AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of UV Spectrophotometric Method for Determination of Cefuroxime in Pharmaceutical Dosage forms

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ABSTRACT

A rapid and sensitive UV-Visible spectroscopic method was developed for the estimation of cefuroxime in pure and its Pharmaceutical formulations. The method was based on the measurement of absorbance of Cefuroxime active moiety of Cefuroxime tablet at 277 nm using methanol as solvent. The absorbance was found to increase linearly with increase in concentration of Cefuroxime which was corroborated by correlation coefficient values. The standard solution of Cefuroxime obeyed Beer's law over the concentration range of 9.20–27.60 µg/mL. The method is linear (from 9.20-27.60 µg/mL) with an R^2 of 0.999, accurate (% recovery 100.56%) and precise (% RSD 0.316%). The method is specific and robust for Cefuroxime.

Key words: Cefuroxime, Spectrophotometric method, validation, accuracy

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Received 3 June 2012, Accepted 24 June 2012

Please cite this article in press as: Asharaful Islam SM *et al*., Development and Validation of UV Spectrophotometric Method for Determination of Cefuroxime in Pharmaceutical Dosage form. American Journal of PharmTech Research 2012.

INTRODUCTION

Cefuroxime (figure 1) is one of the bactericidal second generation cephalosporin antibiotic which is active against a wide range of Gram-positive and Gram-negative susceptible organisms including many beta-lactamase producing strains[1]. It is indicated for the treatment of infections caused by sensitive bacteria. Chemically cefuroxime axetil is (*RS*)-1-hydroxyethyl (6*R*, 7R)-7-[2-(2-furyl) glyoxyl-amido]-3-(hydroxymethyl)-8- oxo-5-thia-1-azabicyclo [4.2.0]-oct-2-ene-2-carboxylate, 72-(*Z*)-(*O*-methyl-oxime), 1-acetate 3-carbamate. The drug is official both in BP and USP[2-3].

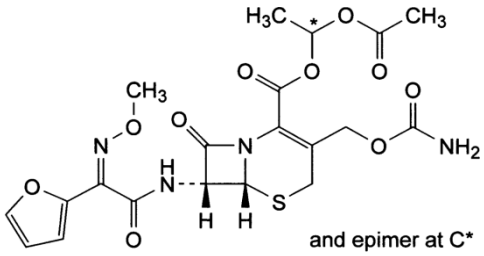

Figure1: Chemical structure of Cefuroxime axetil

Analysis of drug is an important component in design and quality control of dosage form. A simple analytical method must be developed and validated for the analysis of drug(s) in the bulk, in drug delivery systems, from release dissolution studies and in biological samples. The estimation of cefuroxime axetil by mercurimetric method[4], high performance liquid chromatography [HPLC][5], high performance thin layer chromatography [HPTLC][6] and spectroflurimetric method[7-8] has been reported in literature. The British Pharmacopoeia and United States Pharmacopeia describe HPLC method for the assay of cefuroxime axetil[2-3]. HPLC method is more accurate and acceptable. But the instrument is not available in all quality control labs. So, the present work was undertaken with the aim to develop and validate an economic and rapid UV spectroscopic method with high accuracy, precision and linearity according to ICH [9] guideline. The method will be helpful for the routine estimation of cefuroxime in bulk and cefuroxime tablet.

MATERIALS AND METHODS

Materials

Methanol was analytical reagent grade and purchased from E. Merck, Darmstadt, Germany. Water was deionised and double distilled. Working standard of cefuroxime axetil was collected from Eskayef Bangladesh Ltd as gift samples. Marketed formulation was purchased from local drug store in Dhaka city after checking their manufacturing license number, batch number, production and expiry date.

Instrumentation

A double-beam Shimadzu (Kyoto, Japan) UV-Visible spectrophotometer, Model UV-1601 PC, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of +0.5 nm (with automatic wavelength correction) was used. The drug analyses data were acquired and processed using UV Probe software (Version 2.0, Shimadzu, Japan) running under Windows XP on a Pentium PC. For scanning, the wavelength range selected was from 400 nm to 200 nm with medium scanning speed.

Preparation of Stock Standard Solution

Accurately weighted 45 mg of cefuroxime axetil standard was dissolved in a 100-mL volumetric flask. 2 mL of stock standard solution was diluted to 50 mL with methanol and mixed uniformly.

Preparation of Test Sample

Accurately weighted tablet powder equivalent to 45 mg cefuroxime axetil (from cefuroxime 500 Tablet) was taken in a 100-mL of volumetric flask. After adding 60 mL methanol the mixture was sonicated for 10 minutes at ultrasonic bath then cooled at room temperature and finally volume up to the mark with methanol. 2 ml of this solution was diluted to 50 mL with methanol.

Linearity and Range

The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of drug in samples within a given range[10]. Linearity of the method was determined by constructing calibration curves. Linearity was determined from concentration 50-150% of nominal concentration for a total of 7 different concentrations. Aaccurately weighted 22.5 mg, 27.0 mg, 36.0 mg, 45.0 mg, 54.0 mg, 63.0 mg, and 67.5 mg of cefuroxime axetil working standard were taken in separate 100-mL volumetric flask and 60 mL methanol was added to dissolve the drug, finally volume was made up to the mark. 2 mL of this solution was diluted to 50 mL with methanol to achieve the analyte standard of 50% to 150% of nominal concentration. Data was evaluated by plotting the absorbance of cefuroxime axetil (Y-axis) against its respective concentration (X-axis). Each measurement was carried out in six replicates and the absorbances were used to obtain the calibration curves and correlation coefficients. Characteristic parameters for regression equation ($y = a + bx$) of the method were obtained by least squares treatment of the results and these parameters were used to confirm the good linearity of the method.

Accuracy

Accuracy indicates the deviation between the mean value found and the true value. The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by

means of recovery experiments.

Accuracy was determined by the preparation of 5 (five) different concentration of drug along with different amount of placebo. Placebo was prepared by mixing common excipients: maize starch, lactose, purified talc, povidone K-30 and magnesium state. 36.0 mg, 40.5 mg, 45.0 mg, 49.5 mg & 54.0 mg of cefuroxime axetil were taken in five different 100-mL volumetric flask and 23.8 mg, 26.7 mg, 29.7 mg, 32.7 mg and 35.6 mg of placebo were added respectively. Volume was made up to the mark with methanol. 2 mL of this solution was diluted to 50 mL with methanol after filtration. From the absorbance potency was calculated. The accuracy was assessed from the test results as the percentage of the drug recovered by the assay.

% recovery was calculated by the following equation:

% Recovery of Target = {(Experimental Concentration ÷ Theoretical Concentration) x 100}

Percent relative standard deviation was calculated using the following relationship:

%RSD = {(Standard Deviation ÷ Mean Concentration) x 100%}

Precision

The precision of the method was investigated with respect to repeatability (inter assay precision), intermediate precision (inter day precision). Repeatability was determined by performing six repeated analysis of the samples on the same day, under the same experimental conditions. Experiment was done from 9.00 am to 9.00 pm. % RSD was calculated to determine the reparability. Intermediate precision of the method was assessed by carrying out the analysis of standard solutions by two analysts in the same laboratory.

Specificity

Specificity was determined by measuring absorbance of blank and placebo separately. If analyte spectrum does not affected by blank and placebo, the method will be specific.

Robustness

To determine the robustness analysis was performed by two different analysts, on two different days with their own preparation of solutions. Analytical methods are generally known as robust if percent recovery is within 98-102%.

Analysis of market products

The proposed method was used to determine the potency of commercially available tablets. Six replicate determinations (n=6) were carried out.

RESULTS AND DISCUSSION

Linearity, Range, LLOQ and ULOQ

Absorbance was measured for each concentration level. The actual concentrations of the seven standards against the respective absorbance were computed and the linear regression curve was generated. A linear relationship was determined through calculation of a regression line by the method of least squares. A plot of the data as well as the correlation coefficient, y-intercept, slope of the regression line is presented in figure 2. The lower limit of quantitation (LLOQ) was defined as the lowest concentration within the linear range (9.20µg/mL). The upper limit of quantitation (ULOQ) was defined as the highest concentration within the linear range (27.60µg/mL).

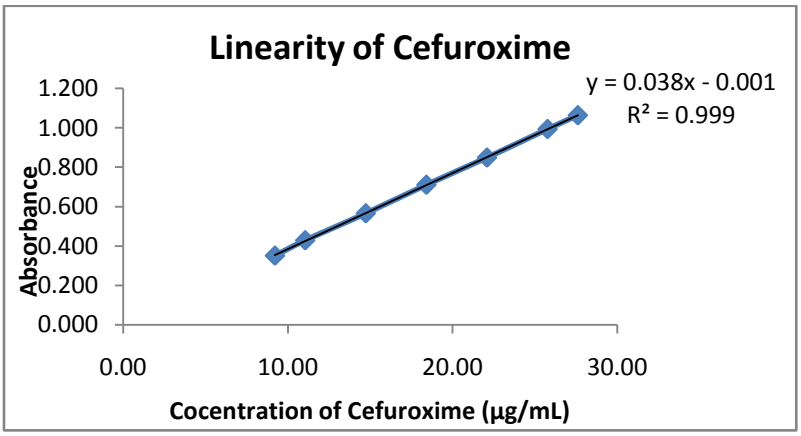

Figure 2: Linearity of cefuroxime

Table-1: Result of Linearity and Range

% of Nominal value	Conc. of Std (µg/mL)	Abs.	Regression coefficient(R^2)		y-intercept	Slope of Regression line	Pass/ Fail
			Limit	Result			
50%	9.20	0.351					
60%	11.04	0.429					
80%	14.72	0.566					
100%	18.40	0.711	NLT 0.995	0.999	0.001	0.038	Pass
120%	22.08	0.849					
140%	25.76	0.994					
150%	27.60	1.064					
Lower limit of quantitation (LLOQ)					9.20 µg/mL		
Upper limit of quantitation (ULOQ)					27.60 µg/mL		

Accuracy:

Accuracy was conducted by adding known amounts of analyte to the sample matrix and five different concentrations of test sample were prepared. Concentration values were calculated from the corresponding absorbance for five concentrations. The accuracy was found to be 100.56%. The data is presented in Table 2 with acceptance criteria.

Table 2: Result of Accuracy

% of Nominal Value	Weight		Abs. of sample	Abs. of Std	Recovery	%Recovery	Limit
	API (mg)	Placebo (mg)					
80%	36.9	23.4	0.588	0.724	37.36	101.24	98.0% to
90%	40.8	26.5	0.650		41.30	101.22	102.0%
100%	45.5	29.7	0.718		45.62	100.26	
110%	49.0	32.6	0.776		49.30	100.62	
120%	53.4	35.7	0.836		53.12	99.47	
Mean						100.56%	
SD						0.738	
RSD						0.734%	

Precision:

Repeatability:

One analyst (Analyst 1) conducted assay by using six samples. Single absorbance was measured for each of six sample preparations. Concentration was calculated from the corresponding absorbance for six samples. Percent relative standard deviations were calculated (% RSD 0.348%) for analyst 1. This percent relative standard deviation proves the repeatability of the method. The data is presented in Table 3 with acceptance criteria.

Table 3: Result of Repeatability

Sample	Label claim (Mg/Tab.)	Std (mg)	Abs. of sample	Abs. of Std	Assay, mg/tablet	Limit (%)
1	500	46	0.839	0.724	501.43	NMT 2.0
2			0.861		503.32	
3			0.876		502.68	
4			0.851		500.01	
5			0.850		505.11	
6			0.816		501.81	
Mean Assay					502.39 mg per tablet	
SD					1.7476	
RSD					0.348%	

Intermediate precision:

Separately a second analyst checked the assay of six sample of the same batch as the analyst 1. Single absorbance was measured for each sample preparation. Concentration was calculated from the corresponding absorbance for each of six samples preparation. The precision of analyst 2 were combined with that of the analyst 1 (n=12) and the combined relative standard deviation (RSD) is calculated to check intermediate precision. The data is presented in Table 3 with acceptance criteria. The intermediate precision of the method was found to be 0.316% (Table 4).

Specificity: As there was no absorption of blank and placebo measurement, it did not contribute anything to the analyte absorption.

Table 4: Result of Intermediate Precision

Sample		Label claim (mg)	Std (mg)	Abs. of sample	Abs. of Std	Assay, mg/tab	%RSD	Limit (%)
Analyst 1	1	500	46	0.839	0.724	501.43	0.348	NMT 2.0
	2			0.861		503.32		
	3			0.876		502.68		
	4			0.851		500.01		
	5			0.850		505.11		
	6			0.816		501.81		
Analyst 2	1	500	47	0.846	0.739	504.89	0.306	
	2			0.871		502.07		
	3			0.875		501.03		
	4			0.818		501.55		
	5			0.854		503.92		
	6			0.849		503.80		
Mean Assay						502.64 mg per tablet		
SD of 12 samples						1.5893		
RSD of 12 samples						0.316%		

Robustness: The validation was performed by two different analysts, on two different days with their own preparation of solutions. % recovery was within range. Therefore, the method is considered to be robust.

Analysis of tablets: Market products analysis results are summarized in Table 5. Potency was within limit.

Table 5: Potency of market products

Drug	Cefuroxime
Label claim (mg) (n=6)	500.00
Observed amount (mg) (n=6)	504.02
Potency (%)	100.80
SD	0.92
% RSD	0.91

CONCLUSIONS:

From the above test parameters, it is established that the proposed method is linear; accurate and precise (repeatability and intermediate) within the acceptable limit. Therefore this method is validated and suitable for the assay of cefuroxime tablet.

ACKNOWLEDGEMENTS

The authors are thankful to Eskayef Bangladesh Ltd for providing cefuroxime as gift.

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