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Application of ninhydrin and ascorbic acid for the determination of cefpodoxime proxetil in pharmaceutical formulations

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ABSTRACT

A simple and sensitive spectrophotometric method by exploiting the importance of the condensation reaction of Ninhydrin[1] indane-1,2,3- trione hydrate and Ascorbic acid[2-4] for the assay of Cefpodoxime proxetil has been described (CEFP), in bulk form and dosage forms. The ninhydrin reaction is one of the most useful reactions of α-amino acids. Ninhydrin along with a reducing agent has been used as a chromogenic reagent. In the present method, the drug CEFP which possesses $-NH_2$, when heated with ninhydrin in the presence of ascorbic acid afforded a blue violet color product. The absorption maximum was exhibited at 590nm. Regression analysis of Beer's law plots showed good correlation in the concentration range 10-60 µg/ml.

Key words: Cefpodoxime proxetil, Ninhydrin, Ascorbic acid, Spectrophotometry.

INTRODUCTION

Cefpodoxime proxetil(CEFP) is an antibacterial drug which has an excellent activity against entero bacteriaceae and other Gramnegative bacilli. Its chemical name is Rs-1 isopropoxy carbonyloxy (+)-(6R,7R) –7-[2-(2- amino-4-thiazolyl)-2-[(Z) methoxy imino] acetamido] -3-methoxy methyl-8-oxo-5 thia –1- azabicyclo[4.2.0] oct –2- ene-2- carboxylate.

A very few physico- chemical methods appeared in the literature for the assay of CEFP in biological fluids (more) and pharmaceutical formulations(less).Among the preferred methods for the regulatory assays HPLC[5-12] for CEFP and also applicability afford simplicity , speed, good specificity and excellent precision and accuracy. However they involve sophisticated instruments which are expensive and pose problems of maintenance. When suitable equipment is not available, an alternate that can be chosen is to combine a precise quantitative assay such as spectrophotometeric (visible or colorimetry[13-18], voltammteric, electrochemical reduction). As part of our continuing efforts to develop simple, sensitive and selective visible spectrophotometric methods for bulk drugs and their formulations, attention was focused on CEFP molecule, keeping in view the relative lack of such methods for its estimations. The present communication describes this sensitive method for the assay of CEFP in bulk and dosage forms. In this method, the drug developed a blue violet color product because of inter molecular oxidation and reduction of the ninhydrin[19] in the presence of AA[20].

EXPERIMENTAL

Apparatus

An ELICO, UV-visible digital spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance

measurements. An ELICO L1-120 digital pH meter was used for pH measurements.

Reagents

Ninhydrin solution (BDH 1% 5.605×10^{-2}M)

Prepared by dissolving 1g of Ninhydrin in 100ml of acetone.

Ascorbic acid solution (BDH 0.1%, 5.678×10^{-3}M)

Prepared by dissolving 50mg of AA in 50ml distilled water.

Buffer solution(pH 5.0)

Prepared by diluting a mixture of 200ml of 0.5M citric acid and 200ml of 1.0M NaOH solutions to 500ml with distilled water and the pH was adjusted to 5.0.

Preparation of Standard drug solution

A 1mg/ml stock solution of CEFP was prepared by dissolving 100mg of the drug in 100ml aldehyde free methanol. The standard stock solution of the drug was further diluted with distilled water to get the working standard solution of concentration 200µg/ml.

Pharmaceutical formulation solution

Tablets were mixed thoroughly and twenty tablets were selected at random and reduced to a fine powder. A portion of the mixed powder, equivalent to 100mg of CEFP was dissolved in aldehyde free methanol (100ml). This stock solution of pharmaceutical formulation was further diluted to the requisite concentration and analyzed as under bulk samples.

Procedure

Aliquots of standard CEFP solution (0.5 ml -2.5 ml; 200 µg/ml) was transferred into a series of calibrated tubes containing 4.0 ml of buffer (pH 5.0), 1.0 ml ninhydrin (5.605×10^{-3}M) solution and 0.5 ml of ascorbic acid 2.839×10^{-3}M) solution. The volumes in each tube were adjusted to 8.0 ml with distilled water and were kept in boiling water bath. After 15minutes tubes were removed and chilled in ice water. The solution in each tube was made up to 10.0 ml with distilled water. The absorbances were measured at 590 nm after 10 min. against a reagent blank prepared similarly. The amount of CEFP was calculated from its calibration graph.

Table 1

Parameter	NIN-AA
λmax (nm)	590nm
Beer's law limits (µg/ml)	10 – 60
Detection limits (µg/ml)	0.0269
Molar absorptivity (1.mol^{-1}cm^{-1})	4.053x10^{5}
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.0976
Optimum photometric range (µg/ml)	25-40
Regression equation (Y=a+bx)	
i) Slope (b)	0.0098
ii) Standard deviation on slope (S_b)	0.0011x10^{-4}
iii) Intercept (a)	0.0118
iv) Standard deviation on intercept (S_a)	0.0037
v) Standard Error of Estimation (S_e)	0.0035
vi) Correlation co-efficient (r)	0.9998
vii) Relative standard deviation (%)*	0.3632
% Range of error (confidence limits)*	
i) 0.05 level	0.3541
ii) 0.01 level	0.4490
% Error in bulk samples**	-0.1047

RESULTS AND DISCUSSION

The optimum conditions for the development of the method were established by varying the parameters one at a time and keeping the others fixed and observing the effect produced on the absorbance of the colored species.

The optical characteristics and figures of merits, such as Beer's law limits, molar absorptivity and Sandell's Sensitivity for the proposed method are presented in Table 1. The precision of each method was estimated by Six replicate samples within the Beer's law limits and the results are incorporated in Table1 Regression analysis using the method of least squares was made to evaluate slope (b), intercept(a),correlation coefficient (r) and standard error of estimation (S_e) for this method and the results are given in Table 1.

Commercial formulations (Tablets) containing CEFP were successfully analysed by the proposed method. The results obtained by the proposed and UV reference methods for dosage forms were compared statistically by means of the F- and t-tests were found not to differ significantly at 95% confidence limit. As an additional check of accuracy of the proposed method recovery experiments were performed by adding a fixed amount of the drug to the preanalysed formulations and the results are presented in Table 2. These results indicate that the commonly used excipients and additives in the dosage forms of CEFP did not interfere in the analysis of tablets.

Table 2

Formulation	Labelled amount (mg)	Amoung found (mg) by proposed methods*	Reference method	% Recovery by proposed methods**
CEEF				
Tablet 1	50	50.25±0.20 F = 2.78 t=0.866	50.1±0.12	100.07±0.13
Tablet 2	50	49.88±0.28 F=1.410 t = 0.3464	49.81±0.32	99.55±0.24
Tablet 3	100	99.72±0.77 F=1.891 t=0.4395	99.55±0.56	99.55±0.60

Tablets from four different pharmaceutical companies.

**Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F=5.05,t=2.57.

CONCLUSION

The method is simple, rapid and sensitive with reasonable precision and accuracy. Various mild reducing agent convert ninhydrin to hydrindantin ,the bimolecular hemiacetal. AA appears to be the reagent of choice. The hydrindantin acts as an antioxidant. The stability of the reduction product hydrindantin, formed in the first step could be enhanced by making use of reducing agent such as AA.

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