RESEARCH ARTICLE

Development of colorimetric method for cephalexin in dosage forms

P Priyanka, P Suresh[1]

Department of Pharmaceutical Analysis, BR Nahata College of Pharmacy, [1]Mandsaur Institute of Pharmacy, Mandsaur, Madhya Pradesh, India

A simple, sensitive, accurate, rapid, and economical colorimetric-spectrophotometric method has been developed for the estimation of cephalexin in capsules. This method is based on the reaction of the drug with ferric chloride and potassium ferricyanide, giving a green-colored chromogen exhibiting maximum absorbance at 791 nm against reagent blank. Beer's law was obeyed in the concentration range of 1-6 μg/ml. Results of the analysis were validated statistically and by recovery studies.

Key words: *Calibration curve, chromogen, correlation coefficient, molar absorptivity, Sandell's sensitivity*

INTRODUCTION

Chemically, cephalexin is {(6R, 7R)-7-[(R)-2-Amino-2-Phenylacetamido]-3-Methyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid monohydrate}[1] is a first generation cephalosporins for oral administration which is bactericidal, and mainly used in the treatment of various bacterial infections caused by gram +ve and gram –ve microorganisms.[2] Cephalexin is official in Indian Pharmacopoeia (IP), United States Pharmacopoeia (USP), and British Pharmacopoeia (BP). The IP[3] method describes titrimetric procedure, whereas USP[4] and BP[5] describe a HPLC method, for estimation of cephalexin from formulations. Literature survey revealed a flourimetry[6] and polarography,[7] high-performance liquid chromatography,[8] flow injection analysis,[9] densitometric method,[10] high-pressure thin layer chromatography[11] and reverse phase high-performance liquid chromatography[12] methods, for determination of cephalexin in pharmaceutical formulations, and in biological fluids. The present communication describes simple, sensitive, accurate, rapid and economical colorimetric-spectrophotometric method for the estimation of cephalexin in capsule dosage forms.

MATERIALS AND METHODS

A Perkin-Elmer EZ301 double-beam UV visible spectrophotometer with 1 cm matched quartz cells were used for the measurement of absorbance. Cephalexin (Rajasthan Drug Pharmaceutical Ltd., Jaipur), ferric chloride solution (0.1 M) and potassium ferricyanide (0.1%w/v) solution and distilled water were used in the study.

Preparation of standard solution

The standard solution of cephalexin was prepared, by dissolving 100 mg of cephalexin in a 100 ml volumetric flask, using distilled water to obtain the final concentration of 1000 μg/ml. This stock solution was further diluted to obtain a working standard solution containing 100 μg/ml of the drug.

Determination of λ_{max}

The standard solution of 1 ml (100 μg/ml) was pipetted out into 10 ml volumetric flask. Ferric chloride solution (0.5 ml) and potassium ferricyanide solution (1 ml) were added into each flask and kept aside at room temperature for 10 min to complete the reaction. Appropriate quantity of distilled water was added to the flask to make up the volume. The absorbance of green-colored complex was scanned in visible range for maximum absorbance which was found at 791 nm.

Calibration curve

Aliquots ranging from 1 to 6 ml of the working standard drug solution (100 μg/ml) were transferred to a series of 10-ml volumetric flasks. To each flask, 0.5 ml of ferric chloride solution and 1 ml of potassium ferricyanide solution were added. After shaking it thoroughly, the flasks were kept aside at room temperature for 10 min to complete the reaction. Appropriate quantity of distilled water was added to each flask to make up the volume. The absorbance of green-colored complex was measured at 791 nm against a reagent blank and the calibration curve was plotted. Similarly, the absorbance of sample solution was measured and the amount of the cephalexin was determined by referring to the calibration curve.

Address for correspondence:
P Priyanka, BR Nahata College of Pharmacy, Mandsaur - 458 001, Madhya Pradesh, India. E-mail: priyankapasrija2007@rediffmail.com

Table 1: Optical Characteristics of the proposed colorimetric method

Parameters	Observations
λ_{max} (nm)	791
Molar extinction coefficient (1 mol^{-1} cm^{-1})	1.32×10^4
Sandell's sensitivity ($\mu g/cm^2$/0.001 absorbance unit)	0.2768×10^{-4}
Regression equation ($y = mx + c$)	
Slope (m)	0.0276
Intercept (c)	0.0293
Standard deviation	±0.0514
Standard error of mean	±0.0219

Table 2: Summary of validation parameters

Parameters	Observations
Linearity range (μg/ml)	1-6
Correlation coefficient (r^2)	0.9998
Precision (% RSD)	
Repeatability (n = 6)	0.630
Intra day (n = 3)	0.920
Inter day (n = 3)	0.932
% Recovery	99.32
Limit of detection (μg/ml)	0.140
Limit of quantification (μg/ml)	0.410

Table 3: Results of recovery study of cephalexin in dosage form

Formulations*	Label claim (mg/cap)	Amount found (mg/cap)	% Of label claim† ± SD	% Recovery ± SD
A	250	249.80	99.93 ± 1.24	99.36 ± 0.75
B	250	247.71	99.08 ± 0.76	99.21 ± 0.61
C	250	249.01	99.60 ± 0.55	99.21 ± 0.71
D	250	246.98	98.79 ± 0.73	99.52 ± 0.97

*Different brands marketed preparations; †Mean of six determinations

Table 4: Comparative analysis of cephalexin in marketed formulations

Dosage form	Amount of drug taken (mg)	Found values (% recovery)	
		IP method	Developed method
A	250	99.50	99.92
B	250	98.50	99.08
C	250	99.00	99.60
D	250	98.50	99.79

Estimation of marketed dosage forms

Twenty capsules of cephalexin (each containing 250 mg of cephalexin) were emptied and weighed. An accurately weighed powder to 100 mg of the cephalexin was taken in small quantity of distilled water and was shaken thoroughly. It was then filtered through a Whatmann filter paper no. 42 into 100-ml volumetric flask and the volume was made up with distilled water. The amount of cephalexin was determined by referring to the respective calibration curve. The analysis procedure was repeated six times for formulations, and the result of analysis of the respective formulations is shown in Table 3.

Validation studies

To study the accuracy, reproducibility and precision of the above proposed method, recovery studies were carried out by addition of known amount of standard drug solution of cephalexin, to the pre-analyzed formulation. The resulting solution was re-analyzed by proposed method. Precision of the method was studied by carrying out interday, intraday analyses and expressed as percent coefficient of variance (% CV).

Limit of detection and limit of quantitation were studied based on standard deviation of the response and the slope.

RESULTS AND DISCUSSION

The present work depicts the quantitative reaction of the drug with ferric chloride and potassium ferricyanide. The reaction is based on the reduction of ferric chloride to ferrous form, which in turn couples with potassium ferricyanide to give a green color with maximum absorbance at 791 nm. It was found that 0.5 ml of ferric chloride solution and 1 ml of potassium ferricyanide solution were sufficient for the development of maximum color intensity. Stability study of the developed chromogen carried out, by measuring the absorbance values at time intervals of 20 min for 1 h, and it was found to be stable for more than 1 h at room temperature. The linearity was found in the concentration of 1-6 μg/ml ($r^2 = 0.9998$). Different validation parameters[13-17] are summarized in Table 2. The percentage recovery was found to be more than 99% as shown in Table 3. The statistical tests were applied and shown in Table 4.

The precision value of the proposed method was good as indicated from the low relative standard deviation (less than 2.0%) calculated from six replicate analyses of cephalexin. The % recovery value in the range of 99.21-99.52% indicates noninterferences from the formulation excipients.

The applicability of the proposed method was examined for the assay of cephalexin in marketed formulations (capsules). The performance of the proposed method was judged by calculating the Student's t-test. At the 95% confidence level, the calculated t-values do not exceed the theoretical values indicating that there is no significant difference between the

Table 5: t-Test: Paired two sample for means

	Variable 1	Variable 2
Mean	99.3475	98.875
Variance	0.257958333	0.229166667
Observations	4	4
Pearson correlation	0.957965924	
Hypothesized mean difference	0	
df	3	
t Stat	6.476300314	
P ($T \leq t$) one-tail	0.003735813	
t Critical one-tail	2.353363016	
P ($T \leq t$) two-tail	0.007471626	
t Critical two-tail	3.182449291	

proposed method and the official method.

In conclusion, the proposed method is simple, sensitive, accurate, precise and economical, and can be successfully employed for the routine analysis of cephalexin in capsule dosage forms.

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