Original Article

Development and validation of spectrophotometric method of cefpodoxime proxetil using hydrotropic solubilizing agents

Abstract

Purpose: To develop and validate specific and accurate UV spectrophotometric method of cefpodoxime proxetil by using different hydrotropic solubilizing agents. **Materials and Methods:** The present study deals with spectrophotometric analysis of cefpodoxime proxetil by utilizing 4 different hydrotropic agents such as ammonium acetate (6 M), sodium citrate (1.25 M), sodium gycinate (1 M), sodium chloride (1 M), and urea (1 M). **Results**: From different hydrotropic agents, urea showed best aqueous solubility of cefpodoxime proxetil. The linearity was observed in the concentration range of 10-120 μg/ml. The method was validated and found to be precise. Accuracy (percent recovery) for cefpodoxime proxetil was found to be 99.82 ± 0.106. **Conclusion:** Urea as hydrotropic agent showed best aqueous solubility of cefpodoxime proxetil, which can be used as solubilizing agent. The proposed method is new, simple, safe, eco-friendly, economic, accurate, and cost-effective and can be successfully employed in routine analysis.

Key words: Eco-friendly, hydrotropic solubilization, safe, spectrophotometric, urea

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INTRODUCTION

Cefpodoxime proxetil is an orally-absorbed prodrug of cefpodoxime, an extended-spectrum, semi-synthetic cephalosporin developed by Sankyo Co. Ltd Japan. Cefpodoxime proxetil, chemically a relatively new broad-spectrum third-generation cephalosporin, has very good *in vitro* activity against *Enterobacteriaceae, Hemophilus spp.*, and *Moraxella spp.*, including fl-lactamase producers and many strains resistant to other oral agents. It also has activity against gram-positive bacteria, especially against streptococci. Cefpodoxime proxetil [Figure 1] chemically is (RS)- 1-(isopropoxycarbonyloxy)-ethyl- (+)-(6R,7R)-7-[2-(2-amino-4-thiazolyl-2- { (Z)-methoxy-imino} acetamido]-3-methoxymethyl-8-oxo-5-thia-l-azabicyclo- [4.2.0]oct-2-ene-2-carboxylate.[1]

It is very soluble in acetonitrile or methanol, freely soluble in dehydrated ethanol, slightly soluble in ether and very slightly soluble in water. Literature survey revealed that RP HPLC[2] and HPTLC[3] re the methods available for its estimation. Cefpodoxime proxetil is slightly soluble in water. Thus, hydrotropy can be used to increase the solubility. The proposed methods utilize solutions of non-toxic, non-volatile hydrotropic agents, which are the substitutes and minimizes the use of organic solvents, which are costlier, toxic, and source of pollutant.

The term "Hydrotropy" has been used to designate the increase in aqueous solubility of various poorly water-soluble compounds due to presence of a large amount of additives. Still the mechanism of hydrotropy is not understood very clearly. The concept of hydrotropy was first introduced in 1916 by Neuberg. According to his definition, hydrotropes are metal salts of organic acids, which at fairly high concentration increase the solubility of poorly water-soluble compounds.[4] On the other hand, Poochikian, Gradock (1979)

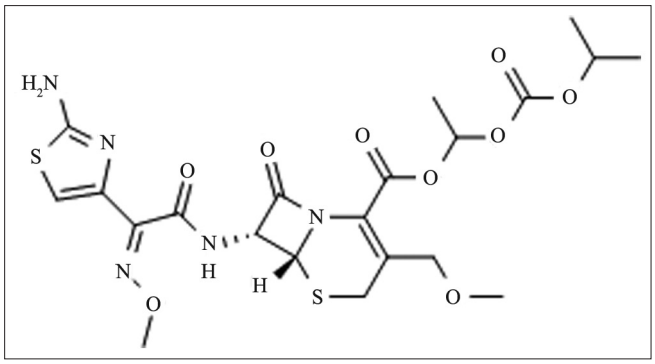

Figure 1: Structure of Cefpodoxime proxetil

studied that planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization.[5] Hence, it seems rational to propose that molecules with a planar hydrophobic part and a polar group, which is not necessarily anionic, can act as hydrotropic agents. Saleh *et al*, in 1985, extended the definition of a hydrotrope and said that it can be cationic, anionic, or a neutral molecule, provided it has a hydrophobic as well as a hydrophilic group.[6] Coffman and Kildsig studied the mechanism of hydrotropic solubilization using the riboflavin–nicotinamide system. They concluded that the complexation of nicotinamide and riboflavin did not occur because nicotinamide is not able to quench riboflavin fluorescence and does not produce significant UV- spectral changes.[7] Literature survey revealed that the hydrotropes can be used to enhance the solubility of poorly-soluble drugs, same as that of surfactants forming a term critical hydrotrope concentration (CHC) has been used in consonant with the critical micelle concentration.[8-13] Hydrotropic solutions can also be used as co-solvents, in solid dispersion technology,[14] nanotechnology, parentral preparations,[15] extraction purpose for solubilize[16] poorly water-soluble drugs. When hydrotropes are added to aqueous surfactants or to polymer solutions, they produce strong synergistic effects.

MATERIALS AND METHODS

Pharmaceutical grade cefpodoxime proxetil was kindly supplied as a gift sample by Orchid Pharmaceutical, Chennai. Tablets of cefpodoxime proxetil were procured from local market. Hydrotropic agents used were ammonium acetate, sodium citrate, sodium glycinate, sodium chloride and were of analytical grade.

Instrument

Instrument used were Schimadzu 1800 double beam UV/Visible Spectrophotometer and schimadzu 1600 analytical balance, Japan

Preliminary solubility study of cefpodoxime proxetil

In the solubility studies, it was found that there was more than 5-fold enhancement in the solubility of cefpodoxime proxetil in 1 M urea solution in comparison to its solubility in distilled water, ammonium acetate (6 M), sodium Citrate (1.25 M), sodium glycinate (1 M), sodium chloride (1 M).

Preparation of standard stock solution

Standard drug solution of cefpodoxime proxetil was prepared by dissolving 10 mg cefpodoxime proxetil in 10 ml 1 M urea. This solution was then sonicated for 15 mins and filtered through Whatmann filter Paper≠41.

Preparation of calibration curve

Aliquots of 1–12 ml portion of stock solutions were transferred to series of 100 ml volumetric flasks, and volume made up to mark with distilled water. Solutions were scanned in the range of 400-200 nm against blank. The absorption maxima were found to be at 231 nm against blank. The calibration curve was plotted. The optical characteristics are summarized in Table 1.

Preparation of sample solution

The proposed method was applied to analyze commercially available cefpodoxime proxetil tablet. Ten tablets were weighed and powdered. The amount of tablet powder equivalent to 10 mg of cefpodoxime proxetil was weighed accurately and transferred to 10 ml volumetric flask, and then, 10 ml 1 M urea was added and kept for sonication for 15 min. The solution was then filtered through

Table 1: Calibration curve of cefpodoxime proxetil

Concentration (µg/ml)	Absorbance
10	0.13
20	0.20
30	0.32
40	0.45
50	0.56
60	0.68
70	0.74
80	0.81
90	0.97
100	1.04
110	1.17
120	1.28

Table 2: Determination of accuracy by percentage recovery method

Ingredient	Tablet amount[a] (µg/ml)	Level of addition (%)	Amount added[a] (µg/ml)	Total amount taken from tablet[a] (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average % recovery ± SD
Cefpodoxime proxetil	100.00	80	80.2	180.2	180.0	99.88	99.82 ±0.106
	100.00	100	100.4	200.4	199.8	99.70	
	100.00	120	119.94	320.94	320.6	99.89	

[a]Amount equivalent to pure drug

Table 3: Reproducibility and Precision data (intraday and interday study)

Validation parameter	Lable Claim/ tablet (mg)	% Lable claim estimated Mean (± SD)	% Coefficient of variance	Standard error
Repeatability	100	99.52 ± 0.170	1.673	0.069
Intraday	100	99.68 ± 0.14	1.001	0.057
Interday	100	99.99 ± 0.46	1.428	0.188

Table 4: Validation parameters

Parameter	Result
Absorption maxima (nm)	231
Linearity range (µg/ml)	10-120
Standard regression equation	Y = 0.010x + 0.010
Correlation coefficient (r2)	0.996
Molar absorptivity	$0.1818 m^{-1} cm^{-1}$
A(1% , 1 cm)	130
Accuracy (% recovery ± SD)	99.82±0.106
Precision (% CV)	1.367
Limit of quantitation (µg/ml)	3.61
Limit of detection (µg/ml)	0.119

Whattman filter paper #41. This filtrate was diluted suitably with 1 M urea to get the solution of 10 µg/ml concentration. The absorbance was measured against blank solution. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is summarized in Table 2.

Method Validation

Linearity

The linearity of the response of the drug was verified at 10–120 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis as shown in Table 1.

Precision

Assay of method precision (intraday precision) was evaluated by carrying out 6 independent assays of test samples of cefpodoxime proxetil. The intermediate precision (interday precision) of the method was also evaluated using two different analysts, systems, and different days in the same laboratory for 6 days. The relative standard deviation (RSD) and assay values obtained were calculated, which are shown in Table 3.

Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in powdered tablets. The recovery was performed at 3 levels, 80, 100, and 120% of Cefpodoxime proxetil standard concentration. The recovery samples were prepared in aforementioned procedure. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated. [Table 2].

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

The LOD and LOQ of cefpodoxime proxetil were determined by using standard deviation of the response and slope. The standard deviations (SD) of responses and the average standard deviations (ASD) were calculated. Detection limit was calculated as (3.3 × ASD)/b, and quantification limit was calculated as (10 × ASD)/b, where 'b' denotes the slope obtained in the linearity study.

Determination of active ingredients in tablets

The validated method was applied for the determination of cefpodoxime proxetil in tablets (6 tablets were assayed, and the amount of active ingredient was calculated by using beer-Lambert's law. (98% – 102% of the label claim). [Table 4].

RESULT AND DISCUSSION

Main criteria for the selection of hydrotropic agents in spectrophotometric methods include sufficient concentration and volume of hydrotropic agents, which completely solubilize content of drug' and these hydrotropic agents should not interfere in analyzes. We have used 5 different hydrotropic solutions, which included ammonium acetate (6 M), sodium citrate (1.25 M), sodium glycinate (1 M), sodium chloride (1 M), and urea (1.0 M) in distilled water. Sufficient volumes of these hydrotropic solutions were used

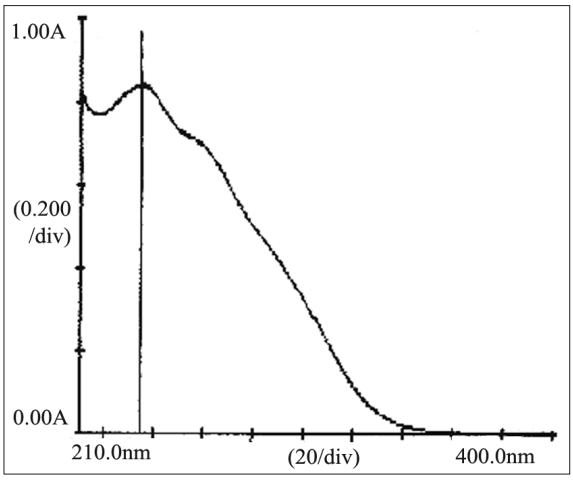

Figure 2: UV spectra of cefpodoxime proxetil standard and test tablet from 400 - 200 nm

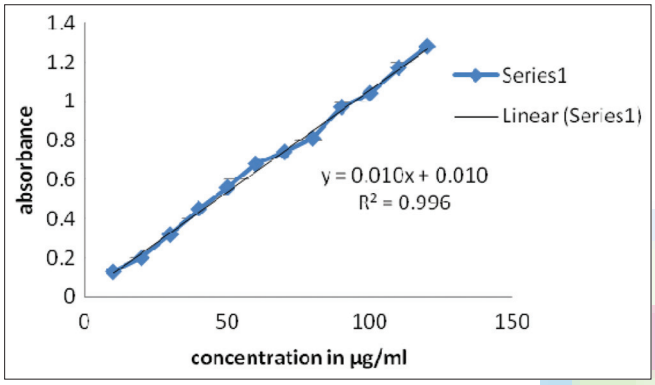

Figure 3: Calibration curve of cefpodoxime proxetil

to solubilize the content of cefpodoxime proxetil completely.

Hydrotropic solutions selected for this work in spectrophotometric methods have not shown any interference. The linearity was found in concentration range of 10 to 120 µg/ml. The mean percentage label claims estimated was 99.01%. The mean percentage recoveries ranged from 99.82 ± 0.10, indicating the accuracy of the proposed method. These values are very close to 100, indicating the accuracy of the proposed method. Low values of standard deviation, coefficient of variation, and standard error further validated the method. Thus, it may be concluded that the proposed method of analysis is new, cost-effective, environment-friendly, safe, accurate, and reproducible. This method can be successfully applied in routine analysis of cefpodoxime proxetil tablet formulation.

The equation of the calibration curve for cefpodoxime proxetil obtained was y = 0.010x + 0.010; the calibration curve was found to be linear in the aforementioned concentrations (the correlation coefficient (r^2) of determination was 0.996) [Figures 2 and 3].

CONCLUSION

Developed spectrophotometric cefpodoxime proxetil by using different hydrotropic agents was found to be the best alternative for estimations of poorly water-soluble drugs and to minimize the use of organic solvents. The proposed method utilizes solution of non-toxic, non-volatile hydrotropic agents, which give a novel, economical, and environment-friendly method for the estimation of cefpodoxime proxetil in tablet dosage forms.

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