day (n=3)	0.213-0.435	0.240-0.460	0.106-0.183	0.306-0.594
Inter-day (n=3)	0.212-0.431	0.237-0.454	0.108-0.185	0.307-0.589
LOD (µg/ml)	0.25	2.6	1.66	0.52
LOQ (µg/ml)	0.77	8.09	5	1.59
Solvent suitability	24hrs	24hrs	24hrs	24hrs

Table 1.2

Statistical Data CEF and LEVO by Simultaneous Method

Parameters	CEF		LEVO	
	240.27nm	**296.28nm**	**240.27nm**	**296.28nm**
Analytical Wavelength(nm)	240.27	296.28	240.27	296.28
Range	3-15(µg/ml)	3-15(µg/ml)	3-15(µg/ml)	3-15(µg/ml)
Slope	0.036	0.037	0.014	0.047
Intercept	-0.005	0.009	0.027	0.026
Regression Coefficient (r2)	0.999	0.999	0.992	0.999

Table1.3

Accuracy Data for CEF and LEVO by Simultaneous Method

% Level	Amount of Drug		Amount of Drug Added		Amount Recovered		% Recovery	
	CEF (µg/ml)	LEVO (µg/ml)	CEF (µg/ml)	LEVO (µg/ml)	CEF (µg/ml)	LEVO (µg/ml)	%CEF	% LEVO
50	4	5	2	2.5	5.9	7.4	98.33	98.66
100	4	5	4	5	8.1	10.10	101.25	101
150	4	5	6	7.5	9.95	12.46	99.50	99.68

Table 1.4

Assay Results of Marketed Formulation

Tablet	Drug	Labeled claim (mg)	Amount found (mg)	% label claim
Cefi-L	Cefixime trihydrate	400	400.30	100.07
	Levofloxacin hemihydrate	500	499.60	99.92

Table 2.1

Summary of Validation Parameters of Q- Absorption Method

Parameters	CEF		LEVO	
	240.27nm	289.39nm	240.27	289.39
Recovery %	98.33-100.75		98.66-99	
Precision				
Intra-day (n=3)	0.213-0.435	0.262-0.507	0.106-0.183	0.264-0.504
Inter-day (n=3)	0.212-0.431	0.260-0.507	0.108-0.185	0.265-0.508
LOD (µg/ml)	0.25	0.50	1.66	0.3
LOQ (µg/ml)	0.77	1.52	5	0.92
Solvent suitability	24hrs	24hrs	24hrs	24hrs

Table 2.2

Statistical Data CEF and LEVO by **Q- Absorption Method**

Parameters	CEF		LEVO	
	240.27nm	**289.39nm**	**240.27nm**	**289.39nm**
Analytical Wavelength(nm)	240.27	289.39	240.27	289.39
Range	3-15(µg/ml)	3-15(µg/ml)	3-15(µg/ml)	3-15(µg/ml)
Slope	0.036	0.042	0.014	0.040
Intercept	-0.005	0.008	0.027	0.028
Regression Coefficient (r2)	0.999	0.999	0.992	0.997

Table 2.3

Accuracy Data for CEF and LEVO by Q- Absorption Method

% Level	Amount of Drug taken		Amount of Drug Added		Amount Recovered		% Recovery	
	CEF (µg/ml)	LEVO (µg/ml)	**CEF (µg/ml)**	**LEVO (µg/ml)**	**CEF (µg/ml)**	**LEVO (µg/ml)**	**%CEF**	**% LEVO**
50	4	5	2	2.5	5.9	7.3	98.33	98.66
100	4	5	4	5	8.06	9.9	100.75	99
150	4	5	6	7.5	9.97	12.3	99.74	98.4

Table 2.4

Assay Results of Marketed Formulation

Tablet	Drug	Labeled claim (mg)	Amount found (mg)	% label claim
Cefi-L	Cefixime trihydrate	400	399.40	99.85
	Levofloxacin hemihydrate	500	500.20	100.04

REFERENCES

1. Genvresse I and Carbon C: Cefixime Trihydrate. International Journal of Antimicrobial Agents, 1993; 3:1-16.

2. Indian Pharmacopoeia Govrnment of India, Ministry of Health & Welfare, 6th edition 2010; 2:857-858

3. The British Pharmacopoeia. London: Her Majesty's Stationery Office, 1998.

4. United States Pharmacopoeia. 30th ed., United States Pharmacopeial Convention, RockVille.2007.

5. Sweetman S. C. and Martindale: The Complete Drug Reference; 33rd ed., Pharmaceutical Press: London, 2002; 219.

6. Fera M, Carbone M and Foch A: Activity of Cefixime Trihydrate against Helicobacter pylori. International Journal of Antimicrobial Agents, 1993; 3:105-108

7. Beckett A. H. and Stenlake JB: Practical Pharmaceutical Chemistry, 4th Edition, Part II, CBS Publications and Distributors, New Delhi, 1997; 1: 275-300

8. Shah P.B. and Pundarikakshudu K: Spectrophotometric, Difference Spectroscopic and High-Performance Liquid Chromatographic Methods for the Determination of Cefixime in Pharmaceutical Formulations, J. AOAC Int.,2006; 89: 987-994.

9. Al-Momani IF: Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. Journal of Pharmaceutical and Biomedical Analysis. 2001; 25: 751–57.

10. Arshad HM, Gauhar S, Bano R and Muhammad IN: Development of HPLC-UV method for analysis of cefixime in raw materials and capsule. Jordan journal of pharmaceutical sciences. 2009; 2(1): 53-65.

11. Falkowski A, Look Z, Noguchi H and Silber M: Determination of Cefixime Trihydrate in biological samples by Reversed-Phase High-Performance Liquid Chromatography. Journal of Chromatography, 1987; 422:145-152.

12. Maheshwari R.K., Kinariwala M., Saxena M., Gahlot M., Chaki R. and Jagwani Y: Spectrophotometric Analysis of Cefixime Trihydrate Tablets using Metformin Hydrochloride as Hydrotropic Solubilizing Agent, Asian J. Chem., 2008; 20: 6047-6050.

13. Zendelovsk D., Stafilov T and Milosevski P:High-performance liquid chromatographic method for determination of cefixime and cefotaxime in human plasma. *Bul.Chem.Tech*., 2003; 22(1): 39–45

14. Prabhu S., Vijay Amirtharaj R. and Senthilkumar N. (2010). Simultaneous RP-HPLC Method Development and Validation of Cefixime and Ofloxacin in Tablet Dosage Form. *Asian J. Research Chem*

15. Janhavirao, Kaminisethy And Savitayadav: Validated hptlc method for simultaneous quantitation of cefixime and ofloxacin in bulk drug and in pharmaceutical formulation" , *Int.J.Pharm.Res*, 2010; 02: 42-45.

16. El-Brashy AM, Metwally ME and El-Sepai FA: Spectrophotometric determination of Some Fluoroquinolone Antibacterials through Charge-transfer and Ion-pair Complexation Reactions. Bull. Korean Chem. Soc., 2004; 25(3): 365-372

17. Burhenne J, Ludwig M and Spiteller M: Polar. photodegradation products of quinolones determined by HPLC/MS/MS. Chemosphere. 1999; 38: 1279-86.

18. Oliphant CM and Green GM: British Pharmacopoeia. Department of Quinolones: A Comprehensive review. Am Health. London: HMSO Publication; Fam Physician February 2002; 65: 455-64.

19. Bottcher S, Baum HV, Hoppe-Tichy T, Benz C and Sonntag HG: An HPLC and microbiological assay to detertmine levofloxacin in soft tissue, bone and serum. J. Pharm. Biomed. Anal., 2001; 25**:** 197.

20. Shirkhedkar AA and Surana SJ: Quantitative Determination of Levofloxacin Hemihydrate in Bulk and Tablets by UV-Spectrophotometry and First Order Derivative Methods. Pak J Pharm Sci 2009; 22: 301-02.

21. Belal F, Al-Majed AA and Al-Obaid AM: Methods Analysis of 4-Quinolone Antibacterials. Talanta 1999; 50: 765-86.

22. Wong a FA, Juzwin SJ and Flor SC: Rapid stereospecific highperformance liquid chromatographic determination of levofloxacin in human plasma and urine. J. Pharm. Biomed. Anal 1997; 15: 765-771.

23. Meyyanathan SN, Ramasarma GVS and Suresh B: Analysis of levofloxacin in pharmaceutical preparations by high performance thin layer chromatography. *J. Seperation Sci., 2003;* 26: 1698-1700.

24. Kothekar KM, Jayakar B, Khandhar AP and Mishra RK: Quantitative Determination of Levofloxacin and Ambroxol hydrochloride in Pharmaceutical Dosage Form by Reversed-Phase High Performance Liquid Chromatography. Eurasian J Anal Chem 2007; 2: 21-31.

25. Validation of analytical procedure, Methodology, ICH Harmonized Tripartite Guidelines. 1996: 1-8.