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Development and Validation of Spectrophotometric Method for Estimation of Cephalexin in Bulk and Tablet Dosage Forms

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ABSTRACT

A spectrophotometric method has been developed for the quantitative estimation of cephalexin in API and in tablets. The method is based on the analysis of complex, formed on the reaction of cephalexin with 1-floro-2, 4-di nitro benzene. The absorbance maxima (I-max) was found to be 441.2 nm. Factors affecting the reaction viz. reaction time, temperature and effect of reagent were optimized. The proposed method was validated as per ICH guidelines. The recovery studies confirmed the accuracy and precision of the method.

Key words: Cephalexin, 1-floro-2, 4-di nitro Benzene, Validation, Spectrophotometry.

INTRODUCTION

Chemically, Cephalexin is 7 -a -D-phenylglycyl amino -3 -methyl -3 -cephem - 4-carboxylic acid monohydrate (1). It is first-generation member of cephalosporins which inhibits mucopeptide synthesis in the bacterial cell wall (2). Cephalexin is official in B.P. but not in I.P. and U.S.P. The method of analysis given in U.S.P. and I.P. is by iodimetry³. The other analytical methods reported in the literatures include HPLC [3,4,5,6], RP-HPLC⁷, Micellar Electrokinetic Chromatography⁸ and Densitometry (9) etc. This paper presents a simple,

accurate, sensitive, reproducible and economic method for determination of Cephalexin in bulk and in tablet formulations.

MATERIAL AND METHODS

Instrument

Elico SL 160 Double beam UV-VIS Spectrophotometer with spectral bandwidth of 1.8 nm, wavelength accuracy of 2 nm and matched quartz cells of 10 mm optical path length, GR 200 Analytical weighing balance (A&D Company) and Sonicator (Enertech Electronics).

Chemicals and Reagents

Methanol A.R grade was procured from Loba Chem. Ltd., Mumbai. 1-floro-2, 4-di nitrobenzene was procured from S. D. fine chem. Ltd. Mumbai. Cephalexin was obtained as gift sample from Lupin Ltd., Bhopal (M.P). Cephalexin tablets; Cephadex tablet (125 mg) (Cipla lab), Phexin tablet (125 mg) (GSK) and Sporen tablet (125 mg) (DAY'S) were procured from local market.

Preparation of standard Cephalexin solution

Cephalexin (10 mg) was accurately weighed and dissolved in 100 ml of distilled water to give a stock solution of concentration 100 mg / ml.

Optimization of reaction parameters

Reaction time

Standard solutions of Cephalexin (5 ml) were pipetted into a series of six, 25 ml volumetric flasks. 1 ml of 1-floro-2, 4-di nitro benzene was added to each flask and mixed. 1 ml of hydrochloric acid was added in each flask to maintain the acidic condition and the volumes in each were adjusted up to the mark with methanol. The final solutions were heated at 45°C for different time intervals (5, 10, 15, 20, 25, and 30 min). The absorbances of the complexes were measured at 441.2 nm against blank. The blank was prepared similarly in which volume of standard solution was replaced by an equal volume of methanol. The results are reported in Fig. 1.

Table 1: Optical and validation characteristics of cephalixin

Parameters	Values
Bear's law limit (mg/ml)	5-25
Sandell's sensitivity (mg/cm ² /0.001 absorbance unit)	0.273
Molar extinction coefficient (1 mole ⁻¹ cm ⁻¹)	1.337 X10 ³
Correlation coefficient (r)	0.999
Regression equation (Y')	
Slope (a)	0.1672
Intercept (b)	0.025
Precision (% CV)	
Repeatability (n=6)	0.022
Intraday (n=3)	0.187-0.555
Interday (n=3)	0.233-0.546
Accuracy (% recovery)	99.70
Limit of detection (LOD)	0.033
Limit of quantification (LOQ)	0.099

$y^* = b + ac$, where C is the concentration in g/ml and Y is absorbance unit;

CV = coefficient of variance; n = No. of measurements

Table 2: Results of assays of tablets of Cephalexin

Formulations	Label/Claim mg/tab	Estimated mg/tab*	% Found	RSD
Cephadex	125	124.58	99.6	1.005
Phexin	125	124.41	99.66	
Sporen	125	124.55	99.7	

*Average of 6 measurement

Effect of temperature

Standard solutions of Cephalexin (5 ml) were pipetted into a series of seven, 25 ml volumetric flasks. 1 ml of 1-floro-2, 4-di nitro benzene was added to each flask and mixed. 1 ml of hydrochloric acid was added in each flask to maintain the acidic condition and the volumes were adjusted up to the mark with methanol. The final solutions were heated at different temperatures (5, 15, 25, 30, 40, 45 and 50 °C) for 20 min. The absorbances of the complexes were measured at 441.2 nm against blank. The blank was prepared similarly in which volume of standard solution was replaced by an equal volume of methanol. The results are reported in Fig. 2.

Effects of concentration of reagent

Standard solutions of cephalaxin (5 ml) were pipetted into a series of six, 25 ml volumetric flasks. Different volumes (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) of 1-floro-2, 4-di nitro benzene were added to each flask, mixed and acidified by the addition of 1 ml hydrochloric acid. The volumes in each were adjusted up to the mark with methanol. The absorbance of the complexes was measured at 441.2 nm against blank. The blank was prepared similarly in which volume of standard solution was replaced by an equal volume of methanol. The results are reported in Figure 3.

Preparation of calibration curve for Cephalexin

Standard solutions of cephalaxin (0.5, 1.0, 1.5, 2.0 and 2.5 ml) were pipetted in series of five, 25 ml volumetric flasks. 1 ml of 1-floro-2, 4-di nitrobenzene was added to each flask and mixed. 1 ml of 1M hydrochloric acid was added to maintain acidic condition. The volume was adjusted with methanol. This mixture was heated at 40°C for 25 min and cooled. The calibration curve was constructed by plotting absorbance V/s concentration (mg/ml). Co-relation co-efficient was also measured.

Estimation of cephalaxin in tablets dosage forms

Twenty tablets (of same respective batch numbers) of three pharmaceutical companies were weighed and ground to fine powder. Quantity of powder (2.487 gm) equivalent to 10 mg of

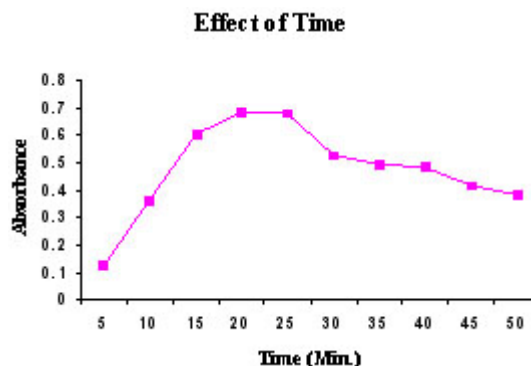


Fig. 1: Effect of different time intervals for heating the final solutions on the absorbance of complexes measured at 441.2 nm

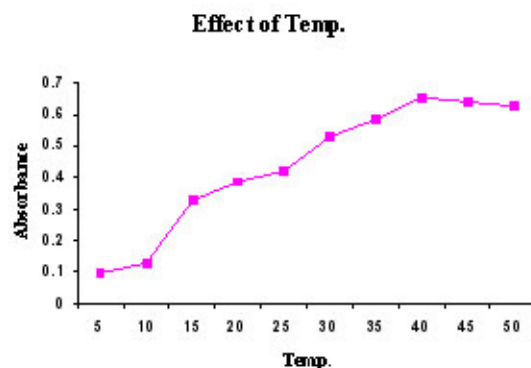


Fig. 2: Effect of different temperatures for heating the final solutions on the absorbance of complexes measured at 441.2 nm

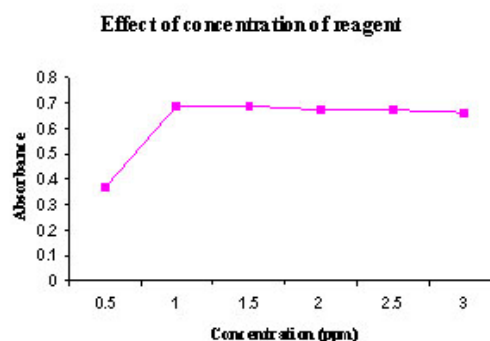


Fig. 3: Effect of different concentrations of reagents on the absorbance of complexes measured at 441.2 nm against the blank

cephalexin for each was transferred to three 100 ml volumetric flasks. The contents were dissolved in 50 ml of distilled water and sonicated for 20 minutes. The solutions were filtered through Whatmann filter paper No. 40 and residues were washed thoroughly with distilled water. The filtrate and washings were combined in a 100 ml volumetric flask and diluted up to the mark with distilled water. From these stock solutions, working sample solutions were prepared by appropriate dilutions and analyzed by proposed method. Recovery studies were carried out those gave satisfactory results.

Recovery studies and validation of the method (As per ICH Q2A Guidelines)

Recovery studies were carried out by adding 1, 2 and 3 mg of pure drug to different samples of tablet powder containing the equivalent of 10 mg of drug. Percentage recovery was calculated from the amount obtained by recovery studies. Precision of the method was studied by carrying out intraday, interday analysis and expressed as % Relative Standard¹⁰.

RESULTS AND DISCUSSION

In this proposed method, the determining conditions were established by varying one parameter at a time and keeping the other fixed by observing the effect produced on the absorbance of

a complex. The parameters involves the maximum complex development viz. concentration of reagent, temperature and time required to yield complex of maximum sensitivity were optimized. Maximum absorbance of Cephalexin was observed in the presence of 1.0 ml of reagent solution. Optimum temperature to obtained maximum complex intensity was found to be 40°C in Cephalexin. Maximum absorbance of Cephalexin was obtained after 20 min.

The optical characteristics such as Beer's law limits, Molar extinction coefficient, Sandell's sensitivity, Correlation coefficient, Regression equation and Accuracy were calculated for the method and the results are summarized in Table 1.

The proposed method was found to be accurate, simple and rapid for routine analysis of cephalexin. Drug when reacts with 1-floro-2, 4-di nitro benzene reagent forms a complex, which was than analyzed. The results of recovery studies were found to be satisfactory and are reported in Table 2.

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