International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

SPECTROPHOTOMETRIC DETERMINATION OF CEFPODOXIME USING 2-HYDROXYNAPHTHALDEHYDE AS A DERIVATIZING REAGENT

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ABSTRACT

A new simple and sensitive spectrophotometric method has been developed for the determination of the cefpodoxime in pharmaceutical formulations. The method is based on the formation of colored compound of cefpodoxime with 2- hydroxynaphthaldehyde which absorbs maximally at λ_{max} 436nm. The Beer's law is obeyed in the concentration range from 02-10 µg/ mL with molar absorptivity $6.6x10^4$L/mole/cm. The optimum experimental parameters for the reactions have been studied. The validity of the procedure was assessed and did not show any change in absorbance up to 24 hrs at room temperature. Statistical analysis of the result has been carried out revealing high accuracy, good precision and suitability for quality control application. The developed procedure is used for the determination of cefpodoxime in pharmaceutical formulations including tablets and suspensions. A good agreement is observed with labeled values Relative standard deviation (RSD) within 1% and recovery of the drug from pharmaceutical preparations was calculated within 95- 99.5% .

Key words: Cefpodoxime, Spectrophotometric method, Relative standard deviation and 2- hydroxynaphthaldehyde

INTRODUCTION

Cefpodoxime (CPD) is a third generation cephalosporin for oral use indicated for the treatment of mild to moderate infections i-e community acquired pneumonia and tonsillitis in children and adults which are caused by susceptible strains of the of the designated micro organism . Cefpodoxime is chemically 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-caboxylic acid ,[6R-[6α,7β(Z)]]-7-[[2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-methoxy-methyl)-8-oxo-,1-[[(1-methylethoxy)-cabonyl]oxy]ethyl. Different analytical methods are reported or the analysis of CPD, based on spectrophotometric [1-5], FIA [6, 7], flourimetric [8], TLC [09, 10], HPLC [11-17], voltametric [18-20], GC [21], and clinical evaluation techniques [22]. For spectrophotometric analysis the determination of CPD is carried out by derivatizing with suitable reagents i.e. Cu, Cd, Zn, oxidation with cerium (IV) [19] and 1-chlorobenzotriazole [2], 1,10-phenanthroline [24]. The derivatization either increases the molar absorptivity or produces bathochromic shift. In present study 2-hydroxynaphthaldehyde is examined for the first time as a derivatizing reagent or spectrophotometric determination of CPD from bulk and pharmaceutical preparations.

EXPERIMENTAL

Apparatus: The spectrophotometric studies were carried out on double beam spectrophotometer (Perkin Elmer, lambda 25 UV/Visible USA), which was controlled by UV winlab 35 software with dual1cm matched quartz cells. For different buffer preparations pH meter (Orion model 420 A) was used. Ethanol and 2-hydroxynaphthaldehyde were of analytical grade, reagents obtained from (E. Merck, Germany). Cefpodoxime was obtained from (Bosch Pharmaceuticals, PVT (Ltd.), Karachi, Pakistan). Ethanolic 1% W/V solution of 2-hydroxynaphthaldehyde was prepared for this study.

Stock solution of Cefpodoxime 100µg / mL was freshly prepared by dissolving 0.01 g of drug in 100 mL of ethanol. Buffer solutions ranging between 1-10 at unit interval were prepared from hydrochloric acid (0.1M), potassium chloride (0.1M), acetic acid (0.1M), sodium acetate (0.1M), sodium bicarbonate (0.1M), sodium carbonate (0.1M), ammonium chloride (0.1M), and ammonia solution(0.1M).

Analytical Procedure: For the spectrophotometric analysis, the aliquots ranging from 0.1 to 0.5ml (50µg) of CPD were transferred to series of 5 ml calibrated volumetric flasks. To each flask 1.5mL of HN (1% in ethanolic) solution was added followed by 0.5 ml of acetate buffer pH 6 and heated the contents on water bath at 95 °C for 20 minutes. The flakes were cooled to room temperature and the volume was adjusted to mark with ethanol. The absorbance of light pink colored solution was measured at 436nm against reagent blank. The calibration curve was constructed by plotting the absorbance versus concentration of drug. The content of unknown was computed either through calibration curve or regression equation.

Analysis of Cefpodoxime From Pharmaceutical Preparations: For the determination of CPD from pharmaceutical preparations the amount of drug 0.1gm was completely and thoroughly dissolved in 20ml of ethanol and filtered using whattman filter paper No 41. The filtrate was made up to 100 ml with ethanol and appropriate aliquots of the drug solution were treated as described in section D.

RESULT AND DISCUSSION

Cefpodoxime CPD reacts with HN reagent in ethanolic medium to form an imine derivative which absorbs maximally at 436 nm with bathochromic shift having molar absorptivity $6.6x10^4$ L $mole^{-1}$ cm^{-1} HN was therefore examined as derivatizing reagent for the spectrophotometric determination of CPD. The amount of HN added, effects of pH, heating time and temperature on the formation of (CPD-HN) derivative along with stability were studied.

Optimization of parameters

Analytical wavelength: For the Quantitative analysis, the wavelength of maximum absorbance plays an important role. It is compulsory to ensure that the derivatizing reagent should not absorb close to the region where the analyte derivative absorbs. This may cause in accuracy in absorption of the drug because the derivatizing reagent is added in excess to complete the reaction quantitatively. To avoid this set back, it is obligatory to select the wave length where the analyte derivative shows maximum absorbance value and the derivatizing reagent indicates maximum absorbance. The absorbance value of 20 µg mL^{-1} CPD as HN derivative was recorded at different wave lengths between 230-550 nm after heating for 20 minutes at 90 °C using buffer pH 6. It was noted that the maximum absorbance occurs at 436 nm against reagent blank; therefore the wavelength of 436 nm was selected as optimal.

Effect of reagent concentration: The effects of various amounts HN (1% ethanolic solution) on the absorbance of 30 µg mL^{-1} CPD was observed. The concentration of 1% ethanolic HN was varied between 0.5-1.5 mL with an interval of 0.5 ml and the absorbance was measured at λmax 436 nm. A similar absorbance was observed with the addition of 1.5 mL and above. Therefore the addition of 1.5 mL (1% w/v) solution was optimized and selected.

Order of mixing of reagent: The order of adding reagents during derivatization process has significant role in precision of results and improvement of absorbance. In this study, it was observed that in 05 mL caliberated volumetric flasks the addition of the buffer pH 6 (0.5 mL) to 10 µg CPD solution (0.1 mL) followed by 1.5 mL reagent (1% HN) resulted in decrease in absorbance value. Taking reagent first and then adding the buffer pH 6, followed by CPD solution also gave lower absorbance value. The maximum absorbance value was achieved when 1.5 mL of reagent was added to the standard solution of CPD followed by buffer (0.5 mL) pH 6. The contents were than heated on water bath and the volume was adjusted to the mark with ethanol.

Optimization of heating time and temperature for the formation of derivative: To obtain the maximum absorbance value for an analyte, the selection of optimum time and temperature for the formation of stable derivative are essential parameters. The effects of time and an absorbance 40µg mL^{-1} CPD solution in the presence of 1% HN solution was checked at 436 nm from 0-30 min with an interval of 05 min at 60-90 °C with an interval of 10 °C maximum absorbance was observed after heating for 20 min at 90 °C was considered as optimal.

Effects of solvent: The effects of various solvents such as methanol, 1-Propanol, 1-butanol, amyl alcohol, acetonitrile, ethyl acetate toluene, nitrobenzene and carbon tetra chloride on the absorbance of 10 µg mL^{-1} CPD was examined. Each of the solvents 0.5and 1mL was added after addition of 1.5 mL 1% ethanolic solution of HN and 0.5 mL

acetate buffer pH 6 followed by heating for 20 min . The ethanol proved to be the best choice in solvents.

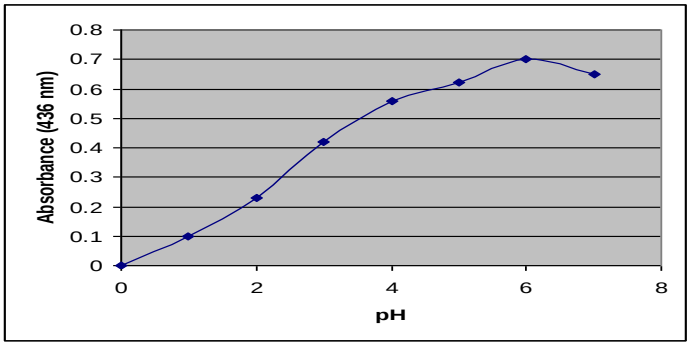
Effect of the pH: The effect of the adding 0.5 mL of 01 M buffers of pH range 1-10 on the absorbance of 20 µg mL^{-1} CPD solutions at optimized conditions was studied. It was observed that the absorbance increased gradually from buffer pH 1 and was maximum at pH 6. Addition of buffer above pH 8 produced precipitation therefore the acetate buffer of pH 6 was selected as optimal.

***Interference study*:** The effect of possible presence of associated material such as mannitol, sorbitol, sucrose, lactose, glucose, glactose and fructose was investigated at 10 times of concentration of CPD and it was observed that none of these substances interfered with any variation in absorbance within ± 0.5%.

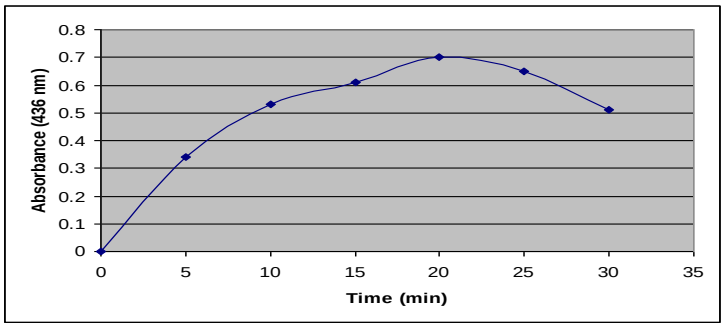
Stability of derivative: The stability of CPD-HN derivative was examined in terms of absorbance at the concentration 10 µg mL^{-1} CPD, but no change in the absorbance of more than 4% was observed within 48 hrs.

Calibration plot: Using the optimized conditions, the effect of variation in the concentration of CPD on its absorbance as derivative CPD-HN was studied. A linear calibration curve was obtained which obeyed the beer's law within the concentration range 02-10 µg $mL^{-1}$of CPD with coefficients of determinations r^2 0.9994. The sandal's sensitivity (0.004) was observed at 0.5 µg mL^{-1} CPD-HN. The validity of calibration curve was obtained by the analysis of test solution of CPD and relative deviation from the labeled values were found 0.79-0.92 % and 0.69-0.94%5 tablets and suspensions respectively. The methods are applied on 13(Thirteen samples of tablets and suspensions) containing CPD, available in the local market to determine the amount of CPD quantitatively. The mean values (g) and % (95% confidence limit) 0.01g tablets and 0.04g suspension 96-98 % and 96.1-99.5 % tablets and suspensions respectively.

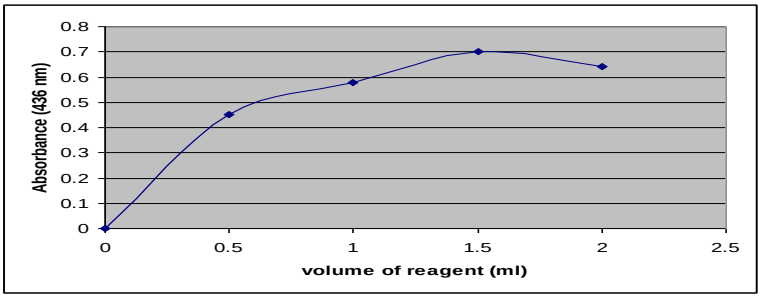
Day to day reproducibility/repeatability: For the determination of intra and inter day reproducibility of the method, the ethanolic standard solution (0.2ml) of 100 µg mL^{-1} CPD was taken in three different calibrated volumetric flasks (05mL) and the procedure was followed as discussed in section B. The above procedure was repeated for three days (n=3). The mean absorbance of intra and interday reproducibility were observed as 0.145-1.48 with (RSD) values 0.69-0.99.5 mean for Tablets and suspensions.



Graph: 01: Effect of pH on derivatization of Cefpodoxime.



Graph: 02: Effect of heating time on derivatization of Cefpodoxime.



Graph: 03: Effect of reagent volume on derivatization of Cefpodoxime.

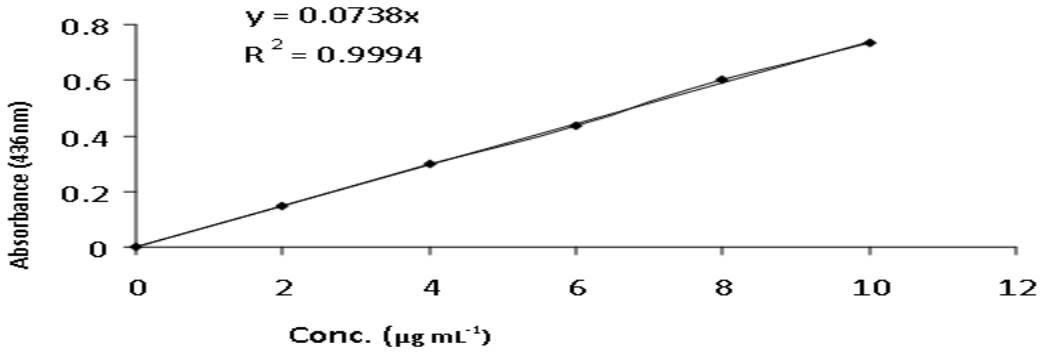
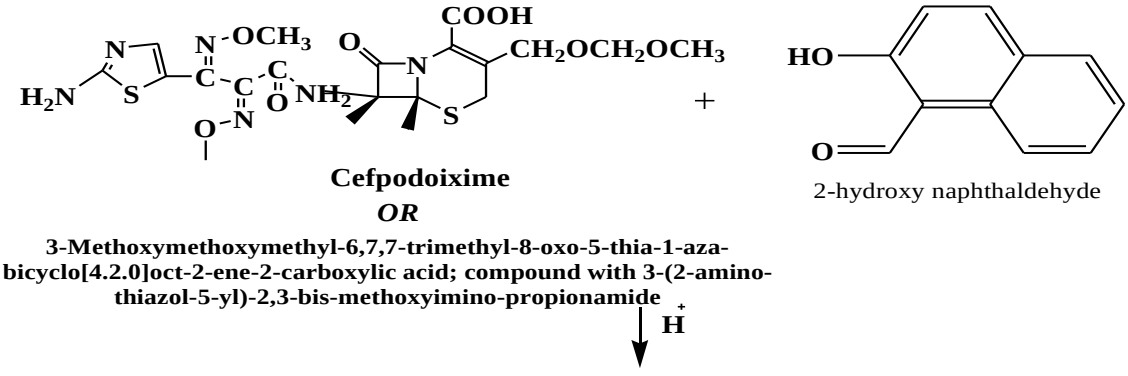
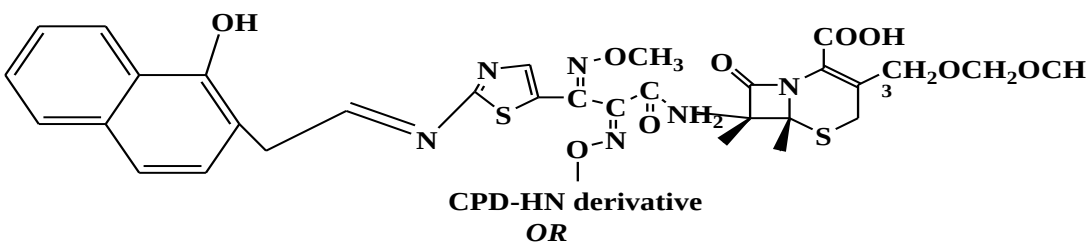


Fig.1 Calibration curve of Cefpodoxime using 2-hydroxynaphthaldehyde as derivatizing reagent





Scheme 1: Reaction of cefpodoxime with 2-hydroxy naphthaldehyde

Table: 01: Optical Characteristic and precision

λ max (nm)	436
Beer's law (µg /mL)	2-10
Molar absorptivity ($L/mol^{-1}cm^{-1}$)	$6.6x10^4$
% RSD	0.69-0.92
Sandells sensitivity (µg /mL/ cm^2 0.004 absorbance unit)	0.5
Regression equation Slope (b)	0.0738
Intercept (a)	0
Correlation coefficient $(R)^2$	0.9994
Mean Value Range of error %at 95 % confidance limit.	
Confidence limits with 0.01 level.	0.249-0.673
Confidence limits with 0.05 level	0.499-0.13515

Table.2: Analysis of CPD from tablet (100mg) dosage form

S.#	Drug.	Amount found (mg) sample	Relative Standard deviation %	Relative deviation %	% Recovery
01.	Oribro	98	(0.79)	0.0.02	98.0
02.	Orelox	97.7	(0.92)	0.023	95.0
03.	Evodoixime	96.5	(0.85)	0.035	98.0
04.	Cef-poo	98.7	(0.89)	0.013	97.0
05.	Cefprox	99.8	(0.91)	0.002	98.0
06.	Nuodoxin	99.0	(0.96)	0.01	96.0
07.	Qink	95.0	(0.80)	0.05	97.0

Table: 03: Analysis of CPD from suspension (400mg) dosage form

S.#	Drug.	Amount found (mg) sample	Relative Standard deviation %	Relative deviation %	% Recovery
01.	Curidoixime	385	(0.69)	0.037	98
02.	Cefpomed	386	(0.92)	0.035	98.5
03.	Cef-so	389	(0.85)	0.027	97.98
04.	Expodox	389	(0.89)	0.027	99.5
05.	Prelox	398	(0.91)	0.005	98.05
06.	Vantin	384	(0.94)	0.04	96.10

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