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Development of Validated Absorption Correction Method for Simultaneous Determination of Levofloxacin Hemihydrate and Cefpodoxime Proxetil in Synthetic Mixture and Tablet Dosage Form

Hemal Vaidya*, Mrs. Henal Chorawala, Dr. Zarna Dedania
Bhagwan Mahavir College of Pharmacy, Surat, INDIA.
Sr. No. 149, Near Ashirwad Villa, New City Light Road, B/H Heena Bunglow's, Vesu, Bharthana, Surat, INDIA.

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ABSTRACT

Simple, precise and accurate method is described for the direct determination of Levofloxacin hemihydrate and Cefpodoxime proxetil in combined dosage form without prior separation. The Absorption Correction method in which measurement was carried out at 234nm and 341.18nm for Levofloxacin hemihydrate and Cefpodoxime proxetil respectively. The linearity for Levofloxacin hemihydrate and Cefpodoxime proxetil were found to be 2.5-10.5µg/ml and 2-10µg/ml respectively. Co-relation co-efficients were found to be 0.9987 and 0.9998 respectively. Method has been validated according to ICH guidelines. Percentage Recoveries for Levofloxacin hemihydrate and Cefpodoxime proxetil were 98.85-101.20% and 99.45-102.91%. The proposed Method was applied successfully for the determination of the two drugs in synthetic mixture and pharmaceutical dosage form.

Key words: *Levofloxacin hemihydrate, Cefpodoxime proxetil, Absorption Correction Method and Method validation.*

INTRODUCTION [1-4]

Levofloxacin hemihydrate is third generation fluroquinolones class antimicrobial agent. It is yellowish white to yellow colour powder. It's Molecular weight is 370.4 gm/mol. It is freely soluble in methanol, slightly soluble in water and GAA. It's chemical name is [(S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methylpiperazin-l-yl)-7- oxo-7H- pyrido [l, .2, 3-de]-1, 4-benzoxazine-6-carboxylic acid hemihydrate]. It's structure is given below.

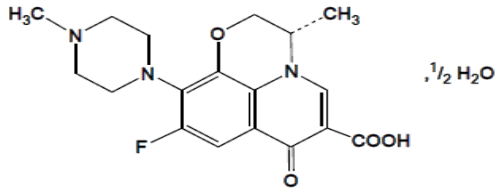

Fig. 1: Structure of Levofloxacin hemihydrate

Cefpodoxime Proxetil is third generation Cephalosporin class antimicrobial agent. It is White to light brownish-white powder. It's Molecular Weight is 557.6gm/mol. It is freely soluble in Dehydrated alcohol, Acetonitrile, Methanol and very slightly soluble in water. It's chemical name is (RS) - 1 (isopropoxy carbonyloxy) ethyl (+) - (6R, 7R) - 7 - [2 - (2 - amino - 4 - thiazolyl) – 2 - [(Z) methoxyimino] acetamido] – 3 – methoxymethyl – 3 – cephem – 4 - carboxy late. It's structure is given below.

***Corresponding author:**
Hemal Vaidya
Bhagwan Mahavir College of Pharmacy, Surat, INDIA.
Sr. No. 149, Near Ashirwad Villa, New City Light Road,
B/H Heena Bunglow's, Vesu, Bharthana, Surat, INDIA.
**E-Mail: hemalvaidya64@yahoo.com*

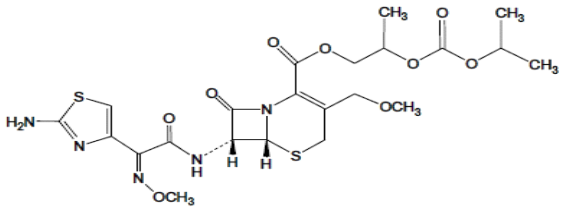

Fig. 2: Structure of Cefpodoxime Proxetil

Referring to literature survey,there is no any HPTLC method was developed for simultaneous estimation [3, 4]. So, I aim to Develop Validated Absorption correction method for simultaneous determination of Levofloxacin Hemihydrate and Cefpodoxime proxetil in synthetic Mixture and tablet dosage form. Validation of the method developed for Accuracy, Precision, Linearity & Range, LOD and LOQ.

MAERIAL AND METHODS

Identification of Drugs:

Identification of Levofloxacin hemihydrate and cefpodoxime proxetil was carried out by Following methods.

Solubility analysis:

For Levofloxacin hemihydrate: 1mg of drug was taken and dissolved in 10,100,150 and 200 ml of water, GAA and methanol.

For Cefpodoxime proxetil: 1mg of drug was taken and dissolved in 10,100,150 and 200 ml of water, acetonitrile and methanol.

Determination of melting point:

Melting Point of pure drugs has been determined using capillary melting point Apparatus. In this a Thiele tube is a glass instrument that is filled with oil that is heated by using an open flame. The sample is placed in the opening in a capillary tube alongside a mercury thermometer and allowed to be heated by the oil as it circulates through the Thiele tube. By using different oils different temperature ranges can be reached and used to determine melting points. The Thiele tube may also be used to determine boiling points, by using a liquid sample instead of a solid sample.

Determination of UV Spectra:

The UV spectra of Cefpodoxime proxetil (25µg/ml) and Levofloxacin hemihydrate (25µg/ml) solutions were taken in the range of 200-400 nm.From these spectra, λ_{max} of each drug was obtained and compared with that available in literature.

Method Development:

Materials:

Apparatus and Instrument were used are Double beam UV-visible spectrophotometer of shimadzu-1800,High Precision balance of wensar, vol. flask-10,25,50 and 100ml of borosil, pipettes-1, 2 and 5ml.

Reagents and Material were used are Cefpodoxime proxetil API, Levofloxacin hemihydrate API. Both are obtained from Kamron laboratory as a gift sample.Methanol of AR Grade is used as a solvent. Marketed formulation Glevo-pod tablet is purchased from local market.

Preparation of standard solution:

Preparation of stock solution (1000 µg/ml):

Cefpodoxime proxetil: Accurately weighed quantity of Cefpodoxime 50 mg was transferred into 50 ml volumetric flask, dissolved and diluted up to mark with methanol.(STOCK-A;1000 µg/ml)

Levofloxacin hemihydrate: Accurately weighed quantity of Levofloxacin hemihydrate 50 mg was transferred into 50 ml volumetric flask, dissolved and diluted up to mark with methanol. (STOCK –A; 1000 µg/ml)

Preparation of working standard solution:

Cefpodoxime proxetil: 100µg/ml of Cefpodoxime proxetil solution was prepared by diluting 10 ml of stock solution-A to 100 ml with methanol and further prepared solution of 10µg/ml. Working std solution of 2, 4, 6, 8, 10µg/ml in a 10 ml volumetric flask were prepared using 10µg/ml stock solution and adjusted upto mark with methanol.

Levofloxacin hemihydrate: 100µg/ml of Levofloxacin hemihydrate solution was prepared by diluting 10 ml of stock solution-A to 100 ml with methanol and further prepared solution of 10 µg/ml. Working standard solution of 2.5, 4.5, 6.5, 8.5, 10.5µg/ml in a 10 ml volumetric flask were prepared using 10µg/ml stock solution and adjusted upto mark with methanol.

Determination of wavelength for measurement:

4 ml of working standard solution of CEF (10µg/ml) and 4 ml of working standard solution of LEVO (10 µg/ml) was prepared. Each solution was scanned between 200-400 nm.Wavelengths were selected from the overlay spectra of CEF and LEVO.

Prepration of synthetic mixture:

The concentration of drugs in synthetic mixture Containing a ratio 2, 4, 6, 8, 10µg/ml of Cefpodoxime proxetil and 2.5, 4.5, 6.5, 8.5 and 10.5 µg/ml of Levofloxacin hemihydrate as per calibration curve make synthetic mixture of CEF and LEVO.

For calibration curve determination:

Cefpodoxime proxetil:

From 1000µg/ml stock solution, 100µg/ml was prepared and from this 10µg/ml solution of CEF was prepared. Then, Appropriate volume of aliquots from CEF of working std solution were prepared in different 10 ml volumetric flask. The volume was adjusted upto mark with methanol to make up the curve to 2, 4, 6, 8, 10µg/ml. This solution were scanned against methanol blank in a range of 200-400nm with medium scan speed. Using UV probe software, the spectra were recorded and wavelength of cefpodoxime proxetil was recorded at 234nm.

Levofloxacin hemihydrate:

From 1000µg/ml stock solution, 100µg/ml was prepared and from this 10µg/ml solution of LEVO was prepared. Then, Appropriate volume of aliquots from LEVO of working std solution were prepared in different 10 ml volumetric flask. The volume with adjusted up to mark with methanol to make up the curve to 2.5, 4.5, 6.5, 8.5, 10.5µg/ml. This solution were scanned against methanol blank in a range of 200-400nm with medium scan speed. Using UV probe software, the spectra were recorded and wavelength of Levofloxacin hemihydrate was recorded at 297.50nm.

Assay procedure for combine dosage form:

Twenty Tablets were weighed and powdered. The average weight of powder was calculated. The tablet powder equivalent to 25 mg of LEVO and 20 mg of CEF was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was filtered through Whatmann filter paper no.42 and first few ml of filtrate were discarded. 1 ml of this solution was further diluted to 10 ml with methanol.1 ml of this solution was further diluted to 10 ml with methanol. The solution was scanned against methanol as blank in a range of 200-400nm with medium scan speed. The spectrum was obtained. The concentration of CEF and LEVO can be obtained by using equation of straight line.

Validation of Proposed Method: [5-7]

Linearity and Range:

The linearity response was determined by analyzing 5 independent levels of calibration curve in the concentration range of 2, 4, 6, 8, 10µg/ml and 2.5, 4.5, 6.5, 8.5, 10.5µg/ml for CEF and LEVO respectively. Plot the calibration curve of absorbance *v/s* concentration and determine co-relation coefficient and regression line equations for CEF and LEVO.

Precision:

- ***Intraday:*** Synthetic mixture of drugs containing CEF and LEVO (µg/ml) equivalent to 4 CEF: 4.5 LEVO ; 6 CEF : 6.5 LEVO ; 8 CEF : 8.5 LEVO were analysed three times a day at a interval of 1 hour and measured % R.S.D.% R.S.D. should be less than 2%.
- ***Interday:*** Synthetic mixture of drugs containing CEF and LEVO (µg/ml) equivalent to 4 CEF: 4.5 LEVO ; 6 CEF : 6.5 LEVO ; 8 CEF : 8.5 LEVO were analysed three consequent day and measured % R.S.D.% R.S.D. should be less than 2%.

Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recoveries for levofloxacin hemihydrate and cefpodoxime proxetil were found out. Recovery between 98% - 102 % justifies the accuracy of the method. Accurately weighed quantity of pre analysed tablet of 25mg LEVO and 20mg CEF was taken in 100ml volumetric flask. To above flask API of both drug in 80%, 100% and 120% were added as shown in the table and then continued assay procedure.

Table No. 1: Accuracy Data for LEVO and CEF

Assay level	Tablet content equivalent to(mg)		Standard added(mg)		Total amount of drug present(mg)	
	LEV	CEF	LEV	CEF	LEV	CEF
BLANK	25	20	-	-	-	-
	25	20	-	-	-	-
	25	20	-	-	-	-
80%	25	20	20	16	45	36
	25	20	20	16	45	36
	25	20	20	16	45	36
100%	25	20	25	20	50	40
	25	20	25	20	50	40
	25	20	25	20	50	40
120%	25	20	30	24	55	44
	25	20	30	24	55	44
	25	20	30	24	55	44

LOD:

The Detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD =3.3 σ/S

Where, σ = Standard deviation of the Intercept and it was calculated from the equation,

$S=s_{yx}\sqrt{\sum x1^2}/n\sum(x_1-x^-)^2$

S= slope obtained from calibration curve

LOQ:

The Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOQ = 10 σ/S

Where, σ = Standard deviation of the Intercept and it was calculated from the same equation that describe above.

RESULT

Solubility analysis:

Table No. 2: Solubility analysis of LEVO and CEF

Sr. No.	Drug	Solvent used	Solubility testing	Solubility
1	Levofloxacin hemihydrate	Water	1mg/150ml	Slightly soluble
		GAA	1mg/150ml	Slightly soluble
		Methanol	10mg/10ml	Freely soluble
2	Cefpodoxime proxetil	Dehydrated alcohol	10mg/10ml	Freely soluble
		Acetonitrile	10mg/10ml	Freely soluble
		Methanol	10mg/10ml	Freely soluble
		Water	1mg/200ml	Very slightly soluble

Determination of melting point:

Melting Point of pure drugs has been determined using capillary melting point apparatus. Melting Points obtained were compared with that available in literature as shown in table.

Table No. 3: melting points of levo and cef

	Levofloxacin hemihydrate	Cefpodoxime proxetil
Standard	228◦C	111-113◦C
Observed	226-228◦C	110-113◦C

Determination of λ_{max}:

Table No. 4: λ_{max} for Cefpodoxime proxetil and Levofloxacin hemihydrate

Sr. No.	Drugs	Reported λ_{max}	Observed λ_{max}
1	CEF	235 nm	234.80
2	LEVO	300 nm	298.40

Selection of Wavelength for Absorption Correction Method: [9-11]

To determine wavelength for measurement, standard spectra of Levofloxacin hemihydrate and Cefpodoxime proxetil were scanned between 400-200 nm against methanol. Overlain spectra of Levofloxacin hemihydrate and Cefpodoxime proxetil are presented in Figure . In absorption correction method overlain spectra showed that,Cefpodoxime proxetil has zero absorbances at 341.18 nm Whereas Levofloxacin hemihydrate has subtantial absorbance. Thus, Levofloxacin hemihydrate was estimated directly at 341.18 nm without interference of Cefpodoxime proxetil. At 234nm which is λ_{max} of Cefpodoxime proxetil, at this wavelength, both drugs shows considerable absorbance. So, selected wavelengths were 341.18 nm and 234 respectively.

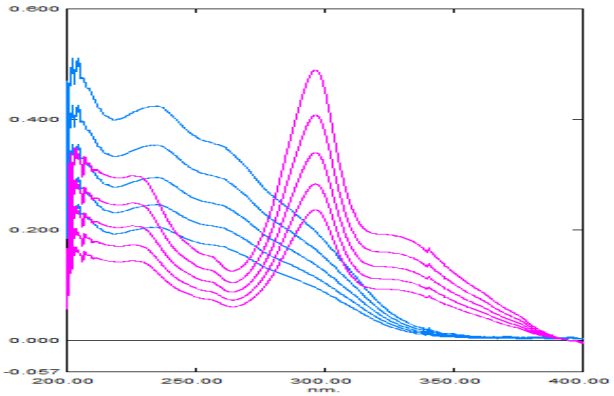

Fig. 3: Overlay Spectra of LEVO and CEF

Table No. 5: Results of validation parameters for Absorption Correction method

Parameter	Cefpodoxime Proxetil	Levofloxacin Hemihydrate
Linearity and range	2-10 µg/ml	2.5-10.5 µg/ml
r^2 Data	0.9988	0.9998
Precision %(R.S.D)		
Intraday precision	0.571%	1.675%
Interday precision	0.779%	0.478%
Accuracy	99.45-102.91%	98.85-101.20%
LOD	0.499 µg/ml	0.2637 µg/ml
LOQ	1.512 µg/ml	0.7992 µg/ml

Table No. 6: Recovery data of marketed formulation

Formulation	Tablet content taken (mg/tablet)		Amount found (mg/tablet)		Assay %estimated (n=3)	
GLEVOPOD	LEV	CEF	LEV	CEF	LEV	CEF
	25	20	24.82	20.45	99.6	102.25

CONCLUSION

Levofloxacin hemihydrate and Cefpodoxime proxetil in combined dosage form is used for the treatment of several infection.

Simple, accurate, rapid and precise Absorption Correction method was developed and validated for simultaneous estimation of both these drugs.

Absorption correction method:

The wavelengths selected were 341.18 nm and 234 nm for Levofloxacin hemihydrate and cefpodoxime proxetil respectively for the absorption correction method. For this method the linearity range were between 2.5-10.5µg/ml for levofloxacin hemihydrate and 2-10 µg/ml for cefpodoxime proxetil. Correlation coefficients of levofloxacin hemihydrate and cefpodoxime proxetil were 0.999 and 0.9988 respectively. The percentage recoveries of Levofloxacin hemihydrate and Cefpodoxime proxetil were in the range of 98.85-101.20 % and 99.45-102.91% respectively. The assay results of

Levofloxacin hemihydrate and Cefpodoxime proxetil was in the range of 98.85-101.20% and 99.45-102.91% respectively. This was comparable to labeled claim. So, the excipients usually present in the pharmaceutical formulation did not interfere with determination of Levofloxacin hemihydrate and Cefpodoxime proxetil. So, It can be used as marketed formulation in Industry.

REFERENCES:

1. O'Neil MO and Heckelmann PE. An Encyclopedia of Chemicals, Drugs and Biologicals The Merck's Index; 14th Edn; Merck Research Laboratory, USA, **2006**; pp 1941,6771.
2. Sean CS. The complete drug reference Martindale; 34th Edn; Pharmaceutical Press, London Chicago, **2005**; pp 178.3,225.
3. Indian Pharmacopoeia; Vol-II, Govt. of India, Indian Pharmacopoeial Commission, Ministry of Health and Family Welfare, Ghaziabad, **2010**; pp 1018-1019, 1579-1580.
4. United State Pharmacopoeia; Vol- III, 31st Edn; USP Convention Rockville, **2008**; pp 2133.
5. ICH guidelines, validation of analytical procedures Q2A; ICH Harmonized Tripartite Guidelines, **1996**.
6. ICH guidelines, validation of analytical procedure: Methodology Q2B; ICH Harmonized Tripartite Guidelines, **1996**.
7. www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_guideline.pdf.
8. Beckett AH, and Stenlake JB. UV-Visible Spectrophotometry: Practical Pharmaceutical Chemistry; 4th Edn; Part-II, C.B.S. Publishers, Delhi, **2001**; pp 285-300.
9. Sharma YR. Ultraviolet and Visible Spectroscopy in Elementary Organic Spectroscopy; 1st Edn; S. Chand and Company, New Delhi, **2004**; pp 9-60.
10. Connors AK. A Textbook of Pharmaceutical Analysis; 3th Edn; A Wiley Interscience Publication, New York, pp 616.
11. Skoog DA., Holler FJ, and Nieman TA. Introduction to UV Spectroscopy in Principle of Instrumental Analysis; 5th Edn; Thomson Brooks-Cole Publication, New Delhi, pp 301, 423.

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