RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND DICLOXACILLIN SODIUM IN COMBINED TABLET DOSAGE FORM

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Abstract: The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first order derivative spectrophotometric method for the simultaneous determination of Dicloxacillin sodium and Cefpodoxime Proxetile in combined tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra was obtained in methanol and the determinations were made at 237.2nm (ZCP of Cefpodoxime proxetil) for dicloxacillin sodium and 307.8 nm (ZCP of Dicloxacillin sodium) for Cefpodoximeproxetil. The linearity was obtained in the concentration range of 10-90 µg/ml for cefpodoxime proxetil and 50-200 µg/ml for Dicloxacillin sodium. The mean recovery was 98.71-102.77% and 98.69-99.62% for cefpodoxime proxetil and dicloxacillin sodium, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of cefpodoxime proxetil and dicloxacillin sodium in pharmaceutical combined tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Cefpodoxime proxetil, Dicloxacillin sodium, First order derivative spectrophotometric method, Methanol

INTRODUCTION

Dicloxacillin sodium [2S - (2α,5α,6β)] - 6 - [[[3 - (2,6 - dichlorophenyl) - 5 - methyl - 4 - isoxazolyl]carbonyl]amino]-3,3-dimethyl- 7 - oxo - 4 - thia - 1 - azabicyclo[3.2.0]heptane - 2 - carboxylic acid monosodium saltis a semi synthetic antibiotic substance which resists destruction by the enzyme penicillinase (beta-lactamase)Dicloxacillin is used to treat infections caused by bacteria such as bronchitis, pneumonia, or staphylococcal (also called "staph") infectionsIt is official in United state Pharmacopoeia (USP) [3]and European Pharmacopoeia(E.P)[4] .It describes High Performance Liquid Chromatography (HPLC) method, and Thin Layer Chromatography (TLC) method. Literature survey also reveals Spectrophotometric [6-10], HPLC with other drugs. Cefpodoximeproxetil (CEF) is chemically 1-(isopropoxycarbonyloxy) ethyl (6R,7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino)acetamido]-3-methoxymethyl-3-ephem-4-carboxylate, is a third generation cephalosporin antibiotic. It is used for infections of the respiratory tract, urinary tract and skin and soft tissues. It has greater activity against staphylococcus aureus. Cefpodoxime is official in IP[12] and USP[13]. IP and USP describe liquid chromatography method for its estimation. Literature survey reveals HPTLC[14] method for the determination of CEF. Literature survey also reveals RP-HPLC[15] and Spectrophotometric[16] methods for determination of CEF with other drugs. The combined dosage forms of DICLO and CEF are available in the market for theFor the treatment of lower RI, skin and soft tissue, bone and joint infection and in adult patient prone to antibiotic induced diarrhoea. The combination of these two drugs is not official in any pharmacopoeia; hence no officialmethod is available for the simultaneous estimation of DICLO and CEF in their combined dosage forms. Literature survey does not reveal any simple Spectrophotometric for simultaneous estimation of DICLO and CEF in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate and economical spectrophotometric method based on First Order Derivative spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Apparatus

A double beam UV/Visible spectrophotometer (shimadzu model 1800, Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. An analytical balance (K.ROY instruments Pvt. Ltd., Varanasi, India), an ultrasonic bath (Janki Impex Pvt. Ltd., Ahmedabad, Gujarat, India) was used in the study.

Reagents and Materials

DICLO and CEF bulk powder was kindly gifted by Baroque Pharmaceutical Ltd., Khambhat, Anand, Gujarat, India respectively. The commercial fixed dose combination product ZEDOCEF DXL200-(DICLO – 500 mg, CEF – 200 mg) was procured from the local market which is marketed by Macleodspharma Mumbai. Methanol solution is used as solvent for the preparation of different concentration of both drugs DICLO and CEF.

Preparation of standard stock solutions

An accurately weighed quantity of DICLO (100 mg) and CEF (100 mg) were transferred to a separate 100 ml volumetric flask and 50 ml methanol is added to both volumetric flask and sonicated for 5 minutes. Volume was adjusted up to the mark with methanol to obtain standard solution having concentration of DICLO (1000µg/ml) and CEF (1000µgThese solutions CEF (1000µg/ml) were diluted up to concentration of CEF (100µg/ml)

Methodology

The standard solutions of DICLO (1000µg/ml) and CEF (100µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 0.1 nm. The two spectra were overlain and it appeared that DICLO showed zero crossing at 275.8 nm, 280.8 nm, 283.0 nm and 307.8 nm, while CEF showed zero crossing at 237.2 nm, 237.3 nm and 237.5 nm. At the zero crossing point (ZCP) of DICLO (307.8 nm), CEF showed a significance first-derivative absorbance, whereas at the ZCP of CEF (237.2 nm) DICLO showed a significance first-derivative absorbance. Hence 237.2 and 307.8 nm was selected as analytical wavelengths for determination of DICLO and CEF respectively. These two wavelengths can be employed for the determination of DICLO and CEF without any interference from the other drug in their combined dosage formulations.

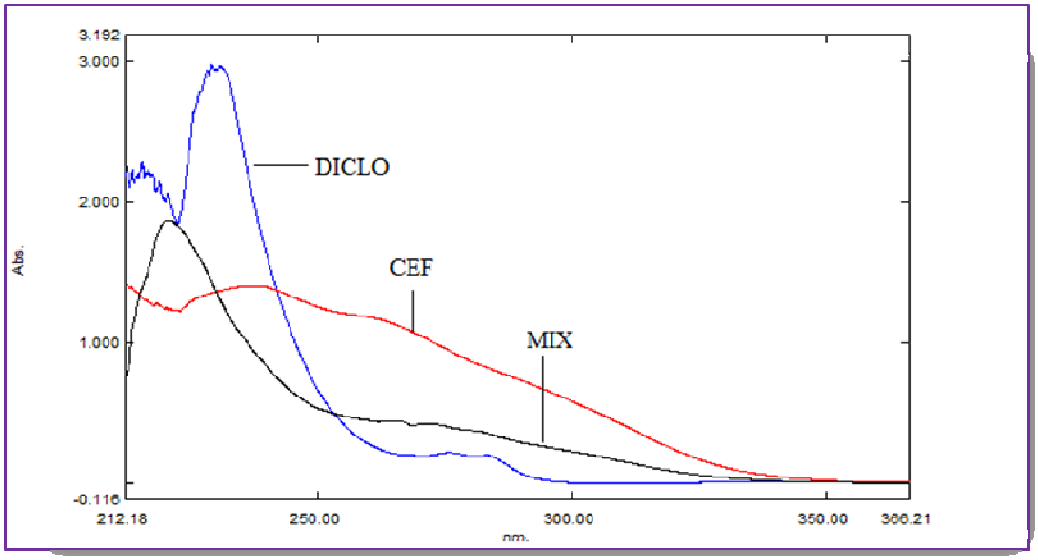

Figure 1. Overlain zero-order absorption spectra of DICLO and CEF and their mixture in Methanol

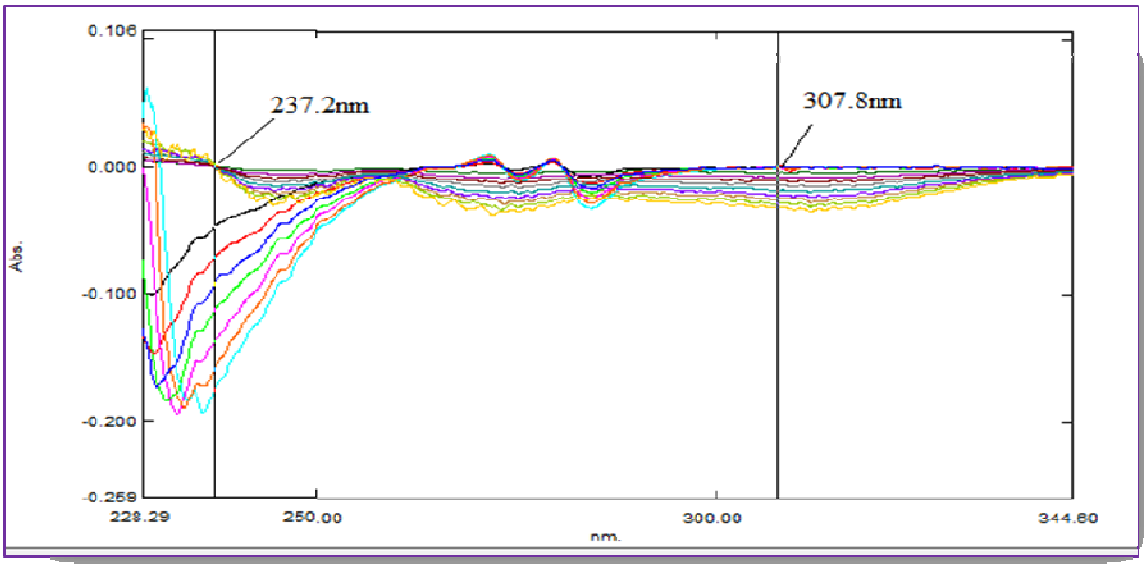

Figure 2. Overlain first-order derivative spectra of DICLO and CEF in 0Methanol

METHOD VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[18]

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 50-200µg/ml for DICLO and 10-90µg/ml for CEF. Accurately measured standard solutions of DICLO (0.5, 0.75, 1, 1.25, 1.5 ,1.75 ,2.0 ml) and CEF (1.0,2.0,3.0,4.0,5.0,6.0,7.0,8.0, and 9.0 ml) were transferred to a series of 10 ml of volumetric flasks from the standard stock solution of DICLO (1000µg/ml) and CEF (100µg/ml) respectively and diluted to the mark with methanol. First-derivative absorbance (D1) was measured at237.2nm for DICLO and 307.8 nm for CEF. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of this method was checked by repeated scanning and measurement of absorbance of solution without changing the parameter of the first-derivative spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of DICLO(50, 75 ,150)and CEF (70, 80, 90)µg/ml for CEF). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of DICLO and CEF by the standard addition method. Known amounts of standard solutions of DICLO and CEF were added at 50, 100 and 150 % level to pre quantified sample solutions DICLO and CEF (50 µg/ml for DICLO and 20µg/ml for CEF).. The amounts of DICLO and CEF were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

Analysis of CEF and DICLO in combined tablet dosage form

Twenty Tablets were weighed and powdered. The powder equivalent to 50 mg of DICLO and 20 mg of CEF was transferred to a 100 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 20 min. The solution was

filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution is expected to contain 500 µg/ml of DICLO and 200 µg/ml of CEF. This solution (1.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of DICLO (50 µg/ml) and CEF (20 µg/ml). The responses of the sample solution were measured at 237.2 nm and 307.8 nm for quantification of DICLO and CEF, respectively. The amounts of the DICLO and CEF present in the sample solution were calculated by fitting the responses into the regression equation for DICLO and CEF in the proposed method

RESULTS AND DISCUSSION

The standard solutions of DICLO and CEF were scanned separately in the UV range, and zero-order spectra (Figure 1) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 0.1 nm. The two derivative spectra showed significance absorbance at 237.2 nm (ZCP of CEF) for DICLO and 307.8 nm (ZCP of DICLO) for CEF. First-derivative absorbance (D1) was recorded 237.2 nm for DICLO and 307.8 nm for CEF (Figure 2). First derivative spectra give good quantitative determination of both the drugs at their respective without any interference from the other drug in their combined dosage formulations.

Linear correlation was obtained between absorbance and concentration of DICLO and CEF in the concentration ranges of 50-200µg/ml and 10-90 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1).The relative standard deviation (less than 2 %) indicate that the proposed method is repeatable. The RSD values of interday (0.018-0.024 %) and intraday (0.019-0.024 %) for CEF and The RSD values of interday (0.08-0.12 %) and intraday (0.08-0.12 %) for DICLO, respectively, reveal that the proposed method is precise (Table 1). LOD values for DICLO and CEF were found to be 15.85 and 2.95µg/ml, respectively and LOQ values for DICLO and CEF were found to be 48.05 and 8.95µg/ml, respectively (Table 1). These data show that proposed method is sensitive for the determination of DICLO and CEF.

The recovery experiment was performed by the standard addition method. The mean recoveries were 99.80 ± 1.50 and 99.90 ± 0.36 % for DICLO and CEF, respectively (Table 2). The results of

recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine DICLO and CEF in their combined dosage form. The results obtained for DICLO and CEF were comparable with the corresponding labelled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of DICLO and CEF in pharmaceutical dosage forms.

CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 50-200µg/ml and 10-50 µg/ml for DICLO and CEF, respectively with co-efficient of correlation, (R^2)=0.999289 and (R^2) = 0.998355 for DICLO and CEF, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of DICLO and CEF. The method can be used for the routine analysis of the DICLO and CEF in combined dosage form without any interference of excipients.

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Table 1

Regression analysis data and summary of validation parameters for the proposed method

Parameters	First-derivative UV Spectrophotometry	
	DICLOXACILLIN SODIUM	CEFPODOXIME PROXETIL
Concentration range (µg/ml)	50 – 200	10 – 90
Slope	0.000888	0.00033
Intercept	0.004267	0.000733
Correlation Coefficient (r^2)	0.999289	0.998335
Accuracy (% recovery) (n = 6)	98.69 – 99.62	98.71 – 102.77
Repeatability (%RSD, n = 6)	1.48	1.82
Interday (n = 3) (%RSD)	0.08-0.12	0.018-0.024
Intraday(n = 3) (%RSD)	0.08-0.12	0.019-0.024
LOD (µg/ml)	15.85	2.95
LOQ (µg/ml)	48.05	8.95

Table 2.

Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml) (n=3)	% Recovery (n = 3)
CEF	0 %	20	0	21.16	100.84
	500 %	20	10	30.55	101.83
	100 %	20	20	39.48	98.71
	150 %	20	30	51.38	102.77
DICLO	0 %	50	0	49.81	99.62
	50%	50	25	74.02	98.69
	100 %	50	50	98.79	98.79
	150 %	50	75	123.37	98.85

Table 3.

Analysis of DICLO and CEF by proposed method

Formulation	Drug	Labelled claim (mg/tab)	Amount Taken (mg)	Amount Found (mg) (n = 3)	% Amount Found
ZEDOCEF DXL200-	CEF	200	20	21.16	100.84%
	DICLO	500	50	49.81	99.62 %

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