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RESEARCH ARTICLE

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

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of dicloxacillin and cefpodoxime proxetil 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 233.8 nm (ZCP of cefpodoxime proxetil) for dicloxacillin and 321 nm (ZCP of dicloxacillin) for cefpodoxime proxetil. The linearity was obtained in the concentration range of 10-80 µg/ml for dicloxacillin and 4-32 µg/ml for cefpodoxime proxetil. The mean recovery was 100.70 ± 0.38 and 99.90 ± 0.36 for dicloxacillin and cefpodoxime proxetil, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of dicloxacillin and cefpodoxime proxetil in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Dicloxacillin, Cefpodoxime proxetil, first order derivative spectrophotometric method, Tablet,

INTRODUCTION

Dicloxacillin(DCX) is chemically 9(2S,5R,6R)-6-[3-(2,6-dichlorophenyl)-5-methyl-1,2-oxazole-4-amido]-3,3-dimethyl-7-oxo-4-thia-1 azabicyclo [3.2.0] heptane-2-carboxylic acid¹, is a penicillinase resistant penicillin, used in the treatment of bacterial infections such as pneumonia and bone, ear, skin and urinary tract infection². It is official in IP and USP. IP³ and USP⁴ describe RP-HPLC method for its estimation. Literature survey reveals HPLC⁵ method for determination of DCX in pharmaceutical dosage forms as well as in biological fluids. Literature survey also reveals spectofluorimetric⁶ and RP-HPLC⁷⁻⁹ methods for determination of DCX with other drugs. Cefpodoxime proxetil (CEF) is chemically 1-(isopropoxy carbonyloxy) ethyl (6R,7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino) acetamido]-3-methoxymethyl-3-cephem-4-carboxylate¹⁰, 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 aureus¹¹. Cefpodoxime proxetil is official in IP and USP. IP¹² and USP¹³ describe liquid chromatography method for its estimation. Literature survey reveals HPTLC¹⁴ method for the determination of CEF. Literature survey also reveals RP-HPLC¹⁵ and spectofluorimetric¹⁶ methods for determination of CEF with other drugs. The combined dosage forms of DCX and CEF are available in the market for the treatment of infections caused by susceptible micro-organisms Vis. Urinary tract infections and gonococcal urethritis. The combination of these two drugs is not official in any pharmacopoeia, hence no official method is available for the simultaneous estimation of DCX and CEF in their

combined dosage forms. Literature survey does not reveal any simple spectrophotometric or other method for simultaneous estimation of DCX and CEF in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate and economical spectrophotometric method based on dual wavelength spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer 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. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Materials

DCX and CEF bulk powder was kindly gifted by Acme Pharmaceuticals Ltd. Ahmedabad, India. The commercial fixed dose combination product was procured from the local market. Methanol AR Grade was procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Preparation of standard stock solutions

An accurately weighed quantity of DCX (10 mg) and CEF (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol

to obtain standard solution having concentration of DCX (100 $\mu\text{g/ml}$) and CEF (100 $\mu\text{g/ml}$).

Methodology

The standard solutions of DCX (10 $\mu\text{g/ml}$) and CEF (10 $\mu\text{g/ml}$) 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 1 nm. The two spectra were overlain and it appeared that DCX showed zero crossing at

321 nm, while CEF showed zero crossing at 233.8 nm. At the zero crossing point (ZCP) of DCX (321 nm), CEF showed a first-derivative absorbance, whereas at the ZCP of CEF (233.8 nm), DCX showed a first-derivative absorbance. Hence 233.8 and 321 nm was selected as analytical wavelengths for determination of DCX and CEF, respectively. These two wavelengths can be employed for the determination of DCX and CEF without any interference from the other drug in their combined dosage formulations.¹⁷

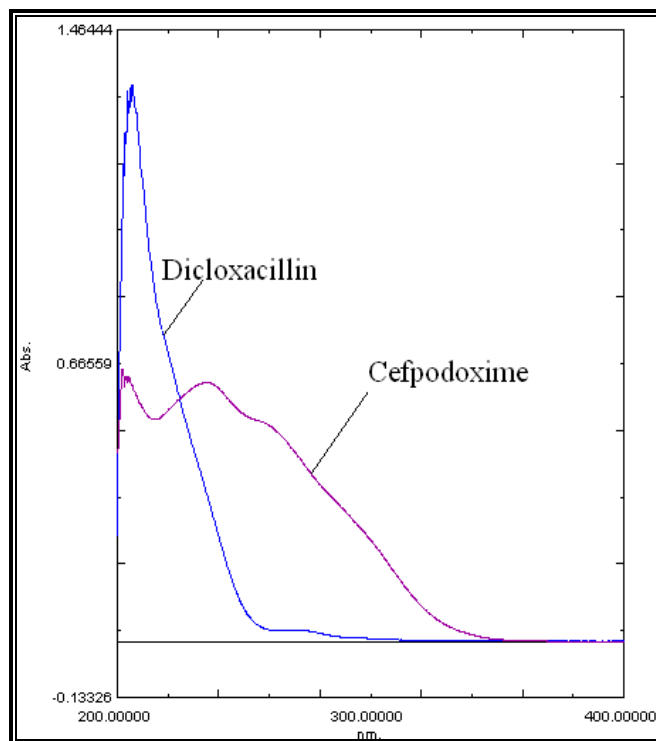


Figure 1: Overlain zero-order absorption spectra of DCX and CEF in methanol

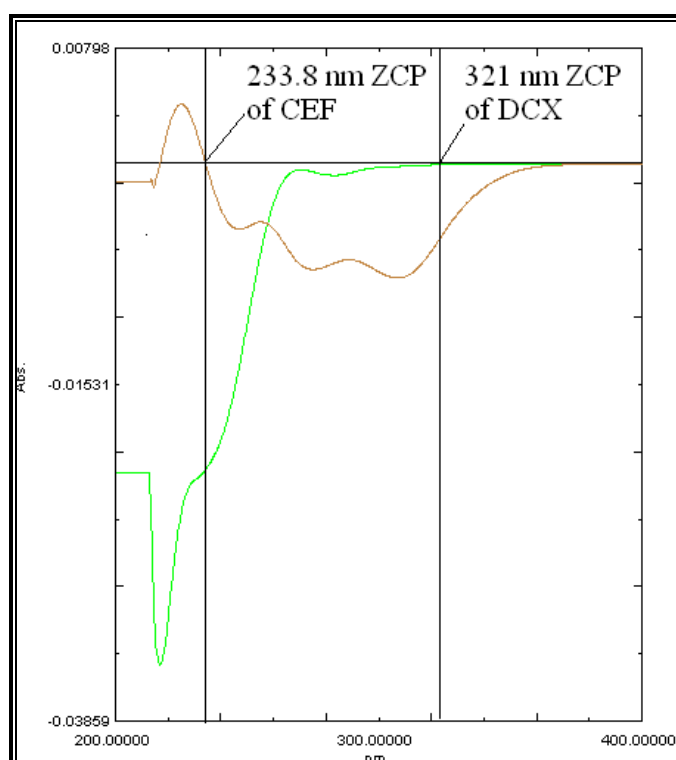


Figure 2: Overlain first-order derivative spectra of DCX and CEF in methanol

Validation of the proposed method

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 10-80 µg/ml for DCX and 4-32 µg/ml for CEF. Accurately measured standard solutions of DCX (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 ml) and CEF (0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8 and 3.2 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with methanol. First-derivative absorbance (D1) was measured at 233.8 nm for DCX and 321 nm for CEF. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.¹⁸

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution ($n = 6$) for DCX and CEF (10 µg/ml) without changing the

parameter of the first-derivative spectrophotometry method.¹⁸

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 DCX and CEF (20, 50, 80 µg/ml for DCX and 8, 20, 32 µg/ml for CEF). The result was reported in terms of relative standard deviation (% RSD).¹⁸

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of DCX and CEF by the standard addition method. Known amounts of standard solutions of DCX and CEF were added at 50, 100 and 150 % level to prequantified sample solutions of DCX and CEF (30 µg/ml for DCX and 6 µg/ml for CEF). The amounts of DCX and CEF were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times.¹⁸

Table 1: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	% Mean recovery ± S.D. (n = 6)
DCX	I	30	15	101.15 ± 0.08
	II	30	30	102.04 ± 0.14
	III	30	45	98.93 ± 0.93
CEF	I	6	3	99.42 ± 0.91
	II	6	6	99.71 ± 1.79
	III	6	9	99.98 ± 1.45

S. D. is Standard deviation and n is number of determinations

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.¹⁸

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of DCX and CEF in combined tablet dosage form

Twenty Tablets were weighed and powdered. The powder equivalent to 50 mg of DCX and 10 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 100 µg/ml of DCX and 100 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 DCX (50 µg/ml) and CEF (10 µg/ml). The responses of the sample solution were measured at 233.8 nm and 321 nm for quantification of DCX and CEF, respectively. The amounts of the DCX and CEF present in the sample solution were calculated by fitting the responses into the regression equation for DCX and CEF in the proposed method.¹⁸

Table 2: Analysis of CEF and DCX by proposed method

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S.D. (n=6)	
	CEF	DCX	CEF	DCX	CEF	DCX
I	100	500	100.8	499	100.80	99.80
II	100	500	101.4	498.6	101.40	99.72

S. D. is Standard deviation and n is number of determinations

Table 3: Regression analysis data and summary of validation parameters for the proposed method

PARAMETERS	First-derivative UV Spectrophotometry	
	DCX at 233.8 nm	CEF at 321 nm
Concentration range ($\mu\text{g/ml}$)	10-80	4-32
Regression equation ($y = a + bc$)	$y = 0.00102x - 0.00069$	$y = 0.00029x + 0.00019$
Slope (b)	0.00102	0.00029
Intercept (a)	-0.00069	0.00019
Correlation Coefficient (r^2)	0.9993	0.9990
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001 \text{ A.U.}$)	0.0333	0.0254
Accuracy (% recovery), (n = 6)	100.70 ± 0.38	99.90 ± 0.36
Repeatability (%RSD ^a , n = 6),	0.058	0.282
Interday (n = 3) (%RSD)	0.576-0.793 %	0.694-1.005 %
Intraday(n = 3) (%RSD)	0.212-0.565 %	0.344-0.827 %
LOD ^b ($\mu\text{g/ml}$)	0.56 $\mu\text{g/ml}$	0.64 $\mu\text{g/ml}$
LOQ ^c ($\mu\text{g/ml}$)	1.70 $\mu\text{g/ml}$	1.93 $\mu\text{g/ml}$

^aRSD = Relative standard deviation. ^bLOD = Limit of detection. ^cLOQ = Limit of quantification

RESULTS AND DISCUSSION

The standard solutions of DCX 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 1 nm. The two derivative spectra showed maximum absorbance at 233.8 nm (ZCP of CEF) for DCX and 321 nm (ZCP of DCX) for CEF. First-derivative absorbances (D1) were recorded 233.8 nm for DCX and 321 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. Second and third-ordered derivative spectra of the drugs were not tested because the first-order spectra give satisfactory ZCPs and good quantitative determination of both the drugs without any interference.

Linear correlation was obtained between absorbances and concentrations of DCX and CEF in the concentration ranges of 10-80 $\mu\text{g/ml}$ and 4-32 $\mu\text{g/ml}$, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 3). The RSD values for DCX and CEF were found to be 0.058 and 0.282 %, respectively (Table 3). The low values of relative standard deviation (less than 2 %) indicate that the proposed method is repeatable. The low RSD values of interday (0.576-0.793 and 0.694-1.005 %) and intraday (0.212-0.565 and 0.344-0.827 %) for DCX and CEF, respectively, reveal that the proposed method is precise (Table 3). LOD values for DCX and CEF were found to be 0.56 and 0.64 $\mu\text{g/ml}$, respectively and LOQ values for DCX and CEF were found to be 1.70 and 1.93 $\mu\text{g/ml}$, respectively (Table 3). These data show that proposed method is sensitive for the determination of DCX and CEF.

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.70 ± 0.38 and 99.90 ± 0.36 % for DCX and CEF, respectively (Table 1). The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine DCX and CEF in their combined dosage form. The results obtained for DCX and CEF were comparable with the corresponding labeled 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 DCX 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 10-80 $\mu\text{g/ml}$ and 4-32 $\mu\text{g/ml}$ for DCX and CEF, respectively with co-efficient of correlation, (r^2)=0.9993 and (r^2) = 0.9990 for DCX 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 DCX and CEF. The method can be used for the routine analysis of the DCX and CEF in combined dosage form without any interference of excipients.

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REFERENCES

- 1) Maryadele, Neil JO. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals. 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co. Inc. Whitehouse station; 2006. P. 523.
- 2) Sweetman SC. Eds. In The Martindale. The Complete Drug Reference. 35th ed. London: Pharmaceutical Press; 2007. P. 237.
- 3) Indian Pharmacopoeia. Vol. III. New Delhi: The Controller Publication. Govt. of India; 2010. P. 1201-1202.
- 4) The United State Pharmacopoeia. USP28-NF23. Rockville MD: United State Pharmacopoeial Convention. Inc; 2005. P. 451-452.
- 5) Alderetea O, Gonzalez-Esquivelb DF, Riveroa LM and Torres NC, "Liquid chromatographic assay for dicloxacillin in plasma" *Journal of Chromatography B*, 2004, 805, 353-356.
- 6) Basilio M, "Second-derivative spectrophotometric assay of mixtures of dicloxacillin sodium and ampicillin sodium in pharmaceuticals" *Journal of Pharmaceutical Sciences*, 1988, 77, 1042-1046.
- 7) Barot T, Patidar K, Kshartri N and Vyas N, "Development and Validation of LC Method for the Determination of Ampicillin and Dicloxacillin in Pharmaceutical Formulation Using an Experimental Design" *E-Journal of Chemistry*, 2009, 6, 955-964.
- 8) Kathiresan K, Murugan R, Hameed SM, Inimai GK and Kanyadhara T, "Analytical Method Development and Validation of Cefixime and Dicloxacillin Tablets by RP-HPLC" *Rasayana Journal of Chemistry*, 2009, 2, 588-592.
- 9) Zhang MJ, Zhang HJ, Guan X and Xiao ZH, "HPLC determination of dicloxacillin and amoxicillin in human plasma" *Yaowu Fenxi Zazhi*, 2006, 26, 228-231.
- 10) Maryadele, Neil JO. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals. 14th ed. New Jersey: Published by Merck Research Laboratories, Division of Merck and Co., Inc. Whitehouse station; 2006. P. 319.
- 11) Sweetman SC, Eds. In The Martindale. The Complete Drug Reference. 35th ed. London: Pharmaceutical Press; 2007. P. 207.
- 12) Indian Pharmacopoeia. Vol. III. New Delhi: The Controller Publication. Govt. of India; 2010. P. 1018.
- 13) The United State Pharmacopoeia. USP28-NF23. Rockville MD: United State Pharmacopoeial Convention. Inc; 2005. P. 397.
- 14) Darji BH, Shah NJ, Patel AT, Patel NM, "Development and validation of a HPTLC method for the estimation of cefdoxime proxetil" *Indian J Pharm Sci*, 2007, 69, 331-333.
- 15) Singh S, Dubey N, Jain D, Tyagi L, Singh M, "Spectrophotometric and RP-HPLC methods for simultaneous determination of cefdoxime proxetil and clavulanate potassium in combined tablet dosage form" *American-Eurasian Journal of Scientific Research*, 2010, 5(2), 88-93.
- 16) Gandhi SV, Patil UP, Patil NG, "Simultaneous spectrophotometric determination of Cefdoxime proxetil and Potassium clavulanate" *Hindustan Antibiot Bull*, 2009, 51(1-4), 24-28.
- 17) Beckett H, Stenlake JB. *Practical Pharmaceutical Chemistry*. 14th ed. CBS Publishers and distributors; 1997. P. 297-299.
- 18) The International Conference on Harmonization, Q2 (R1), "Validation of Analytical Procedure, Text and Methodology" 2005.