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Simultaneous determination of Cefixime trihydrate and Ofloxacin in pharmaceutical dosage form by second order derivative UV spectrophotometry

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Abstract

Derivative spectrophotometry offers a useful approach for the analysis of drugs in multi-component formulation. In this study a second order derivative spectrophotometric method is applied for the simultaneous determination of Cefixime Trihydrate and Ofloxacin in Tablet dosage form. The measurements were carried out at wavelengths of 307 and 298 nm for Cefixime Trihydrate and Ofloxacin respectively. The method was found to be linear (r^2=0.999) in the range of 4-20 µg/ml for Cefixime Trihydrate in the presence of 20 µg/ml of Ofloxacin at 307 nm. The linear correlation (r^2=0.999) was obtained in the range of 4-20 µg/ml for Ofloxacin in the presence of 20 µg/ml of Cefixime Trihydrate at 298 nm. The method was successfully used for simultaneous determination of Cefixime Trihydrate and Ofloxacin in tablet dosage form without any interference from excipients and prior separation.

Keywords: Cefixime Trihydrate, Ofloxacin, UV visible spectrophotometry, Method Validation, Second order derivative method.

1. Introduction

Cefixime Trihydrate (CEF) chemically is (6R, 7R)-7-[[(z)-2-(2-aminothiazol-4-yl) 2-[(carboxy methoxy) imino] acetyl]-amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2ene-2-carboxylic acid. It is a oral third generation of cephalosporin and is used as an antibacterial and especially against gram negative, gram positive and anaerobic bacteria pathogens including β- lactamase producing strains.[1,2] It consists of high affinity for penicillin binding proteins with deceitful site of activity. It acts by inhibition of bacterial cell-wall synthesis. It is clinically used in the treatment of susceptible infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary-tract infections. [3] Literature survey revealed the estimation methods of cefixime trihydrate or with other drugs by UV spectrophotometry[4], HPLC [5] calorimetric method, flow injection analysis, and HPTLC.

Ofloxacin (OFL) a fluorinated carboxyaquinolone, chemically is a racemate (+) -9-fluro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid.[5,6] It is a synthetic broad spectrum antibacterial agent official in BP, USP and EP. Ofloxacin have much greater antibacterial activity towards urinary tract infections. [7] It acts by inhibiting DNA gyrase of microorganisms. Literature survey reveals UV spectrophotometric method [8], atomic absorption spectrometry, spectroflurometry, HPLC [9-12] and microbiological method for its determination.

Application of derivative technique of spectrophotometry offers a powerful tool for quantitative analysis of multi-component mixtures. When derivatised, the maxima and minima of the original function take zero values, and the inflections are converted into maxima or minima, respectively. The derivative curves are more structured than the original spectra, thus enabling very tiny differences between the original spectra to be identified. Derivative spectrophotometry provides selectivity and offers a solution in resolving the overlapping spectra in multi-component analysis without previous chemical separation. In the last decades, this technique has rapidly gained application in the field of pharmaceutical analysis to overcome the problem of interference, due to substances other than analytes, commonly present in pharmaceutical formulations or for combination of two or more drug substances.[13] Lack of any published method for simultaneous spectrophotometric determination of Cefixime Trihydrate and Ofloxacin, therefore, provoked us to investigate the application of derivative spectrophotometry for simultaneous determination of these compounds in pharmaceutical dosage forms using zero-crossing method.

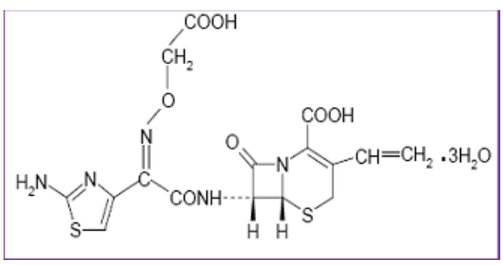

Fig. 1: Chemical structure of Cefixime Trihydrate

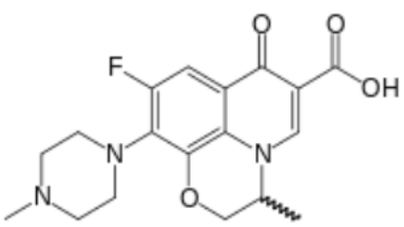

Fig. 2: Chemical structure of Ofloxacin

2. Materials and Methods

2.1 Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the

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solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India).Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.2 Materials

Reference standard of Cefixime Trihydrate and Ofloxacin API was supplied as gift sample by Cipla Pharmaceutical company,Goa, India. The commercial formulation ABIXIM*O was purchased from the local market Mangalwedha, Dist: Solapur, Maharashtra, India.

2.3 Method development

2.3.1 Preparation of standard stock solution

Stock solution was prepared by diluting 10 mg of each drug in sufficient quantity of methanol in separate volumetric flask and volume was made up to 100 ml to get the concentrations of 100 µg/ml for each drug. Dilutions from stock solution were prepared in the range of 4-20 µg/ml for Cefixime Trihydrate and 4-20 µg/ml for Ofloxacin. Methanol was used as a blank solution.

2.3.2 Spectrophotometric Measurements

Zero-order spectra of standard solutions of Cefixime Trihydrate (20µg/ml) and Ofloxacin (20 µg/ml) versus their solvent blank were recorded in the range of 200-400 nm (Figure 3). The second order derivative spectra of these solutions were obtained in the same range of wavelength against their blanks (Figure 4). The values of second derivative amplitudes for Cefixime Trihydrate in the presence of Ofloxacin and vice versa were measured at 307 nm (zero-crossing of Cefixime Trihydarte) and 298 nm (zero-crossing of Ofloxacin), respectively. The calibration curves for derivative spectrophotometry were constructed by plotting the drug concentration versus the absorbance values of the second derivative spectrum, at 307 nm for Cefixime Trihydrate and at 298 nm for Ofloxacin.

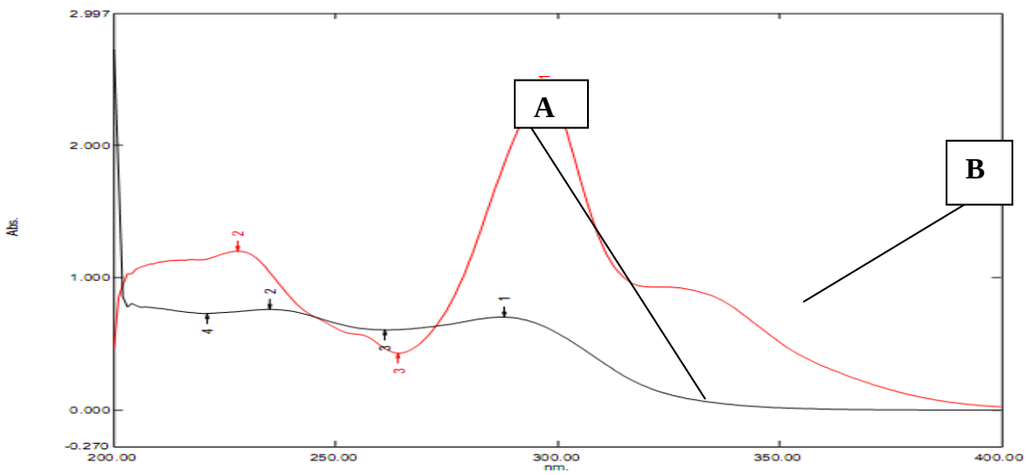

Figure 3: Zero order spectra (overlain) of Cefixime Trihydrate 20 µg/ml (A) and Ofloxacin 20 µg/ml (B)

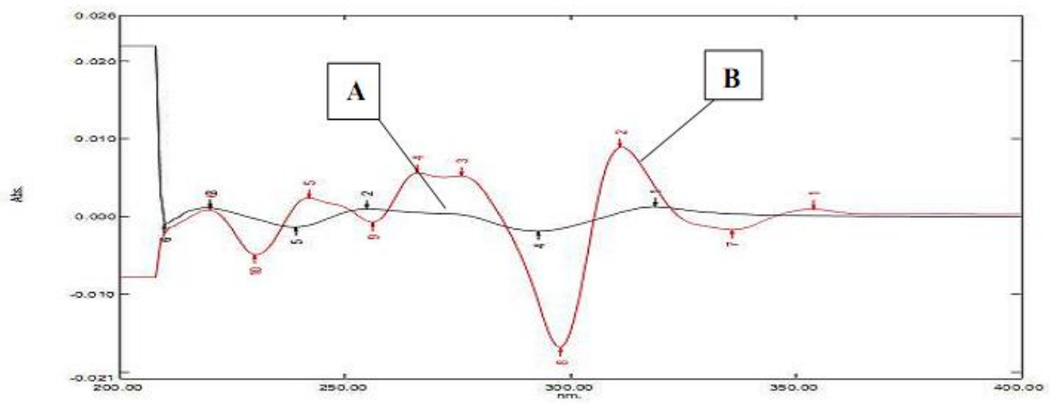

Figure 4: Second order derivative spectra (overlain) of Cefixime Trihydrate 20 µg/ml (A) and Ofloxacin 20 µg/ml (B)

2.3.3 Analysis of commercial tablet formulation

Contents of 20 tablets were weighed and their average weight was determined and powdered. Accurately weighed powder equivalent to fill weight of one tablet was transferred to 100 ml calibrated flask containing 50 ml of methanol and sonicated for 30 minutes. The volume was then made up to the mark with methanol. The resulting solution was then filtered through whatmann filter paper (#41). From this solution, 1 ml was transferred to another 10 ml calibrated flask and diluted up to 10 ml which gives 200µg/ml concentration of solution. Then 1 ml of this solution was further diluted to 10 ml to get approximate concentration 20 µg/ml of Cefixime Trihydrate and 20 µg/ml of Ofloxacin.

Table 1: Assay of tablet dosage form

Sr. No.	Sample Solution Concentration (µg/ml)	Amount found (%)*	Mean % found	%RSD
1	20	101.39		
2	20	98.07	99.37	0.4987
3	20	98.65		

*n=3, % RSD = % Relative Standard Deviation.

3. Results and Discussion

3.1 Linearity and Range

3.2 Linearity

Calibration curves were constructed using six replicates of Cefixime Trihydrate solutions between 4-20 µg/ml in the presence of 4-20 µg/ml of Ofloxacin. The same procedure was used for solutions containing Ofloxacin 4-20 µg/ml in the presence of 4-20 µg/ml of Cefixime Trihydrate. The calibration curves were constructed (Fig. 5 and Fig. 6) and statistical analysis was performed. The regression equations of calibration curves were y=0.024x+0.005 (r^2=0.999) at 307 nm for Cefixime Trihydrate and y=0.029x+0.001 (r^2=0.999) at 298 nm for Ofloxacin for second order derivative spectrophotometry methods. The range was found to be 4-20µg/ml for both drugs for second order spectrophotometry methods. [14, 15]

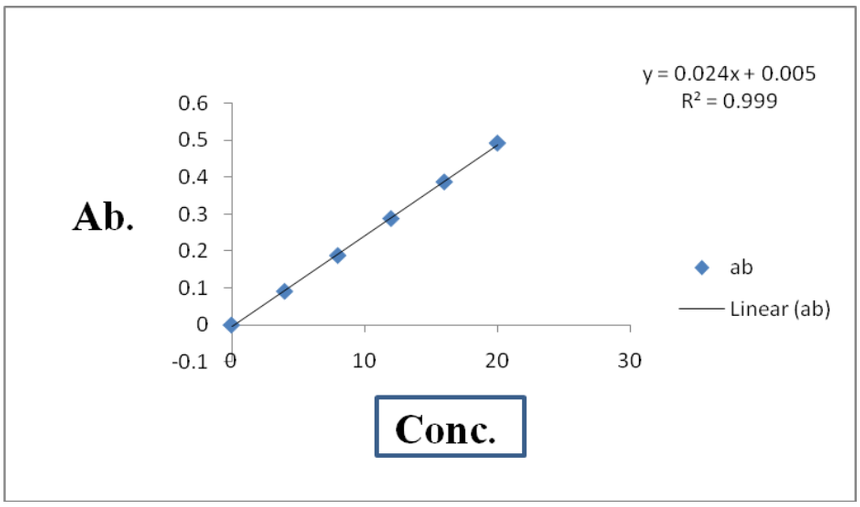

Fig.5 Calibration curve for Cefixime Trihydrate at 307 nm

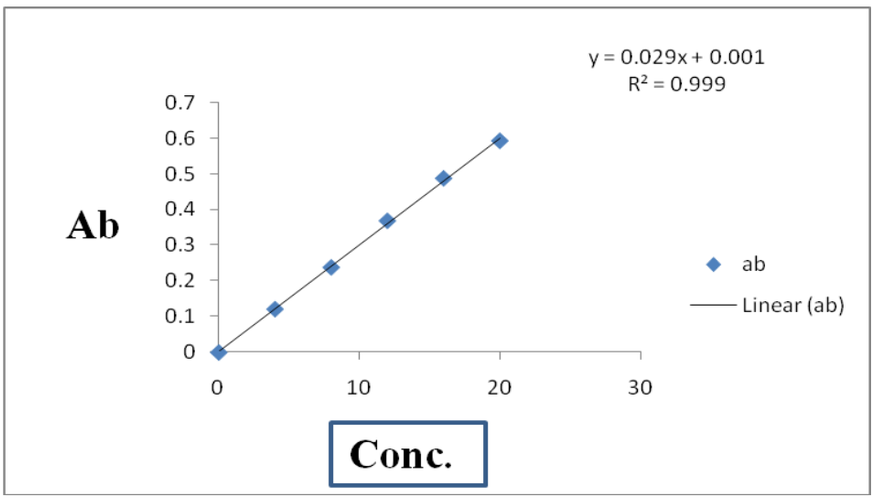

Fig.6 Calibration curve for Ofloxacin at 298 nm

Table 2: Stastical data for the calibration graphs for determination of Cefixime Trihydrate and Ofloxacin by Proposed methods.

Parameters	Cefixime Trihydrate	Ofloxacin
Linearity range (µg/ml)*	4-20	4-20
r^2± S.D*	0.999	0.997

3.3 Accuracy

For accuracy determination, the analysed samples were spiked with extra 80%, 100% and 120% of the standard solution of both drugs and the mixtures were reanalysed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the commercial tablet formulations. The mean recoveries and %RSD are illustrated in Table 3. The data indicates that the proposed derivative spectrophotometric method is highly reproducible during one run and between different runs. [14, 15]

Table 3: Results of drug content and analytical recovery of Cefixime Trihydrate and Ofloxacin

Parameters	Cefixime Trihydrate	% R.S.D	Ofloxacin	% R.S.D
Labelled claim	400 mg	-	400 mg	-
% Drug content ± S.D	100.98 ± 0.4297	0.34	100.04 ± 0.1237	0.54
Analytical recovery at 80 % ± S.D	98.58 ± 0.2145	0.17	98.97 ± 0.1840	0.15
Analytical recovery at 100 % ± S.D	101.08 ± 0.2042	0.22	101.97 ± 0.1258	0.26
Analytical recovery at 120% ± S.D	100.15 ± 0.1043	0.18	98.94 ± 0.1942	0.29

3.4 Precision

To determine the precision of the method, Cefixime Trihydrate and Ofloxacin solutions at a concentration of 20µg/ml were analysed each three times for second order spectrophotometric method. Solutions for the standard curves were prepared fresh everyday. [14,15]

Table 4: Results of Intra and Inter Day Precision

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Cefixime Trihydrate	0.0077	0.6584	0.0048	0.4207
Ofloxacin	0.0074	0.6580	0.0042	0.4209

3.5 Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/ S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.5927 µg/ml and 1.7786 µg/ml respectively of Cefixime Trihydrate for second order derivative and 0.6047µg/ml &1.8176 µg/ml for area under the curve methods respectively. [14, 15]

3.6 Analysis of the Marketed Formulation

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.37% second order spectrophotometric methods. It may therefore be inferred that degradation of Cefixime Trihydrate and Ofloxacin had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Cefixime Trihydrate and Ofloxacin in pharmaceutical dosage form.[14, 15]

Table 5: Summary of validation parameters

Parameter	Cefixime Trihydrate	Ofloxacin
λ range	200-400 nm	200-400nm
Regression Equation (y=mx+c)	Y=0.024x+0.005	Y=0.029x+0.001
Measured wavelength	307 nm	298 nm
Linearity range	4-20µg/ml	4-20µg/ml
Slope	0.024	0.029
Intercept	0.005	0.001
Correlation coefficient (R^2)	0.999	0.999
Limit of Detection (LOD) µg/ml	0.5927	0.6047
Limit of Quantitation (LOQ) µg/ml	1.7786	1.8176
Accuracy (Mean % Recovery)	100.98	100.04
Precission (%RSD)	0.34	0.54

4. Conclusion

From the results of this study it can be concluded that the proposed second order derivative spectrophotometric method can be used for simultaneous determination of Cefixime Trihydrate and Ofloxacin. This method is simple, rapid, practical, reliable and inexpensive and can be used for routine analysis of simultaneous determination of these compounds without any prior separation in quality control laboratories.

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