

SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND LINEZOLID IN COMBINE PHARMACEUTICAL FORMULATION

DISHA THAKKAR*, RAJSHREE MASHRU

Quality Assurance Laboratory, Center of Relevance and Excellence in Novel Drug Delivery System, Pharmacy Department, G.H.Patel Building, Donor's plaza, The Maharaja Sayajirao University, Fatehgunj, Vadodara, Gujarat, India 39002.
Email: dishathakkar282@gmail.com

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ABSTRACT

Three simple, rapid, accurate and precise spectrophotometric methods have been developed for simultaneous estimation of Cefixime Trihydrate (CEF) and Linezolid (LINE) in their combine dosage form. Method I, zero crossing second derivative spectrophotometry involves measurements of absorbance at 276.6 nm (for LINE) and 301 nm (for CEF) in second derivative spectra. Method II, simultaneous equation method (Vierodt's method), involves formation and solving of simultaneous equation at 257.4 nm (λ max of LINE) and 289.4 nm (λ max of CEF). Method III, ratio derivative spectrophotometry, involves division of spectra of LINE by one selected standard spectrum of CEF and then measuring absorbance at 268.2 nm in ratio derivative spectra for estimation of LINE. Similarly, spectra of CEF are divided by one selected standard spectrum of LINE and then absorbance at 233.3 nm in ratio derivative spectra are measured for estimation of CEF. Developed methods were validated according to ICH guideline Q2(R1). The calibration graph follows Beer's law in the range of 5-20 $\mu\text{g/ml}$ for LINE and 2-20 $\mu\text{g/ml}$ for CEF with R^2 value greater than 0.999. Accuracy of all methods was determined by recovery studies and showed % recovery between 98 to 102%. Intraday and interday precision was checked for all methods and mean %RSD was found to be less than 2 for all methods. The methods were successfully applied for estimation of LINE and CEF in marketed formulation.

Keywords: Linezolid, Cefixime Trihydrate, Zero crossing second derivative spectrophotometry, Simultaneous equation method, Ratio derivative spectrophotometry.

INTRODUCTION

Cefixime (as Trihydrate or anhydrous) 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 (Fig. 1)^{1,2}, clinically used in the treatment of susceptible infection including gonorrhoea, otitis media, pharyngitis, lower respiratory tract infection such as bronchitis and urinary tract infection. It acts by inhibiting cell wall synthesis. It binds to one of the penicillin binding proteins (PBPs) which inhibit the final transpeptidation step of the peptidoglycan synthesis in the bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death. Linezolid, (S)-N-[[3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl]methyl]acetamide (Fig. 2)³, is a synthetic antibiotic belonging to a new class of antimicrobial called the oxazolidinones. It is active against most gram positive bacteria including streptococci, methicillin resistant staphylococcus aureus and vancomycin resistant enterococci. It acts by inhibiting initiation of bacterial protein synthesis.

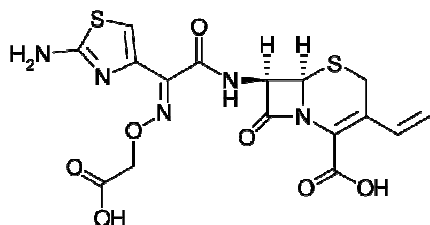


Fig. 1: Structure of Cefixime

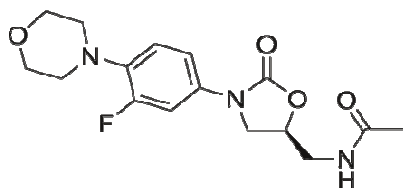


Fig. 2: Structure of Linezolid

Objective of study

Spectrophotometric and RP-HPLC methods for simultaneous determination of Cefixime with Ofloxacin^{4,5,6}, Potassium clavulanate⁷ and with Moxifloxacin⁸ have been reported. Spectrofluorimetric method of Cefixime through derivatization with 2-cyanoacetamide⁹ and stability indicating analytical method¹⁰ has been available in literature. RP-HPLC for determination of Linezolid in plasma¹¹, Chiral-HPLC¹² and stability indicating LC for enantiomeric separation of Linezolid and its impurity¹³ have been reported. The objective of present study was to develop rapid, accurate, economic, precise and specific analytical methods for simultaneous estimation of Cefixime and Linezolid in their combined pharmaceutical formulation.

MATERIAL AND METHODS

Apparatus and Software

Shimadzu UV-1700 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.10 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm. The samples were weighed on electronic analytical balance (A \times 120, Shimadzu).

Reagent and Chemical

Methanol for UV spectroscopy (Spectrochem Pvt. Ltd, Mumbai, India) was used as solvent. Single distilled water was used throughout work. Linezolid and Cefixime combination tablets (ZIFITURBO, 600 mg Linezolid and 200 mg Cefixime, manufactured by Akum drugs and pharmaceuticals, Haridwar, India) were purchased from local market.

Preparation of solution

Accurately weighed Linezolid and Cefixime Trihydrate equivalent to anhydrous Cefixime (in quantity of 25mg and 28mg respectively) were transferred to two separate 25ml volumetric flask, dissolved with the use of methanol and volume was made up to mark with methanol to obtain stock solution of LINE(1000 $\mu\text{g/ml}$) and CEF(1000 $\mu\text{g/ml}$). From these stock solutions LINE(100 $\mu\text{g/ml}$) and CEF(50 $\mu\text{g/ml}$) were prepared by transferring 5ml and 2.5ml

aliquots respectively to other 50ml volumetric flask and make up the volume with methanol. From this, 6-18 µg/ml of LINE and 2-6 µg/ml of CEF were prepared in 10ml volumetric flask using methanol.

Method I - Zero crossing second derivative spectrophotometry

The solution of standard LINE and CEF were prepared in the range of 6-18 µg/ml and 2-6 µg/ml respectively. The absorption spectra of the solution of LINE and CEF were recorded in the range of 200-400 nm

and stored in the memory of the instrument and transformed to second derivative spectrum with $\Delta\lambda=16$ nm and scaling factor 100. At 276.6 nm CEF is having zero crossing point and LINE can be estimated (Fig. 3). The absorbance at 276.6 nm was plotted against respective concentration of LINE for preparation of calibration graph (Fig. 4.1). At 301 nm LINE has zero crossing point so CEF can be estimated. The absorbance at 301 nm was plotted against respective concentration of CEF for preparation of calibration graph (Fig. 4.2).

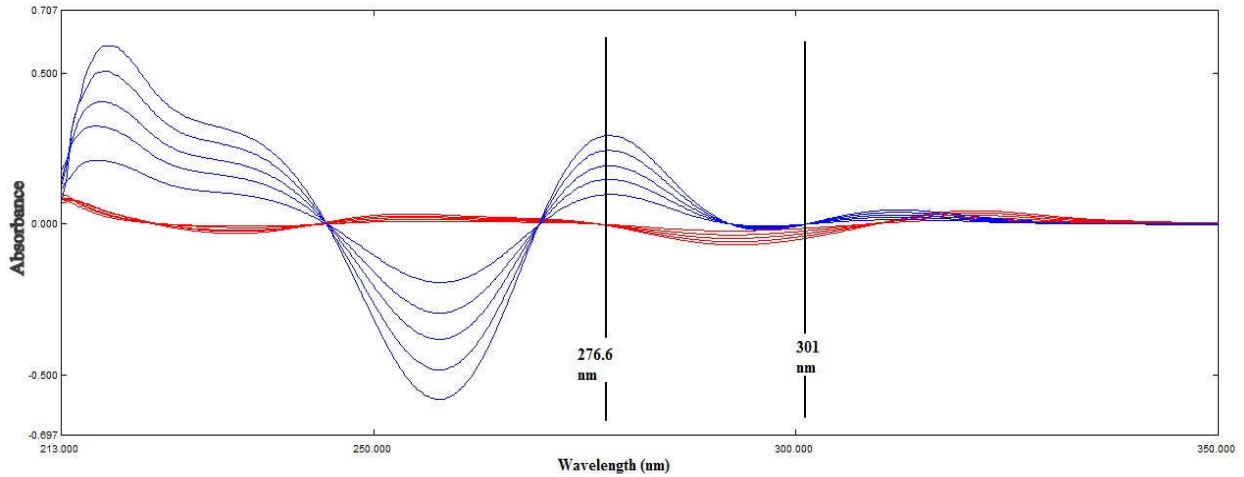


Fig. 3: Second derivative overlain spectra of LINE (6-18 µg/ml, blue) and CEF (2-6 µg/ml, red)

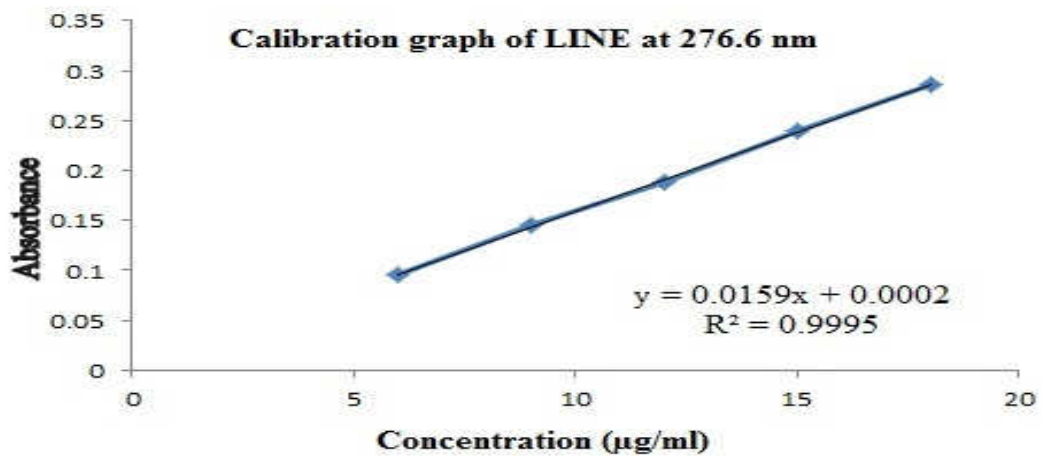


Fig. 4.1: Calibration graph of LINE by zero crossing second derivative method

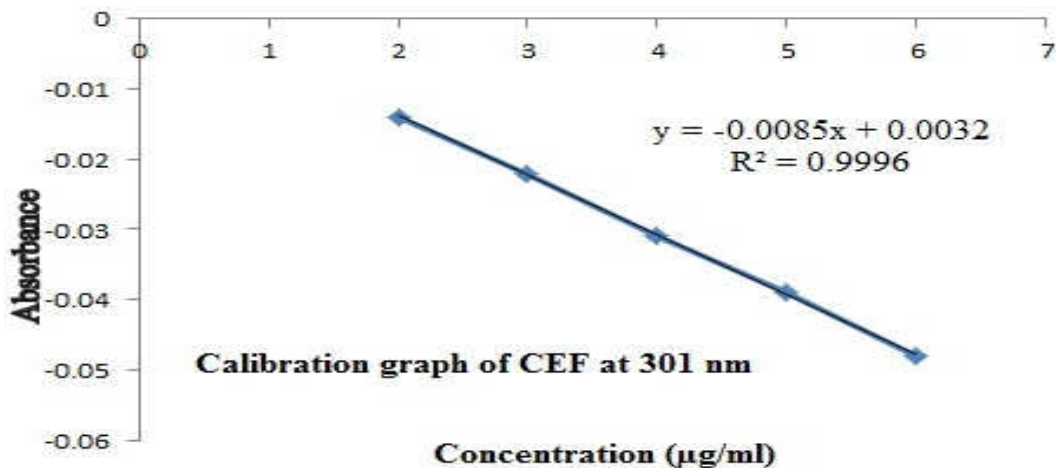


Fig. 4.2: Calibration graph of CEF by zero crossing second derivative method

Method II – Simultaneous equation method (Vierodt’s method)

If a sample containing two absorbing drugs (X and Y) each of which absorb at the λ_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equation¹⁴. From stock solution, solutions with concentration of LINE (6-18 $\mu\text{g/ml}$) and CEF (2-6 $\mu\text{g/ml}$) were prepared by appropriate dilution and were scanned in entire UV range 200-400 nm. From overlain spectra (Fig. 5) λ_{max} for LINE 257.4 nm and λ_{max} for CEF 289.4 nm were selected for formation of simultaneous equation of two drugs. The absorbance at 257.4 nm and 289.4 nm for LINE and CEF were measured respectively

to plot a calibration graph of both the drugs (Fig. 6.1 and 6.2). The absorbance and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs,

$$C_{(LINE)} = (A_2 a_{Y1} - A_1 a_{Y2}) / (a_{X2} a_{Y1} - a_{X1} a_{Y2})$$

$$C_{(CEF)} = (A_1 a_{X2} - A_2 a_{X1}) / (a_{X2} a_{Y1} - a_{X1} a_{Y2})$$

Where, A_1 and A_2 are absorbance of sample at 257.4 nm and 289.4 nm respectively, a_{X1} and a_{X2} are absorptivity of LINE at 257.4 nm and 289.4 nm, a_{Y1} and a_{Y2} are absorptivity of CEF at 257.4 nm and 289.4 nm.

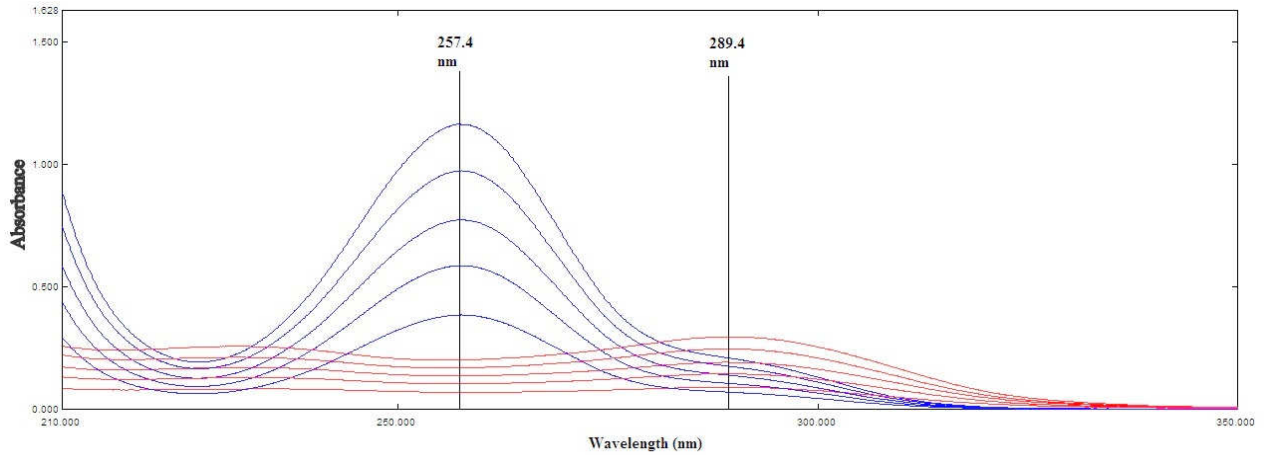


Fig. 5: Zero order overlain spectra of LINE (6-18 $\mu\text{g/ml}$, blue) and CEF (2-6 $\mu\text{g/ml}$, red)

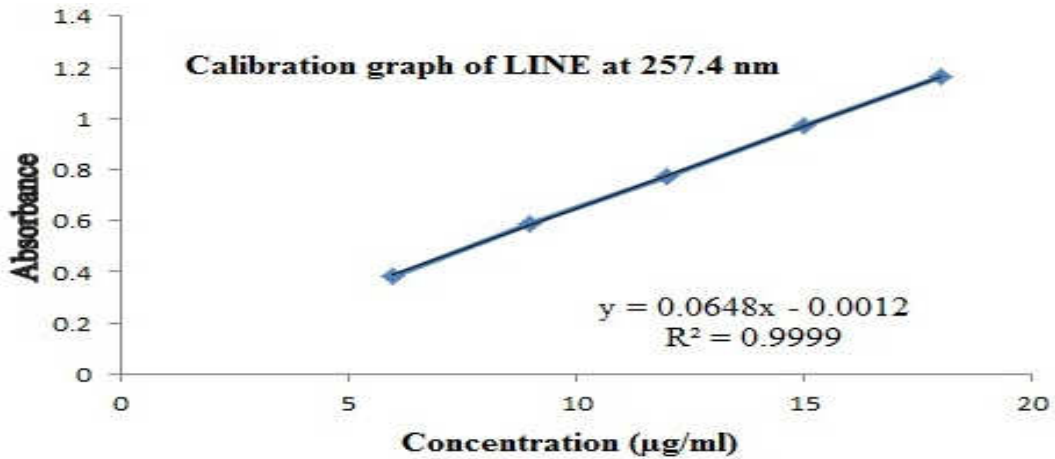


Fig. 6.1: Calibration graph of LINE by simultaneous equation method

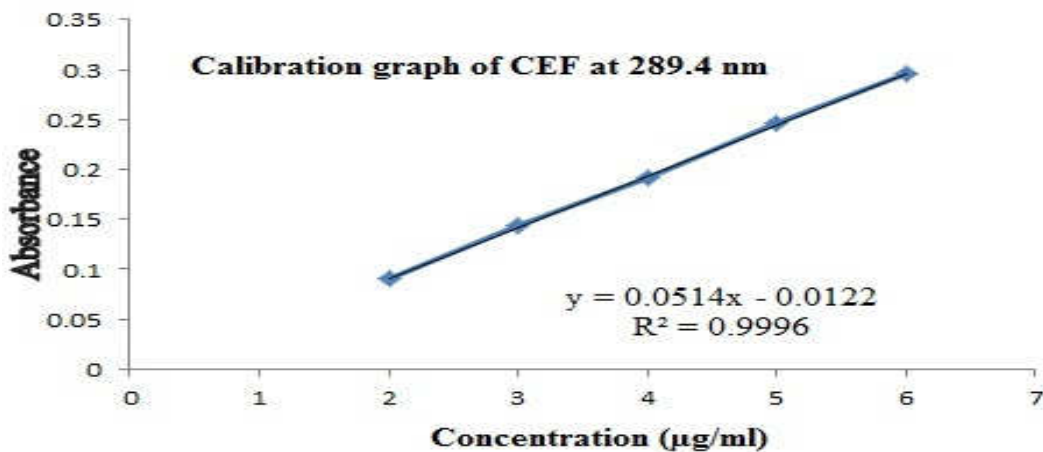


Fig. 6.2: Calibration graph of CEF by simultaneous equation method

Method III – Ratio derivative spectrophotometry

In this method, the spectra of LINE and CEF were divided by one standard spectrum of CEF and LINE respectively. For selecting the divisor concentration appropriate concentration of LINE and CEF were tested and based on better signal to noise ratio 15 µg/ml of LINE and 4 µg/ml of CEF was selected as divisor concentration. The spectra of LINE ranging from 6-18 µg/ml were recorded in the region of 200-400 nm and were divided by 4 µg/ml of CEF to obtain ratio spectra. These ratio spectra were derivatised with $\Delta\lambda=4$ nm

and scaling factor 25. Overlain ratio derivative spectra of LINE are shown in (Fig. 7.1). Analytical wavelength of 268.2 nm was selected for estimation of LINE. Calibration graph at 268.2 nm is plotted and shown in (Fig. 8.1). Similarly, the spectra of CEF ranging from 2-6 µg/ml were recorded and divided by standard spectrum of 15 µg/ml of LINE. These ratio spectra were derivatised with $\Delta\lambda=4$ nm and scaling factor 25. For estimation of CEF analytical wavelength 233.3 nm was selected. Ratio derivative spectra of CEF (Fig. 7.2) and calibration graph (Fig. 8.2) are shown below.

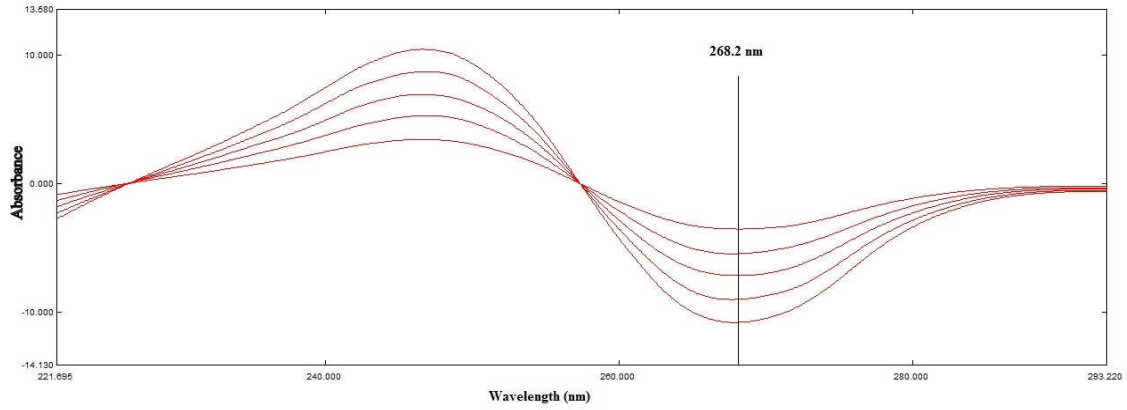


Fig. 7.1: Overlain ratio derivative spectra of LINE(6-18 µg/ml), 4 µg/ml of CEF used as divisor concentration

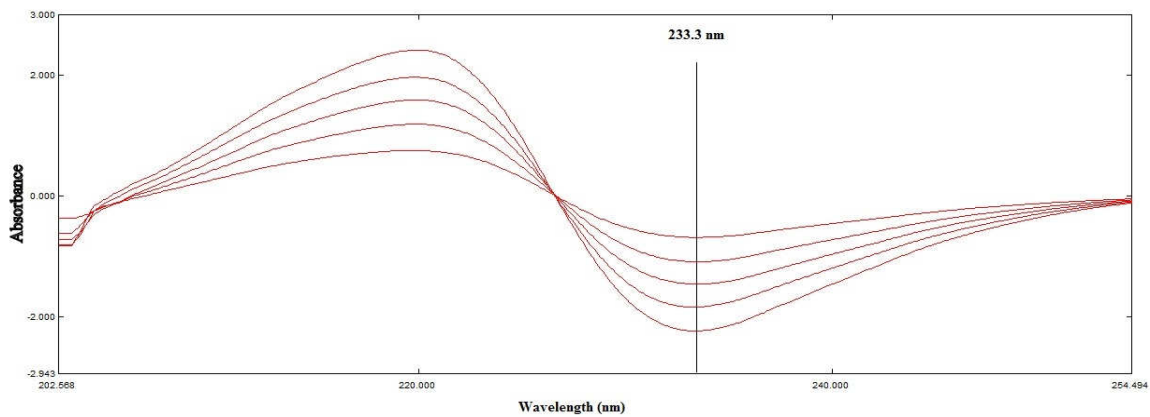


Fig. 7.2: Overlain ratio derivative spectra of CEF (2-6 µg/ml), 15 µg/ml of LINE used as divisor concentration

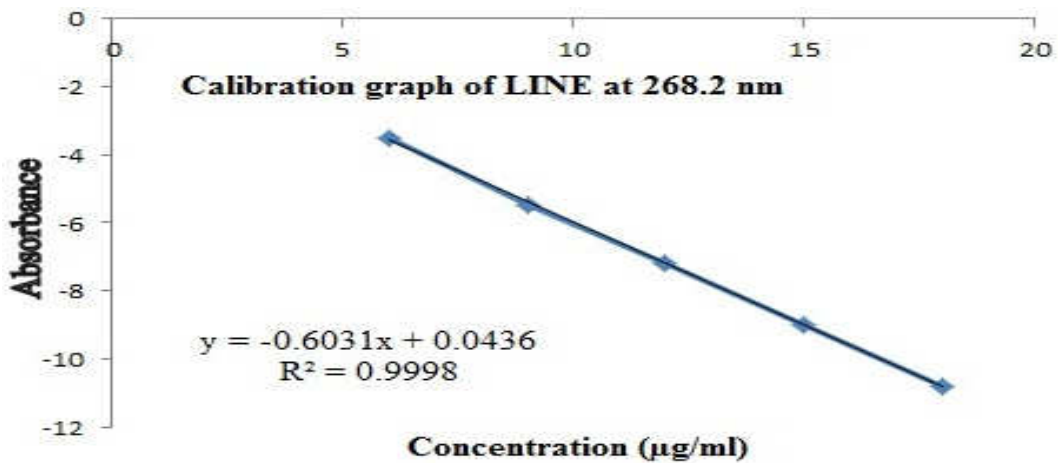


Fig. 8.1: Calibration graph of LINE by ratio derivative spectrophotometry

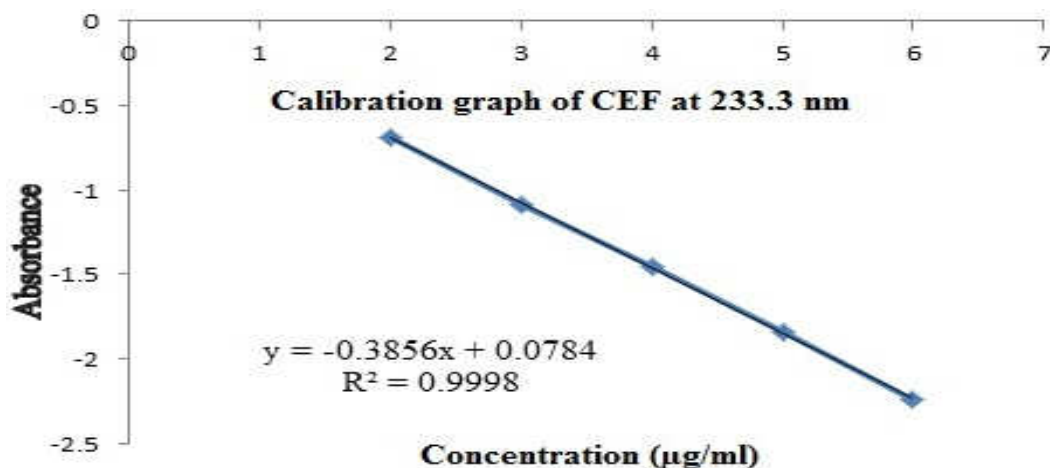


Fig. 8.2: Calibration graph of CEF by ratio derivative spectrophotometry

Assay of marketed formulation by Method I, II and III

Twenty tablets were powdered and an amount equivalent to 200 mg CEF and 600 mg LINE was weighed and dissolved in 25 ml methanol.

Solutions were filtered using whatmann filter paper grade 1. Appropriate dilutions were prepared in methanol taking suitable aliquots of the clear filtrates and subjected to analysis using all the three methods described above. The result of analysis is reported in Table-1.

Table 1: Results of simultaneous estimation of marketed formulation for method I, II and III

Formulation : ZIFITURBO		
Label claim : LINE : CEF (600mg : 200mg)		
Method	*%LINE±S.D	*%CEF±S.D
I	100.33±0.0551 %	100.83±0.0134 %
II	100.69±0.0280%	100.58±0.0244 %
III	100.70±0.0805%	100.24±0.0556 %

*Mean value of five determinations.

Table 2: Summary of validation parameter by proposed methods

Parameter	Method I		Method II		Method III	
	LINE	CEF	LINE	CEF	LINE	CEF
Analytical wavelength (nm)	276.6 nm	301 nm	257.4 nm	289.4 nm	268.2 nm	233.3 nm
Beer's range (µg/ml)	6-18	2-6	6-18	2-6	6-18	2-6
Correlation coefficient	0.9995	0.9996	0.9999	0.9996	0.9998	0.9998
Intraday precision (%RSD)	0.3667	0.7259	0.1987	0.6915	0.5444	0.6193
Inter day precision (%RSD)	0.6359	1.1759	0.2106	0.9825	0.5471	1.1313
LOD (µg/ml)	0.1931	0.3466	0.2435	0.2877	0.114	0.368
LOQ (µg/ml)	0.5852	1.0505	0.7380	0.8719	0.348	1.116
% Recovery*50%Std addition	100.00±.06	101.43±.06	99.7±.00	98.56±.00	100.74±.03	100.77±.06
*100% Std addition	100.42±.05	100.79±.01	100.73±.01	100.49±.03	100.05±.07	99.48±.00
*150% Std addition	99.47±.07	100.39±.05	99.05±.05	100.91±.03	98.75±.04	99.88±.03

*Mean value of three determinations.

RESULT AND DISCUSSION

Three developed spectrophotometric methods for the simultaneous estimation of both the drugs were validated according to ICH guideline Q2(R1)¹⁵. The results of validation parameters for three methods are reported in Table-2

CONCLUSION

Three spectrophotometric methods were developed for simultaneous estimation of LINE and CEF in their combined formulation without prior separation. Methods were found to be simple, rapid, economic, accurate and precise. The results of validation tests were found to be satisfactory and therefore, these methods can be applied successfully for routine quality control analysis of LINE and CEF in bulk and pharmaceutical formulation.

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