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METHOD DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF CEFIXIMETRIHYDRATE AND LINEZOLID IN THEIR COMBINED TABLET DOSAGE FORM

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Abstract

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of cefixime Trihydrate (CEF) and Linezolid (LNZ) in combined tablet dosage form. The method is based on the Q analysis method and Simultaneous equations for analysis of both the drugs using methanol as solvent. First method is Q analysis method based on absorbance ratio at two selected wavelengths 278.72nm (iso-absorptive point) and 256.70nm (λmax of linezolid).Second method is based on the simultaneous equations and wavelengths selected for analysis were 288.72 nm (λmax of Cefixime Trihydrate) and 256.70nm (λmax of Linezolid) respectively, in methanol. The linearity was obtained in the concentration range of 2-10 µg/ml and 5-25 µg/ml for both Cefixime Trihydrate and Linezolid. The results of the analysis have been validated statistically and by recovery studies. Method was found to be accurate, precise and reproducible. This method was applied to the assay of the drugs in marketed formulation, which were found in the range of 99.70% to 100.03% of the labeled value for both Cefixime Trihydrate and Linezolid in Q analysis method and 100.10% and 99.91% of the labeled value for both Cefixime Trihydrate and Linezolid in Simultaneous equation method. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.

INTRODUCTION

Cefixime Trihydrate (CEF) is an oral third generation cephalosporin antibiotic. 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 infections1. Linezolid (LNZ) is a synthetic antibacterial agent of the oxazolidinone class of antibiotics. Linezolid ischemicallyN-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl) phenyl]-2-oxo-1, 3-oxazolidin-5-yl] methyl} acetamide. Clinically used for the treatment of infections caused by multi-resistant bacteria including streptococcus and methicillin-resistant Staphylococcus aureus (MRSA). The drug works by inhibiting the initiation of bacterial protein synthesis. Both the drugs are marketed as combined dose tablet formulation in the ratio of 200:600 mg CEF: LNZ. Literature survey reveals that cefixime Trihydrate can be estimated by spectrophotometrically3, HPLC44-8 and byHPTLC9 individually or with other drugs in bulk drugs and in human plasma, while Linezolid can be estimated byspectrophotometrically10-11, HPLC412-13 in combination with other drugs. However, there is no analytical method reported for the estimation of CEF and LNZ in a combined dosage formulation. Present work describes two methods for simultaneous estimation of CEF and LNZ in tablet formulation.

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 Linezolid (gift sample from Zydus Cadila Pharmaceuticals Ltd., Ankleshwar,

Gujarat, India). The pharmaceutical dosage form used in study was Zifi-turbo (label claim CEF 200 mg, LNZ 600 mg) manufactured in India by F.D.C spectra healthcare Pharma.

Reagent used

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

Preparation of standard stock solution

Accurately weighed 10mg of quantity of Cefixime Trihydrate & Linezolid was transferred into 10 ml volumetric flask and dissolved in methanol and diluted up to the mark with methanol to give a stock solution having strength 1mg/ml (1000µg/ml). 100 µg/ml of Cefixime Trihydrate & Linezolid working standard solution was prepared by diluting 1 ml of stock solution with methanol in 10 ml volumetric flask up to the mark.

METHODS

(1)Q ABSORBANCE RATIO

It uses the ratio of absorbances at two selected wavelengths, one which is anis absorptive point and other being the λ-max of one of the two components. From the CEF and LNZ show an isoabsorptivepoint at 278.72 nm. The second wavelength used is 256.70 nm, which is the λ-max of LNZ. Six working standard solutions having concentration 2,4,6,8,10µg/ml for CEF and 5,10,15,20,2 µg/ml for LNZ were prepared in methanol and the absorbances at278.72nm (isoabsorptive point) and 256.70 nm (λ-max of LNZ) 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.

CX =

[(QM – Qy) / (QX –QY)] × A1/ax1..... (1)

CY =

(A1/ax1) – CX …… (2)

Where, A1 and A2 are absorbances of mixture at 278.72 nm and 256.70 nm;

aX1and aY1 are absorptivities of CEF and LNZ at 278.72 nm;

aX2and aY2 are absorptivities of CEF and LNZ respectively at 256.70 nm;

QM = A2 / A1,

QA = aX2 / aX1 and

QI = aY2 / aY1.

(2)SIMULTANEOUS EQUATION METHOD:

For the simultaneous equation method wavelengths selected were λmax of both the drugs, at the λmax of the LNZ, CEF shows the considerable absorbance and at the λmax of CEF, LNZ shows considerable absorbance. The study of spectra also reveals that CEF and LNZ have λmax at 288.72 nm and at 256.70nmrespectively.Both the drugs were found to have considerable absorbance at λmax of each other. The wavelengths selected for analysis were 288.72 nm and 256.70nm respectively for CEF and LNZ.A series of standard solutions ranging from 2-10µg/ml for CEF and from 5-25µg/ml for LNZ both were prepared and the absorbance of solutions was recorded at selected wavelengths. Calibration curve of absorbance versus concentration was plotted. The Calibration curves were found to be linear in the concentration range under study. The concentration of two drugs in mixture was calculated by using following equations:

CX =

(A2ay1 – A1ay2) / (ax2ay1 – ax1ay2)…(3)

CY=

(A1ax2 – A2ax1) / (ax2ay1 – ax1ay2…(4)

Where A1 and A2 are the absorbances of mixture at 288.72nm and 256.70nm and ax1, ay1, ax2 and ay2 were absorptivity of CEF and LNZ at 288.72nm and 256.70nm respectively.

VALIDATION OF THE Q ABSORPTION RATIO AND SIMULTANEOUS EQUATION 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.

Linearity

Linearity was taken for Cefixime Trihydrate and Linezolid in the concentration range of 2-10 µg/ml &5-25 µg/ml respectively to both methods. The calibration curve was obtained by plotting absorbance →concentrations.

Precision

For Intraday precision, it was carried out by preparing 3 replicates of 4, 6 and 8µg/ml&10, 15&20 µg/ml of CEF and LNZ

concentrations, within the linearity range in both method and measuring the absorbance of each solution. % RSD (% relative standard deviation) was calculated. For Interday precision, 3 different concentration solutions within the linearity range were measured for 3 different days in both methods. % RSD (% relative standard deviation) was calculated.

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

They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y- intercept) and S, is the mean of the slope of calibration plot.

Accuracy

To study accuracy of the method, recovery studies were carried out by addition of standard drug in a tablet sample at 0%, 50%, 100% and 150%. The percentage of recovery was calculated in both methods.

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 200mg of Cefixime Trihydrate was transferred to 50mlolumetricflask 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. From the Concentrate 100 µg/ml of sample stock solution take 0.6 ml of solution of Cefixime Trihydrate and 1.5ml of Linezolid diluted up to the mark in 10 ml volumetric flask. So the final solution was made which contains 6 µg/ml Cefixime Trihydrate and15µg/ml Linezolid both. absorbances were measured at 288.78 nm, 256.70 nm and 278.72 nm against blank. The concentrations of two drugs in sample were determined by using equations 1 and 2 Q-absorbance analysis and equation 3 and 4.in simultaneous equation method.

RESULTS AND DISCUSSION

Method I: Simultaneous Equation Method

UV-spectrophotometric method using simultaneous equation was developed. CEF showed absorbance maxima at 288.72nm and LNZ at 256.70 nm. Linearity was observed in the concentration rage of 2-10 µg/ml for CEF and 5-25 µg/ml for LNZ correlation coefficient was found to be 0.997 and 0.996 at 288.72 nm and 256.70nm respectively. The proposed method was applied for pharmaceutical formulation and % label claim for CEF and LNZ was found to be 100.10 and 99.91 respectively. The method is accurate and precise and can be used for routine pharmaceutical analysis.

Method II: Absorbance Ratio Method

UV-spectrophotometric method by using absorbance ratio method was developed. Absorbances selected were 278.72 nm(isoabsorptive point) and 256 nm (λ max of Linezolid) Linearity was observed in the concentration range of 2-10µg/mland5-25 µg/ml correlation coefficient was found to be 0.998 and 0.996 respectively The proposed method was applied for pharmaceutical formulation; % label claim for CEF and LNZ was found to be 99.70 and 100.03**,** respectively.

CONCLUSION

The proposed Q absorption method and simultaneous equation method provides simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of CEF and LNZ 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 reprocibility. The method can be used for routine analysis of CEF and LNZ in combined dosage form.

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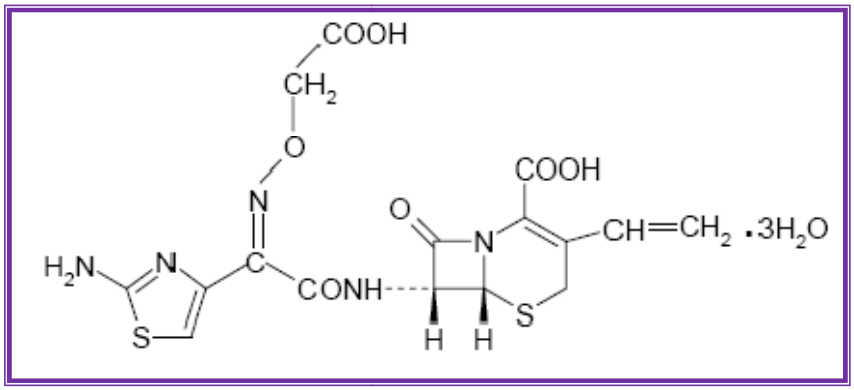

Figure 1 Chemical structure of cefixime trihydrate

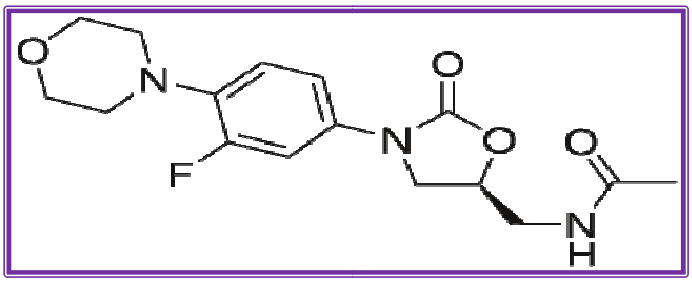

Figure 2 Chemical structure of Linezolid

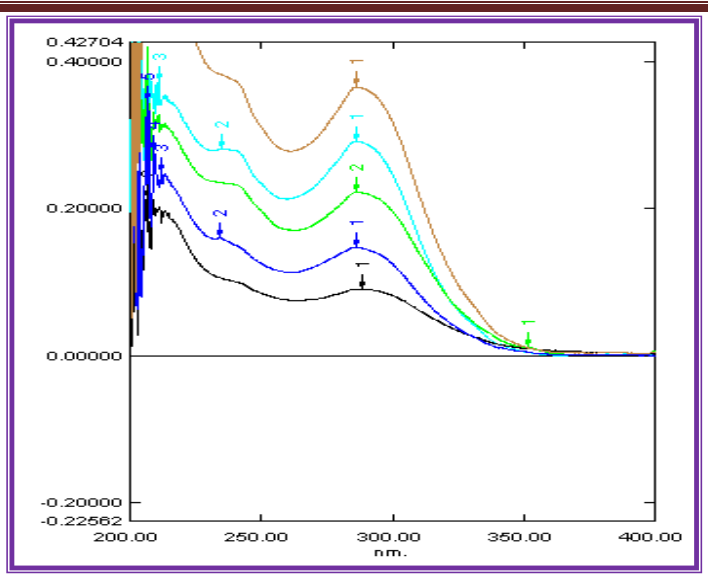

Figure 3 Overlay spectra of CEF at 288.72nm

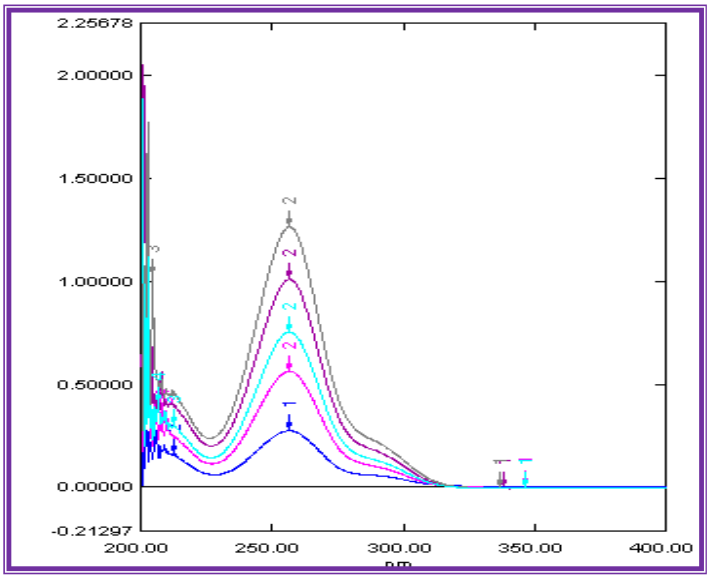

Figure 4 Overlay spectra of LNZ at 256.70nm

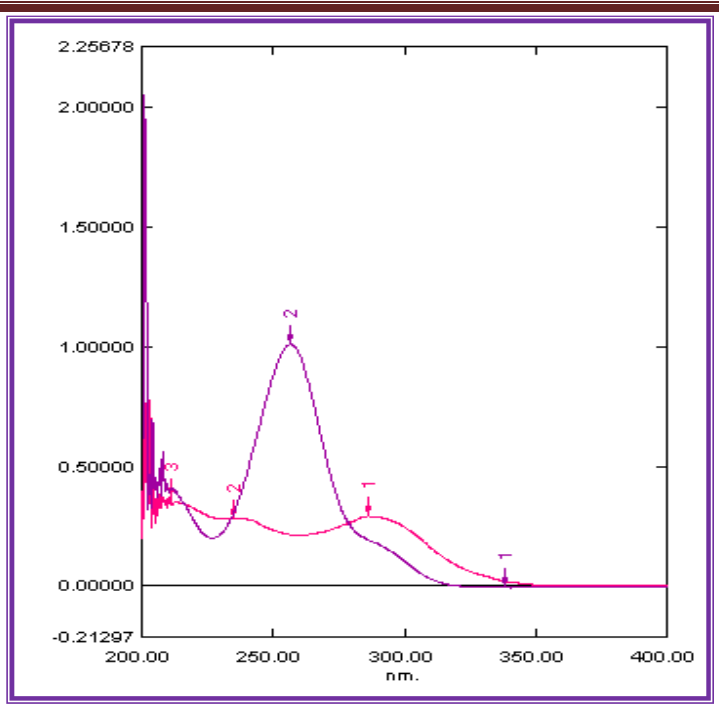

Figure 5 Overlay spectra of CEF (6 µg/ml) and LNZ (15 µg/ml)

Table 1.1 Summary of Validation Parameters of Q- Absorbance ratio

Parameters	CEF		LNZ	
	278.72nm	256.70nm	278.72	256.70
Recovery %	99.72-100.44	98.95-100.22	99.55-100.27	98.05-100.22
Precision				
Intra-day (n=3)	0.13-0.26	0.11-0.21	0.14-0.26	0.11-0.21
Inter-day (n=3)	0.13-0.27	0.11-0.21	0.17-0.26	0.57-1.01
LOD (µg/ml)	0.2	0.11-0.21	0.1	0.8
LOQ (µg/ml)	0.6	3	0.5	2.5
Solvent suitability	24hrs	24hrs	24hrs	24hrs

Table 1.2 Statistical Data CEF and LNZ by Q- Absorbance ratio

Parameters	CEF		LNZ	
	278.72	256.70	278.72	256.70
Analytical Wavelength	278.72	256.70	278.72	256.70
Range	(2-10µg/ml)	(2-10µg/ml)	(5-25µg/ml)	(5-25µg/ml)
Slope	0.031	0.025	0.012	0.048
Intercept	0.013	0.021	0.12	0.049
Regression Coefficient (r^2)	0.998	0.991	0.996	0.996

Table 1.3 Accuracy Data for CEF and LNZ by Q- Absorbance Method

% Level	Amount of Drug taken		Amount of Drug Added		Amount Recovered		% Recovery	
	CEF	LNZ	CEF(µg/ml)	LNZ(µg/ml)	CEF(µg/ml	LNZ(µg/ml)	%CEF	% LNZ
50	3	9	1.5	4.5	4.51	13.51	100.22	100.07
100	3	9	3	9	6.01	17.9	100.16	99.44
150	3	9	4.5	13.5	7.47	22.4	99.6	99.55

Table 1.4 Assay Results of Marketed Formulation

Tablet	Drug	Labeled claim (mg)	Amount found (mg)	% label claim
Zifi-turbo	Cefixime trihydrate	200	199.40	99.70
	Linezolid	600	600.20	100.03

Table 2.1 Summary of Validation Parameters of simultaneous equation method

Parameters	CEF		LNZ	
	288.72	256.70	288.72	256.70
Recovery %	98.95-100.22		98.05-100.22	
Precision				
Intra-day (n=3)	0.146-0.296	0.11-0.21	0.102-0.181	0.11-0.21
Inter-day (n=3)	0.118-0.224	0.118-0.214	0.1150.187	0.575-1.013
LOD(µg/ml)	0.3	0.9	1.1	0.16
LOQ (µg/ml)	1.14	3	1.42	0.5
Solvent suitability	24hrs	24hrs	24hrs	24hrs

Table 2.2 Statistical Data CEF and LNZ by simultaneous equation Method:

Parameters	CEF		LNZ	
	288.72	256.70	288.72	256.70
Analytical Wavelength	288.72	256.70	288.72	256.70
Range	2-10µg/ml	2-10µg/ml	5-25µg/ml	5-25µg/ml
Slope	0.034	0.025	0.008	0.049
Intercept	0.013	0.021	0.015	0.049
Regression Coefficient (r2)	0.997	0.991	0.997	0.996

Table 2.3 Accuracy Data for CEF and LNZ by simultaneous equation Method

% Level	Amount of Drug Taken		Amount of Drug Added		Amount Recovered		% Recovery	
	CEF(µg/ml)	LNZ(µg/ml)	CEF(µg/ml)	LNZ(µg/ml)	CEF(µg/ml	LNZ(µg/ml)	%CEF	% LNZ
50	3	9	1.5	4.5	4.45	13.4	98.88	99.25
100	3	9	3	9	5.96	17.29	99.33	99.44
150	3	9	4.5	13.5	7.51	22.6	100.13	100.44

Table 2.4 Assay Results of Marketed Formulation

Tablet	Drug	Labeled claim (mg)	Amount found (mg)	% label claim
Zifi-turbo	Cefixime trihydrate	200	200.20	100.10
	Linezolid	600	599.50	99.91

REFERENCES

1. Khan Abbas, Iqbal Zafar, Imran Muhammad, Javed Khalid, Khan Abad, Ahmed Lateef and Shah Yasar: Simultaneous Determination of Cefdinir in human plasma by rp-hplc/uv detection method: method development, optimization, validation, and its application to a pharmacokinetic journal of chromatography b. 2011; 879(24): 2423-2429.

2. Jain Rajeev, Gupta Vinod, N. Jadon and K. Radhapyari: Voltammetric determination of cefixime in pharmaceutic and biological. Analytical biochemistry. 2010; 407(1): 79-88.

3. Seric Jovanovic, D agbaba, D zivanov-stakic and S vladimirov: HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage form. Journal of pharmaceutical and biomedical analysis. 1998; 18(4): 893-898.

4. MV Kumudhavalli, Sahu Sandeep, K Abhiteja and Dr. B. Jayakar: Development and validation of rp-hplc method for

simultaneous determination of cefixime and potassium clavulanate in tablet dosage form. International journal of pharma recent research. 2010; 2: 57-60.

5. ICH Q2 (R1) Validation of Analytical Procedure: Text and Methodology, Geneva, International Conference on Harmonisation (2005).

6. Muhammad hafiz, Arshad, Gauhar Shahnaz, Bano Raheel and Iyad Naeem Muhammad: Development of hplc-uv method for analysis of cefixime in raw materials and in capsule. Jordan journal of pharmaceutical sciences. 2009; 2(1): 53.

7. Patel Satish, Patel PU, Patel NJ and Patel MM: High performance thin layer chromatographic method for estimation of linezolid in tablets. Indian journal of pharmaceutical science. 2007; 69(4):571-574.

8. Gaur Gaurav, Prof. Kukkar Vipin and Singh Ramaniranjan: a validated rp- hplc method for estimation of linezolid in linezolid infusion. International journal of pharmaceutical research and development. 2012; 4:132-135.

9. Mohapatra Sharmistha, M. Mathrusri Annapurnam, Anwar Mohammed and Akhter Sohail: validated stability indicating rp-hplc method for the estimation of linezolid in a pharmaceutical dosage form. Journal of liquid chromatography & related technologies. 2011; 34(18):2185-2195.

10. Baietto Lorena, D'avolio, Antonio De Rosa, Francesco Giuseppe and Garazzino: Simultaneous quantification of linezolid, rifampicin, levofloxacin, and moxifloxacin in human plasma using high-performance liquid chromatography with UV. Journal of therapeutic drug monitoring. 2009; 31(1): 104-109.

11. Dhoka Madhura, Gawande Vandana, Joshi Pranav: Simultaneous estimation of Cefixime Trihydrate and Erdosteine in pharmaceutical dosage form by using reverse phase - high performance liquid chromatography. International journal of chem tech research. 2010; 2(1): 79-87.

12. Patel Satish A and Patel Natavarlal J: Development and validation of Rp-hplc method for simultaneous estimation of Cefixime Trihydrate and Ofloxacin in tablets. International journal of

pharmatech research. 2009; 3(4): 1958-1962.

13. Tank manan, Thumar Kashyap and Tanna rupa: Method development and validation for simultaneous estimation of Cefixime Trihydrate and Dicloxacillin sodium in combined dosage form by high performance thin layer chromatograph. Journal of pharmaceutical analysis and quality assurance. 2012; 5: 354-360.

14. Pasha Khaja, Patil CS, Ali Sadath and Chimkod VB: Reverse phase Hplc method for determination of Cefixime in Pharmaceutical Dosage form. Research journal of pharmaceutical, biological and chemical science. 2010; 1(3): 26.

15. Kalsi PS: spectroscopy of organic compounds; ultraviolet and visible spectroscopy; new age international (p) limited; New-Delhi. 2004: 9-63.

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

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

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

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

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

21. Shah PB and Pundarika 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.

22. Maheshwari RK, 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.