VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CEFADROXIL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive, accurate, precise and economical visible spectrophotometric method was developed and validated for the estimation of cefadroxil in tablets. The method is based on the reaction of cefadroxil with ninhydrin reagent in methanol giving blue color chromogen, which shows maximum absorbance at 578 nm against reagent blank. The chromogen obeyed Beer's law in the concentration range of 5-50 µg/ml for cefadroxil. The results of the analysis have been validated statistically and by recovery studies.

Key words: Cefadroxil, Chromogen, Ninhydrin reagent, Visible spectrophotometric, Tablet.

INTRODUCTION

Cefadroxil (CFD) is chemically, (7R)-7-(α-D-4-Hydroxyphenylglycylamino)-3-methyl-3-cephem-4-carboxylic acid[1]. Cefadroxil is a first-generation cephalosporin antibacterial that is para-hydroxy derivative pf cephalexin and is used similarly in the treatment of mild to moderate susceptible infections[2]. Cefadroxil is official in IP, USP and BP. IP[3], USP[4] and BP[5] describes liquid chromatography method for the estimation of cefadroxil. Literature survey reveals chemiluminescence[6], spectrophotometric[7-10] and HPLC[11-13] methods for the estimation of cefadroxil in biological fluids and in pharmaceutical formulations. The present communication describes simple, sensitive, accurate, precise and economical visible spectrophotometric method using ninhydrin reagent for the estimation of cefadroxil in tablet dosage form.

MATERIALS AND METHODS

Apparatus

A Shimadzu model 1601 double beam UV/Vis. spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells was used to measure absorbance of the resulting solutions. A Sartorius CP224S analytical balance (Germany), an ultra sonic cleaner (Frontline FS 4, Mumbai, India), water bath (Cintex Industrial Corporation, Mumbai, India) were used in the study.

Reagents and Materials

Cefadroxil powder was procured as a gift sample from Mann Pharmaceuticals Ltd, Mehsana, Gujarat, India. The commercially available tablets of cefadroxil were procured from local market. Ninhydrin reagent and methanol (AR Grade, S.D. Fine Chemicals Ltd., Mumbai) were used in the study.

Preparation of reagent and standard stock solution

Accurately weighed ninhydrin powder (500 mg) was transferred to a 100 ml volumetric flask, dissolved in and diluted to the mark with methanol (0.5% w/v). Accurately weighed cefadroxil (20 mg) was transferred to a 100 ml volumetric flask, dissolved in and diluted to the mark with methanol. (200 µg/ml).

Methodology

Standard stock solution of cefadroxil (1.0 ml) was transferred to a 10 ml corning volumetric flask. Ninhydrin reagent (2.0 ml) was added and mixed. The flask was immersed in a water bath at 92 ± 1^0 C for 15 minutes, cooled to room temperature and the volume was adjusted to 10 ml with methanol. The absorbance of the colored solution was scanned in the range of 400 to 800 nm against reagent blank, prepared similarly in which volume of standard solution was replaced by an equal volume of methanol. Maximum absorbance was obtained at 578 nm.

Optimization of different conditions

Effect of concentration of ninhydrin reagent

Standard stock solution of cefadroxil (1.0 ml) was transferred to a series of 10 ml corning volumetric flasks. To each flask, different volumes of ninhydrin reagent (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml) was added and mixed. The flasks were immersed in a water bath at 92 ± 1^0 C for 15 minutes, cooled to room temperature and the volume in each flask was adjusted to 10 ml with methanol. The absorbance of the resulting solutions was measured at 578 nm against reagent blank. Maximum absorbance was observed in the presence of 2.0 ml of 0.5% w/v ninhydrin reagent, which remained constant with increase in the volume of the reagent solution.

Effect of temperature

Standard stock solution of cefadroxil (1.0 ml) was transferred to a series of 10 ml corning volumetric flasks. To each flask, 2.0 ml of ninhydrin reagent was added and mixed and heated at different temperatures (40^0, 50^0, 60^0, 70^0, 80^0, 90^0, 92^0, 94^0 and 96^0 C) for 15 minutes, cooled to room temperature and the volume in each flask was adjusted to 10 ml with methanol. The absorbance of the resulting solutions was measured at 578 nm against reagent blank. Optimum temperature to obtain maximum absorbance was found to be 92 ± 1^0 C.

Time for maximum color development and color stability

Standard stock solution of cefadroxil (1.0 ml) was transferred to a series of 10 ml corning volumetric flasks. To each flask, 2.0 ml of ninhydrin reagent was added and mixed. The flasks were immersed in a water bath at 92 ± 1^0 C for different time interval (5, 10, 15, 20, 25, 30, 45 and 60 minutes), cooled to room temperature and the volume in each flask was adjusted to 10 ml with methanol. The absorbance of the resulting solutions was measured at 578 nm against reagent blank. Maximum absorbance was obtained after 15 minutes, which remained constant for 1 h.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[14].

Linearity

Calibration curve was plotted over a concentration range of 5-50 µg/ml for cefadroxil. Accurately measured standard stock solutions of cefadroxil (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 ml) were transferred to a series of 10 ml corning volumetric flasks. To each flask, 2.0 ml of ninhydrin reagent was added and mixed. The flasks were immersed in a water bath at 92 ± 1^0 C for 15 minutes, cooled to room temperature and the volume in each flask was adjusted to 10 ml with methanol. The absorbance of the resulting solutions was measured at 578 nm against reagent blank. Calibration curve was constructed for

cefadroxil by plotting concentration versus absorbance at 578 nm. Each reading was an average of five determinations.

Accuracy (% Recovery)

The accuracy of the method was performed by calculating recovery of cefadroxil by the standard addition method. Known amounts of standard solutions of cefadroxil were added at 50, 100 and 150% levels to prequantified sample solutions of cefadroxil (20 μg/ml). Each sample was prepared in triplicate at each level. The amount of cefadroxil was estimated by applying obtained values to regression equation.

Method Precision (% Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of cefadroxil (20 μg/ml) without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (%RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was performed by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for different concentrations of standard solutions of cefadroxil (5-50 μg/ml). The results were reported in terms of relative standard deviation (%RSD).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug was derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines[14].

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

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

Estimation of cefadroxil from pharmaceutical tablet dosage form

Twenty tablets were accurately weighed and powdered. A quantity of powder equivalent to 20 mg of cefadroxil was transferred to a 100 ml volumetric flask and mixed with methanol (50 ml) and sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol. The solution (2.0 ml) was transferred to a 10 ml corning volumetric flask. Ninhydrin reagent (2.0 ml) was added and mixed. The flask was immersed in a water bath at 92 ± 1^0 C for 15 minutes, cooled to room temperature and the volume was adjusted to 10 ml with methanol. The absorbance of the resulting solution was measured at 578 nm against reagent blank. The amount of cefadroxil present in tablet solution was determined by fitting the responses into the regression equation. The analysis procedure was repeated five times with tablet formulation.

RESULTS AND DISCUSSION

Ninhydrin reagent is mainly used for the detection of free amino and carboxyl groups in proteins and peptides, yielding a blue color under the proper condition[15]. Ninhydrin (triketohydrinden hydrate) reacts with amino group containing substances like amino acids, proteins and peptides and when heated under proper conditions, produce ammonia, carbon dioxide and blue purple complex[16-19]. Literature survey reveals spectrophotometric method for the determination of lisinopril using ninhydrin reagent[20]. Therefore it was thought of interest to extend the application of ninhydrin reagent in the estimation of amino group containing drugs like cefadroxil.

In the proposed method, reagent solution and standard stock solutions of drugs were prepared in methanol. Various reaction conditions were established by varying one parameter at a time and keeping the others fixed by observing the effect produced on the

absorbance of the colored species. The parameters involved for maximum color development viz. concentration of ninhydrin reagent, temperature and heating time required to yield chromogen of maximum color intensity and stability were optimized. In this method all these parameters were strictly followed.

The blue colored complex formed having wavelength of maximum absorbance at 578 nm (Figure 1). In proposed method, it was found that 2.0 ml of 0.5% w/v ninhydrin reagent (Figure 2), 92 ± 1^0 C heating temperature (Figure 3) and 15 minutes heating time (Figure 4) was sufficient for the development of maximum color intensity. Stability study of the developed chromogen was carried out by measuring the absorbance values at a time intervals of 20 minutes for 3 h and it was found to be stable for more than 2 h for the drugs at room temperature.

The linearity was found in the concentration range of 5 to 50 μg/ml ($r^2 = 0.9994$). The reproducibility, repeatability and precision of method are very good as shown by the low values of standard deviation and relative standard deviation (%RSD). The % recovery value in the range of 98.65 to 101.7 % for tablet indicates non-interferences from the formulation excipients. The data of recovery studies and assay results are given in Table 1 and Table 2, respectively. Optical characteristics of method and summary of validation parameters for cefadroxil was given in Table 3.

CONCLUSION

The proposed visible spectrophotometric method was found to be, simple, sensitive, accurate, precise and economic for determination of cefadroxil in tablet dosage form. Hence it can be conveniently adopted for routine quality analysis of the drug in pharmaceutical dosage form.

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TABLE 1: RESULTS OF RECOVERY STUDIES IN TABLET AND INJECTION DOSAGE FORMS

Formulation	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % recovery ± S. D.[*] (n = 3)
Tablet	I	20	50	101.7 ± 0.87
	II	20	100	98.65 ± 1.15
	III	20	150	99.34 ± 0.64

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

TABLE 2: RESULTS OF ANALYSIS OF TABLET FORMULATION

Formulation		Label claim (mg)	Amount found (mg)	% Label claim ± S. D.[*] (n = 5)
Tablet	Brand I	250	246.9	98.75 ± 1.25
	Brand II	500	497.8	99.56 ± 0.89

S. D. is standard deviation n is number of determinations

TABLE 3: OPTICAL CHARACTERISTICS AND SUMMARY OF VALIDATION PARAMETERS

Parameters	Results
λ max (nm)	578
Linearity range (µg/ml)	5 - 50
Sandell's sensitivity (µg/cm^2/0.001 A.U.)	0.0407
Molar extinction coefficient (l/mol.cm)	8940
Correlation coefficient (r^2)	0.9994
Regression equation (y* = b + ac)	
Slope (a)	0.0226
Intercept (b)	0.0401
Standard deviation (S. D.)	± 0.003764
% Relative standard deviation (% RSD)	± 0.867
Standard error of mean (S.E.M)	± 0.001681
Repeatability (% RSD, n = 6).	0.85
Intermediate Precision (% RSD)	
Interday (n = 3)	0.38 - 1.94
Intraday (n = 3)	0.40 - 1.73
Accuracy (% Recovery) (n = 5)	98.65 - 101.7
Limit of detection (LOD) (µg/ml)	1.08
Limit of quantification (LOQ) (µg/ml)	3.58

y* = b + ac where 'c' is the concentration and y is absorbance unit. n is the number of determinations, RSD is relative standard deviation and S.E.M is standard error of mean.
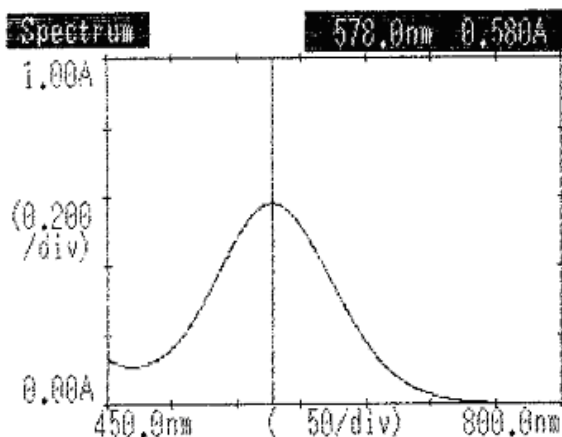

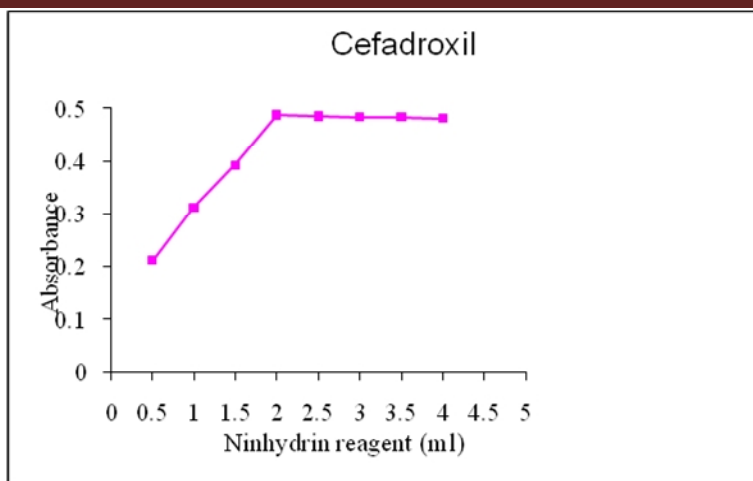**Figure 1: Spectra of cefadroxil with ninhydrin reagent at 578 nm in methanol**

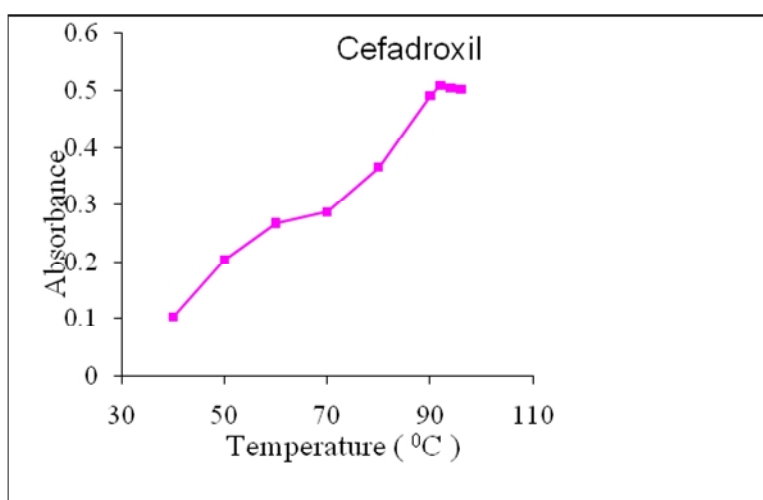**Figure 2: Optimization of volume of 0.5% w/v ninhydrin reagent (ml)**

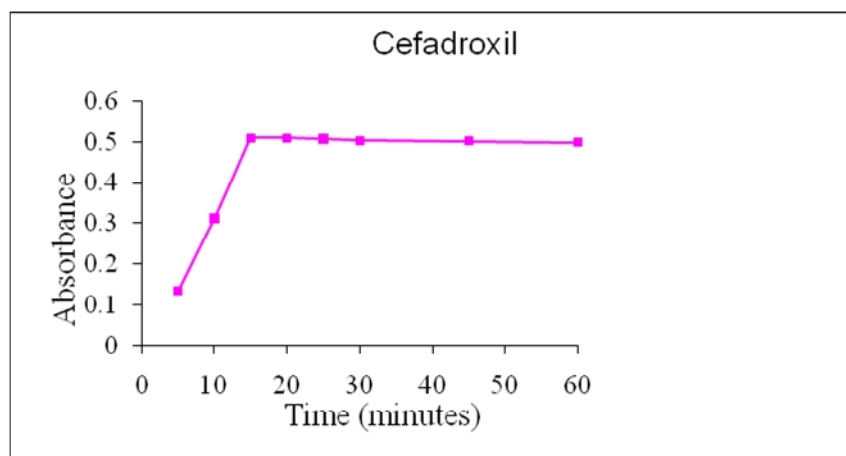**Figure 3: Optimization of heating temperature (^{0}C)**

Figure 4: Optimization of heating time (minutes)

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