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SPECIFIC STABILITY INDICATING ASSAY METHOD FOR THE DETERMINATION OF CEFUROXIME SODIUM IN PHARMACEUTICAL FORMULATION BY UV-VIS SPECTROPHOTOMETER

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Abstract

The present study describes a simple, accurate, precise and cost effective UV-Vis Spectrophotometric method for the estimation of cefuroxime sodium, a second generation cephalosporin anti-biotic in dry powder injection and drug substances. The solvent used throughout the experiment was distilled water. The λmax or the absorption maxima of the drug was found at 275 nm. Beer's law obeyed in the range of 2.0-16.0 µg/ml. The developed method was successfully validated with respect to linearity, accuracy and precision. The sample concentrations are measured on weight basis throughout the experiment in UV-Vis Spectrophotometric method. The method was validated and shown linear in the mentioned concentrations. The correlation coefficient for cefuroxime sodium was 0.9999. The recovery values for cefuroxime sodium ranged from 100.0-100.3. The relative standard deviation of six replicates of assay was less than 2 %. The percent relative standard deviation of inter-day precision ranged 1.0-1.4 % and intra-day precision 1.2-1.6 % of cefuroxime sodium. The limit of detection and limit of quantification of cefuroxime sodium was 0.11 µg/ml and 0.39 µg/ml. The developed method was cross checked with high performance liquid chromatography for six replicate assays as per USP 32, the mean assay was 100.3% and %RSD was 0.35%. The degradation study of cefuroxime sodium was studied for the purpose of stability indicating method. Hence proposed method was precise, accurate and cost effective. This method can be applicable for

quantitative determination of the titled drug with respect to assay from their new commercial formulation of injection in quality control laboratories.

Keywords: UV-Vis Spectrophotometer, Method development, Method validation, Cefuroxime sodium.

Introduction

Cefuroxime sodium chemically, sodium salt of (6R, 7R)-3-carbamoyloxymethyl-7-[Z-2-methoxyimino-2-(fur-2-yl) acetamido] ceph-3-em-4-carboxylate.Cefuroxime sodium, a second generation cephalosporin broad-spectrum beta-lactam antibiotic, used for parental administration. Also it is used to treat the skin and skin structure infections, lower respiratory tract infection, bone and joint infection, central nervous system infection and bacterial septicemia. Cefuroxime sodium is a sterile, dry powder mixture. However no UV Spectrophotometric method was proposed for the estimation of cefuroxime sodium without using hydro tope in bulk and pharmaceutical dosage forms. The aim of the work was to develop and validate an analytical method by using UV-Vis Spectrophotometer for the estimation of cefuroxime sodium in bulk and pharmaceutical dosage forms. The formulation available in Indian market under the trade name of "FORCEF" of 750 mg per vial, manufactured by Aristo Pharmaceuticals Ltd. Literature survey revealed that few analytical methods are available for the individual estimation of cefuroxime sodium in bulk drug and dosage formulations by HPLC in different pharmacopeia [1-3] .Few methods were reported for individual estimation of cefuroxime sodium by HPLC [4-10], and some method in visible region by UV-Vis Spectrophotometer [11-22]. After development, analytical method was validated to ensure their quality and suitability as per ICH guideline [23]. Yet there is no method reported in the literature for the estimation of cefuroxime sodium in ultraviolet range of pharmaceutical dosage forms. In the present research work a simple, accurate and economical UV Specrophotometric method has been developed for the estimation of cefuroxime sodium in dry powder injection and bulk drug substances.

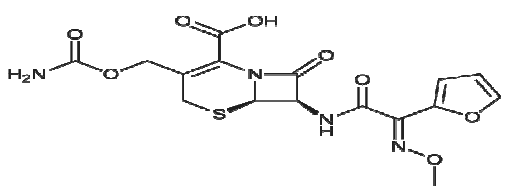

Figure-1: Chemical Structure of cefuroxime sodium.

Experimental

Materials and Reagents

Cefuroxime sodium used as working standard kindly provided by Bharati Vidhypeeth, (Pune, Maharashtra State (M.S.),India). The injection formulation available in Indian market under the trade name of "FORCEF" of 750 mg per vial, manufactured by Aristo Pharmaceuticals Ltd, Mumbai, India. Distilled water was used throughout the experiment. Other chemicals were analytical grade.

Instrumental condition

The instrument used was an UV-Vis double beam spectrophotometer, of Shimazu make (Mode: 2501PC) with matched pair quartz cell for this study.

Method Development

Solubility Test

Solubility test of the drug cefuroxime sodium was performed by using various solvents. The solvents include water, methanol, ethanol, acetonitrile, 0.1N hydrochloric acid (HCl), 0.1 N sodium hydroxide (NaOH) and chloroform. However, the drug is freely soluble in water hence water was chosen as a solvent for developing the method and cost of water is low as compare other solvent.

Determination of λ max

Preparation of Stock Solution

Weigh and transfer accurately, equivalent to 100 mg of cefuroxime sodium as working standard into 100 ml volumetric flask, dissolved and diluted up to mark with distilled water. Transfer 10 ml solution from the stock to 100 ml volumetric flask with distilled water to produce a concentration of 100 μg/ml, use this as standard stock solution.

Preparation of Working Standard Solution:

From the above stock solution, 10 ml pipetted into a 100 ml volumetric flask, the volume was made up with distilled water to produce a concentration of 10μg/ml. The solution was scanned in UV-Vis Spectrophotometer in the range 400-200 nm using distilled water as a blank. The wavelength corresponding to maximum absorbance (λmax) was found at 275 nm (shown in fig. 2).

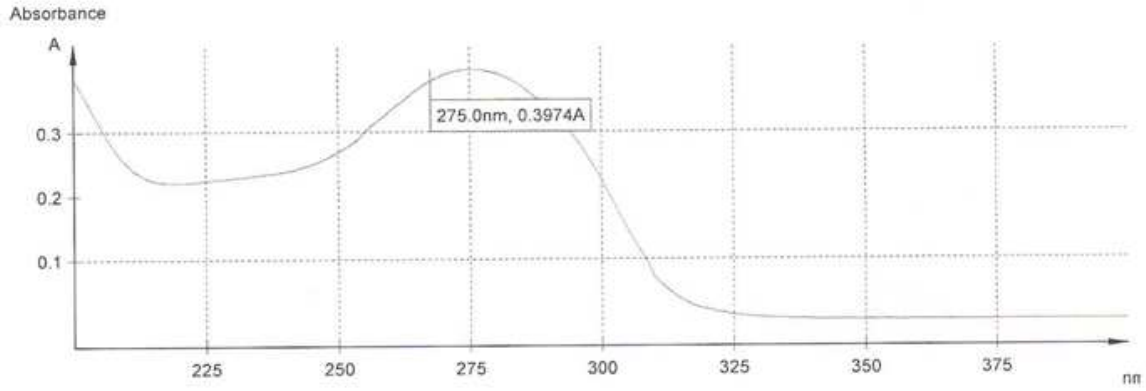

Figure-2: UV Spectrum of cefuroxime sodium (λmax determination).

Preparation of Calibration Curve:

2 ml solution of the 100 µg/ml was diluted to 100 ml to produce 2µg/ml solution. 4ml, 6ml, 8ml, 10ml, 12ml, 14ml, and 16ml of 100µg/ml solution were diluted to 100 ml with distilled water to produce 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml, 14µg/ml, and 16µg/ml solutions respectively. The calibration curve was construction by taking the solution concentrations ranged from 2-16µg/ml. The calibration curve was plotted by taking concentration on x axis and absorbance on y axis (shown in fig.3). The curve showed linearity in the concentration range of 2-16µg/ml. This straight line obeyed linearity in the concentration range of 2-16µg/ml. The method is validated and shown to be linear in the mentioned concentrations. The correlation coefficient for cefuroxime sodium was 0.9999.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like linearity, accuracy, precision, specificity, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

Various aliquots were prepared form the stock solution (100µg/ml) ranging from 2- 16µg/ml. Linearity of the method for cefuroxime sodium was tested from 20-160 % of the targeted level of the assay concentration in triplicate. The solutions were scanned in UV-Vis Spectrophotometer using distilled water as blank. It was

found that the selected drug shows linearity between the 2-16µg/ml. The correlation coefficient (r^2) was found y=0.044x+0.001, R^2=0.9999. (Shown in fig. 3).

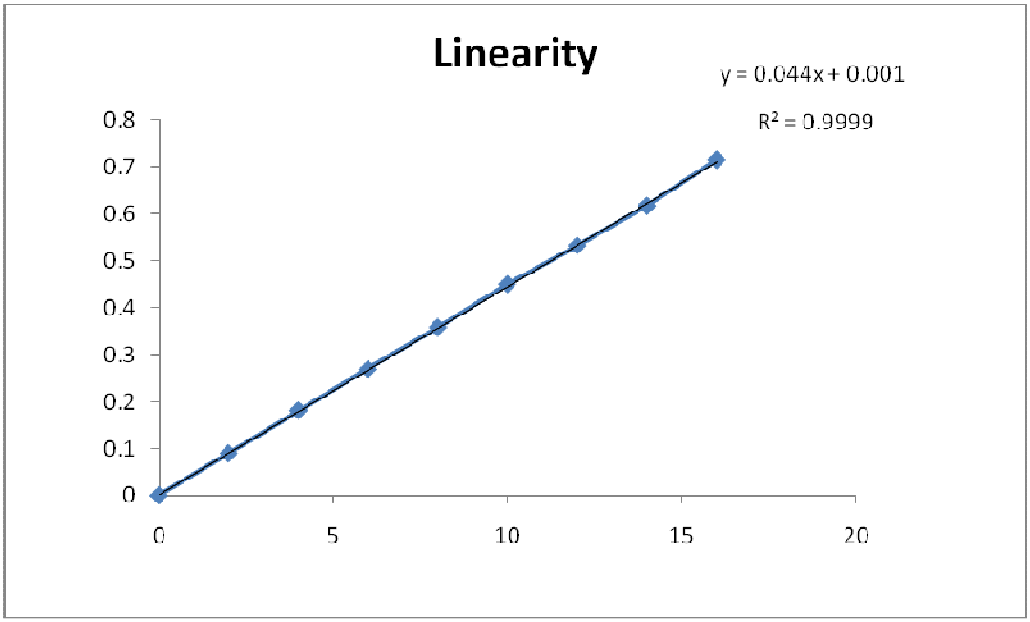

Figure: 3. Calibration curve of cefuroxime sodium

Table 1: Summary	
Parameter	Result
Linearity (Correlation coefficient)	0.9999
Precision (% RSD)	0.72
Accuracy (%Recovery)	100.1 %
Limit of Detection (LOD)	0.11 µg/ml
Limit of Quantitation (LOQ)	0.39 µg/ml
Range	2-16 µg/ml
Linear regression equation	0.044x+0.001
Ruggedness & Robustness (% RSD)	0.07-0.28 %
Assay UV-Vis (% recovery)	100.1
Assay HPLC	100.3

Table 2: Linearity of Cefuroxime Sodium by UV-Vis Spectrophotometer	
Concentration (µg/ml)	Absorbance
0	0
2	0.0895
4	0.1811
6	0.2689
8	0.3577
10	0.4497
12	0.5319
14	0.6164
16	0.7146

Table 3: Optical Characteristic by UV-Vis Spectrophotometer	
Parameter	Result
Molar extinction coefficient	341
Correlation coefficient	0.9999
Regression equation	y=0.044x+0.001
Slope	0.044x
Intercept	0.001

Accuracy (Recovery Test)

Accuracy of the method was studied by performing recovery experiments. To perform the recovery experiment, add a known amount of the drugs in the placebo or blank. The solution were prepared in triplicate at three different levels 80 %, 100 % and 120 % of the test concentration using cefuroxime sodium as working standard, and absorbance was measured of each solution. Recovery values ranged from 99.8-100.4 % at 80% recovery, 99.9 %-100.9 % at 100% recovery and 99.6 %-100.2 % for 120 % recovery. (Shown in table 4) The average recoveries at three levels were 100.1 %, 100.3 % and 100.0 % respectively. The recovery results showed that the proposed method had acceptable level of accuracy for cefuroxime sodium.

Table 4: Accuracy by UV-Vis Spectrophotometer.						
% Recovery	Concentration (µg/ml)			% Recovery	Avg. Recovery	% RSD
	Formulation	Drug added	Drug found			
80	10	8	7.98	99.8	100.1	0.24
80	10	8	8.01	100.1		
80	10	8	8.03	100.4		
100	10	10	10.16	100.2	100.3	0.42
100	10	10	9.99	99.9		
100	10	10	10.09	100.9		
120	10	12	11.95	99.6	100.0	0.26
120	10	12	12.01	100.1		
120	10	12	12.02	100.2		

Method Reproducibility (Precision)

The system precision is measured of method variability, by measuring the absorbance of five replicates of the same working solution. The percent relative standard deviation is less than 2. Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study three different solutions of same concentration, 10µg/ml was analyzed for three times in a day i.e. zero hours six hours and 12 hours and the absorbance is measured. From the absorbance obtained %RSD was calculated. In the inter-day precision, solution of same concentration, 10µg/ml was analyzed for three times, percent relative standard deviation for inter-day assay was 1.0 % - 1.4 % and for intra-day assay 1.2- 1.6 % (shown in table 5).

Table 5: Inter-day and intra-day precision of assay by UV-Vis Spectrophotometer			
Inter-day precision			
Parameter	0 Hour	6 Hours	12 Hours
Mean concentration (µg/ml) n = 3	10	10	10
% RSD	1.0	1.3	1.4
Intra-day precision			
Mean concentration (µg/ml) n = 3	10	10	10
% RSD	1.2	1.4	1.6

Limit of Detection and Quantitation

For determination of limit of detection (LOD) and limit of quantitation (LOQ) the method is based on the residual standard deviation of a regression line and slope. To determine the LOD and LOQ, a specific calibration curve was studied using samples containing the analyte in the range of detection limit (DL) and quantitation limit (QL). The limit of detection for cefuroxime sodium was 0.11 µg/ ml and limit of quantitation was 0.39 µg/ ml.

Ruggedness and Robustness

Ruggedness and **r**obustness of the method was determined by carrying out the analysis by two analysts at two different temperatures i.e. at 25 °c and at 20°c. The absorbance was measured and assay was calculated for six times. The percent relative standard deviation of six replicates was less than 2 for both the analyst at the mentioned temperature condition (shown in table 6).

Table 6: Robustness and ruggedness of the method by UV-Vis Spectrophotometer

	% Assay (25**oC)**	% Assay (20**oC)**		% Assay (25**oC)**	% Assay at 20**oC**
Analyst 1	100.7	100.2	Analyst 2	100.2	100.7
	100.5	100.7		100.7	100.9
	100.9	100.3		100.2	100.1
	100.7	100.7		100.9	101.5
	99.2	100.1		100.7	100.9
	100.7	99.9		101.2	99.7
Mean	100.4	100.3	Mean	100.7	100.6
% RSD	0.35	0.71	% RSD	0.14	0.56

Assay of Cefuroxime Sodium (**FORCEF 750 mg).**

Ten injection vials were weighed and mixed properly. A quantity equivalent to 100 mg of Cefuroxime sodium was weighed in to 100 ml volumetric flask. To this flask, 20 ml distilled water was added and sonicate for 5 minutes with continuous shaking, the solution was cooled to ambient temperature and dilute up to mark with the same solvent. The solution was then filtered through whatman filter paper No.41. From the filtrate, appropriate dilutions were made in distilled water to obtain the desired concentration of 10µg/ml. Measure the

absorbance at 275 nm of standard and sample solution of same concentration and calculate the percent purity of sample.

To get better assurance with respect to assay, the test was conducted as per the procedure of assay mentioned in US Pharmacopeia (USP33) by HPLC method in injection formulation. All parameters used to perform the assay was as per the mentioned pharmacopeia (shown in table 8 and Fig.4).The percent relative standard deviation of six replicates assay was less than 2 (shown in table 9).

Table 8: Assay by HPLC (As per USP33 Method)

Sr. No.	% Assay
1	100.6
2	99.9
3	100.9
4	99.6
5	100.5
6	100.1
Mean	100.3
% RSD	0.35

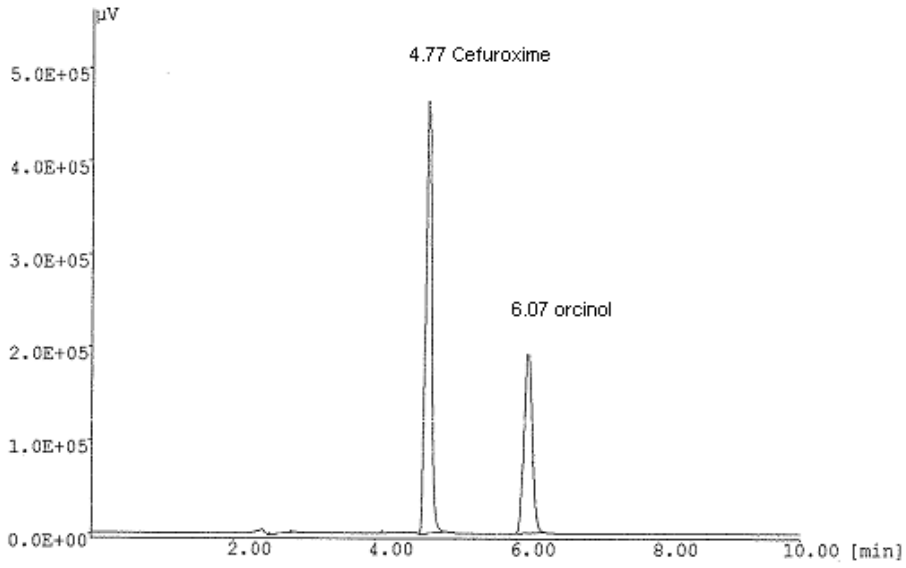

Figure-4: A typical HPLC chromatogram of the injection containing cefuroxime sodium and orcinol as an internal standard.

Table 9: System Suitability Parameters of assay by HPLC Method	
Parameters	Result
Theoretical plates[1]	9199
Resolution	3.46
Tailing factor	0.93
% RSD	0.72
[1] :per column length %RSD: percent elative standard deviation	

Degradation Studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and drug products requires stress testing to elucidate the inherent stability characteristics of the active pharmaceutical ingredients. The purpose of the work was to perform the stress degradation studies of cefuroxime sodium (shown in table 7).

Table 7: Degradation Study by UV-Vis Spectrophotometer			
Sr. No.	Degradation Type	Duration	% Degradation
1	Acid Degradation	90 mim	λmax shifted
2	Alkaline Degradation	90 mim	λmax shifted
3	Oxidative Degradation	30 mim	λmax shifted
4	Thermal Degradation	24 hours	9.7 %
5	Photolytic Degradation	180 min	7.9 %

Acid Degradation (Hydrolytic Degradation under Acidic Condition)

To 1 ml of stock solution (1000 µg/ml) of cefuroxime, 1 ml of 0.1N HCl was added in 100 ml of volumetric flask, the volume was made up with distilled water, the solution was kept at room temperature for the period of 90 minutes. Pipetted 10 ml solution after 90 minute interval, neutralized and diluted with distilled water in 100 ml volumetric flask. Run the uv spectra of the solution between 200 to 400 nm and

calculate the percent of cefuroxime sodium present.

Alkaline Degradation (Hydrolytic Degradation under Alkaline Condition)

To 1 ml of stock solution (1000 µg/ml) of cefuroxime, 1 ml of 0.1N NaOH was added in 100 ml of volumetric flask, the volume was made up with distilled water, the solution was kept at room temperature for the period of 90 minutes. Pipette out 10 ml solution after 90 minute interval, neutralized and diluted with distilled water in 100 ml volumetric flask. Run the uv spectra of the solution between 200 to 400 nm and calculate the percent of cefuroxime sodium present.

Oxidative Degradation

To 1 ml of stock solution (1000 µg/ml) of cefuroxime, 1 ml of 30 % V/v H2O2 added in 100 ml of volumetric flask, the volume was made up with distilled water and the solution was kept at room temperature for the period of 90 minutes. Pipetted 10 ml solution after 90 minute interval and diluted with distilled water in 100 ml volumetric flask. Run the uv spectra of the solution between 200 to 400 nm and calculate the percent of cefuroxime sodium present.

Thermal Degradation

Sample of cefuroxime was kept in a Petri dish and exposed at a temperature of 70°c for 24 hours in an oven. After 24 hour weigh and transfer accurately about 100 mg of cefuroxime sodium and diluted with distilled water. Prepared a solution having final concentration of 10 µg/ml of cefuroxime sodium from the above solution. Run the uv spectra of the solution between 200 to 400 nm and calculate the percent of cefuroxime sodium present.

Photolytic Degradation

Sample of cefuroxime was exposed under ultra violet lamp in photostablity chamber providing illumination of not less than 1.2 million lux per hour. Weigh and transfer accurately about 100 mg of cefuroxime sodium and diluted with distilled water. From the above solution prepared a solution having final concentration of 10 µg/ml of cefuroxime sodium. Run the uv spectra of the solution between 200 to 400 nm and calculate the percent of cefuroxime sodium present.

Result and Discussion

The developed method was found to be precise as the %RSD values for intraday and inter-day were found to be less than 2%. Good recoveries (100.0 % to 100.3 %) of the drug were obtained at each concentration level, indicating that the method was accurate. Assay of cefuroxime sodium by UV Spectrophotometer and HPLC was very close to each other 100.1% and 100.3%.Hence the developed method was also found to be specific.

Solution Stability

The stability of the sample solutions was performed at intervals of zero hour, 6 hours and 12 hours. The stability of solution was determined in terms of the assay of the drugs in sample solutions against the freshly prepared standard solutions. The relative standard deviation for the assay values determined up to 12 hours. The relative standard deviation is less than 2 % up to 12 hour. The results indicate that the solutions were stable to 12 hours at an ambient temperature.

Conclusion

The proposed method development and validation of UV-Vis Spectrophotometric method was to determination of cefuroxime sodium in the pharmaceutical dosage form and bulk drug substances. The developed method was validated and shown accurate, precise and cost effective. It can be used in the quality control department for the estimation of assay of titled drug in pharmaceutical dosage form and in cleaning validation.

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