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Research Article

DEVELOPMENT AND VALIDATION OF UV AND RP-HPLC METHODS FOR THE ESTIMATION OF CEFTRIAXONE SODIUM IN PHARMACEUTICAL FORMULATIONS

Shanta K Adiki[1]*, Baishakhi Dey[2], Nureddin AA Arebi[3], Fauzi El Melad[3], Prakash Katakam[3], Babu Rao Chandu[3]
[1]Nirmala College of Pharmacy, Guntur, India
[2]School of Medical Science and Technology, IIT Kharaghpur, India
[3]Faculty of Pharmacy, University of Zawia, Az Zawiyah, Libya
*Corresponding Author Email: pkatakam9@gmail.com
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ABSTRACT
The current research paper reports a validated UV and RP-HPLC method for routine estimations of CFTX in bulk and unit dosage formulations. For the UV estimation of CFTX, using ammonium acetate buffer as the solvent, λ_{max} was set at 241.5 nm and linearity range obtained in the concentration range of 2–10 µg/mL. The optimized RP-HPLC conditions for CFTX estimation were obtained with isocratic separation mode in a C_{18} inertsil column (150 mm × 4.6 mm, 3 µm) using a degassed mixture of buffer: methanol in the ratio of 74:26, injection volume (20 µL), flow rate (1 mL/min) and run time (20 minutes), at ambient column temperature with UV detector set at 254 nm. The linearity of the method was demonstrated over the concentration range of 80–120 µg/mL. The percent assay of CFTX determined by UV and RP-HPLC method were 99.8 ± 0.001 and 101.5 ± 0.001 respectively. The recovery CFTX determined by UV and RP-HPLC were 99.6–99.8 % and 101.1 % respectively with % RSD values of peak areas 0.2 and 0.3 respectively. Values of all other parameters of method validations in both methodologies were within the acceptance limits.
Keywords: Ceftriaxone, Dosage formulations, UV estimation, RP-HPLC method, validation.

INTRODUCTION

With the advancements in pharmaceutical researches, adoption of novel drug delivery technologies, development of both small and large scale pharmaceutical industries worldwide, the number of drugs and drug formulations are increasing in the market day by day which may be entire new entities or partial modifications of the existing drugs or novel dosage formulations[1–3]. Analytical method development plays a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities and simultaneously an integral part of pre formulation and formulation development research. Quality assurance and quality control departments of Pharmaceutical industries are largely responsible in bringing out safe, effective dosage formulations. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of analytical methodologies which are simple, rapid, cost effective and robust and provide results with great accuracy and precision. Sophisticated hyphenated techniques are in vogue but they are relatively expensive; many methods necessitate analyte extraction from respective sample matrices and hence complicated sample preparation steps, become time consuming, difficult in operation and error in recovery. More rapid, robust and precise the developed analytical methodologies are, more are the chances to bring newer products to the international markets faster[4–8]. Ceftriaxone (CFTX), chemically known as disodium (6R, 7R) -3[(acetyl-oxy) methyl] -7-[(2Z) - (2-amino- 4- thiazolyl) (methoxy amino) - acetyl] amino] -8- oxo- 5-thia-1- azabicyclo [4.2.0.] Oct- 2- ene- 2- carboxylic acid, is a cephalosporin β-lactam antibiotic used in the treatment of bacterial infections caused by susceptible usually gram-positive organisms (Figure 1). Bactericidal activity of CFTX is mostly by inhibition of mucopeptide synthesis in the bacterial cell wall and by binding to one or more of the penicillin-binding proteins (PBPs) which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus hindering cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested[9–11]. Literature surveys have shown that the UV and HPLC methods developed estimates CFTX mostly in combined formulations[1–8,12–21]. However the current research aims to develop a validated UV and RP-HPLC method for routine estimations of CFTX alone in bulk and unit dosage formulations.

MATERIALS AND METHODS

Chemicals and reagents

Pure ceftriaxone sodium was gratis sample from Medreich Ltd, Hyderabad, India. HPLC grade water, methanol, AR grade ammonium acetate, ortho-phosphoric acid were purchased from Merck, Mumbai, India.

Instrumentation

HPLC (Shimadzu model, LC 10 ADVp, Japan with UV detector); UV-visible spectrophotometer (Thermo Scientific, Aquamate Plus, India), Electronic balance (Shimadzu, Japan), Sonicator (Cyber labs, India), pH meter (Datla instruments, DI-45, India)

UV method development

For the UV estimation of CFTX, ammonium acetate buffer was used as the solvent for study. Standard and stock solutions of CFTX were prepared using ammonium acetate buffer as the solvent. A solution of 10 µg/mL concentration was scanned in 200–400 nm wavelength range and the λ_{max} was set at 241.5 nm. The standard calibration curve was prepared with aliquots in the concentration range of 2–10 µg/mL using buffer as the blank.

Validation of the method

The above method was validated as per ICH guidelines in terms of linearity, accuracy, precision, robustness, LOD and LOQ[22]. The linearity range for the estimation of CFTX by UV was determined by preparing aliquots in the concentration range of 2–10 µg/mL and absorbance's measured at 241.5 nm. Calibration curves (concentrations vs absorbance) were plotted and R^2 value not less than 0.99 was regarded as acceptance criterion. Accuracy of the proposed method was ascertained by recovery studies using standard addition method where known quantity of standard drug was mixed with formulation sample in concentrations of 50, 100 and 150 % and percent recovery for CFTX in the range of 95–105 % were set as the acceptance criterion. The precision was studied by inter and intra-day variations in the test method of CFTX and expressed as percent relative standard deviation (% RSD) where these values should not be greater than 2 %. Repeatability or system precision was studied by measuring absorbance of standard solution (prepared as per test method) five times. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the process parameters. As a measure of robustness of the method, intentional alterations were made in the absorbance maximum to evaluate the impact of the method. A 4 µg/mL of CFTX was prepared by above procedure and the absorbance was observed at 239.5 and 243.5 nm[22].

Assay of CFTX

The equivalent weight of marketed brands of CFTX with a labeled claim of 1000 mg was determined accurately. 59.81 mg of CFTX powder equivalent was dissolved in buffer and diluted subsequently to prepare stock and standard solutions. Absorbance of a 4 µg/mL solution of CFTX was measured at 245.1 nm using buffer solution as blank and the amount of CFTX was determined using the formulae:

$$\text{Amout present} = \frac{\text{Test OD} \times \text{Standard Conc} \times \text{dilution factor}}{\text{Standard OD}}$$

$$\% \text{ Purity} = \frac{\text{Amount present}}{\text{Label claim}} \times 100$$

Estimation of CFTX by RP–HPLC

The current research represents a new stability indicating, validated RP–HPLC method development.

Optimization of mobile phase

Before proceeding to the optimized RP–HPLC chromatographic conditions for CFTX estimations, three trails were conducted with different mobile phase compositions. Amongst three trials injection volume (20 µL), flow rate (1 mL/min), and detector wavelength (254 nm) were kept constant. Run time varied between 20–30 minutes. Using buffer: methanol (70:30) as mobile phase, a long retention time of 30 minutes with peak tailing was observed. On changing buffer: methanol ratio to 80:20, peak shape and resolution was not satisfactory. But with buffer: methanol ratio 74:26, satisfactory peak shape with optimum resolution was achieved. The optimized chromatographic conditions for CFTX estimation were obtained with isocratic separation mode in a C_{18} inertsil column (150 mm × 4.6 mm, 3 µm) using a degassed mixture of buffer: methanol in the ratio of 74:26, injection volume (20 µL), flow rate (1 mL/min) and run time (20 minutes), at ambient column temperature with UV detector set at 254 nm. The working standard solution was prepared by dissolving 30 mg of CFTX in 50 mL mobile phase, well sonicated, filtered through 0.45 µ or finer porosity membrane filter and further volume adjustments made with the mobile phase. The sample solution was prepared by mixing 250 mg tablet weight equivalents of CFTX taken in 100 mL volumetric flask, to which 50 mL mobile phase is added, sonicated, filtered through 0.45 µ filter and volume adjusted with mobile phase.

System suitability

The CFTX standard solution (20 µL) was injected into the HPLC system six times to evaluate the system suitability parameters from standard chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections. The limit of % RSD and tailing factor were set below 2.0 %. The theoretical plate count was considered if less than 2000.

Method Validation

The proposed RP–HPLC method was validated as per ICH guidelines[22]. Linearity of the method was determined using solutions prepared in the concentration range of 80–120 % from CFTX working standard. Accuracy of the proposed method was ascertained by recovery studies using standard addition method, where the drug substance was spiked with placebo in concentrations of 75, 100 and 125 % and the acceptable criteria of percent recovery for CFTX was set between 95–105 %. Precision was studied in terms of repeatability (system precision), where 20 µg/mL of standard solution was injected for six times into the HPLC system as per test procedure. For method precision, from sample and stock solution, six replicates of standard and sample of 20 µg/mL were prepared and injected into the HPLC system and % RSD was calculated. Intermediate precision study or ruggedness of experimentation was carried out by different analyst, on different instrument and on different days. From the sample and stock solutions, six replicates of 20 µg/mL were prepared and injected into the HPLC system and % RSD was calculated. As a measure of robustness of the method, deliberate alterations in the flow rate (1.4 mL/min and 1.6 mL/min) was made to evaluate the impact of the method. The tailing factor of CFTX standard, % RSD of asymmetry and retention time of CFTX standard should not be more than 2 % due to the intentional alterations in the flow rate. The stability of the analytical solutions being used for analysis was determined by analyzing the homogenous sample solution at room temperature and 2–8°C at 6 h intervals and % RSD values of the results calculated with the specification limit that final assay value should be within 2 % of the initial value. Thus the final assay values of both the standard and sample solutions after 24 h lie within the specified limits[22].

Assay of CFTX

Prior to the injection of drug (CFTX) solutions, the column was equilibrated for at least 30 minutes with the mobile phase with a flow rate of 1 mL/min. Then 20 µL of standard and sample solutions were injected for six and two times respectively and the drug content was determined using the formula:

$$\text{Assay}\,\% = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label claim}} \times 100$$

Where, AT is absorbance of test, AS is absorbance of standard, DS is dilution factor of standard, DT is dilution factor of test and P is potency of standard

RESULTS AND DISCUSSION

UV spectroscopy

The results of linearity parameters of CFTX estimated by UV spectroscopy are summarized in Table 1. The percent assay of CFTX determined by UV spectroscopy was found to be 99.8 ± 0.001. Results of UV spectroscopic method validations in terms of accuracy (recovery studies), precision (inter and intraday, repeatability), and robustness are provided in Table 2. The results of UV spectroscopy showed high linearity correlation coefficient ($R^2 = 0.99$). The percent assay of CFTX was found appreciable. Since there was no interference, the methods can be said to be specific. High values of percent recovery, and the % RSD values of accuracy, precision and robustness all being within the specified acceptance limits the developed UV spectroscopic method was found to be simple, rapid, specific, accurate, and precise with enough robustness to be applied in the routine estimations of CFTX.

RP-HPLC

The linearity of the method was demonstrated over the concentration range of 80–120 µg/mL with R^2 value not less than 0.99 and RSD of peak areas of the solution not more than 2.0 %. Percent assay of CFTX was found 101.3 ± 0.001 which is appreciable. There was no interference due to blank and placebo at the retention time of the analyte. The recovery of the spiked drug was found 100.1 % with 0.3 % RSD. Regarding precision, the system precision or repeatability of the method showed 0.29 % RSD for the peak areas. Method precision, for intra and inter day assay showed 0.3 % and 0.2 % RSD for peak areas. For intermediate precision or ruggedness of the method, 0.33 % RSD was found for the peak area. Thus all parameters of precision studies were less than 2.0. Results of robustness of the method as determined by deliberate alterations in the flow rate (1.4 mL/min and 1.6 mL/min) showed 0.2 % RSD for peak areas. Thus alterations in flow rate didn't have any effect on the system suitability. A summary of all method validation parameters of the RP-HPLC method was presented in Table 3. All parameters of method validation studies lying within the specified acceptance limits, the developed RP-HPLC method can be applied for routine estimations of CFTX. Results of stability studies of sample solutions at room temperature and at 2–8°C with initial assay value of 101.3 % showed at difference of 0.99 and 1.5 respectively after 24 h interval and standard solutions with initial assay value of 89.8 % showed a difference of 1.7 in both cases after 24 h interval. All parameters of method validation studies lying within the specified acceptance limits, the developed RP-HPLC method can be regarded as accurate, precise, and specific with enough robustness to be applied for routine estimations.

Table 1: Linearity data of ceftriaxone by UV spectroscopy

Parameters	UV method
95 % confidence intervals	
Slope	0.07811 to 0.08729
y-intercept	–0.02504 to 0.03584
x-intercept	–0.4564 to 0.2884
Goodness of Fit	
R^2	0.99
P value	0.0001
Equation	Y = 0.0827*X + 0.005400
Best fit values	
Slope	0.0827 ± 0.001442
y-intercept	0.005400 ± 0.009567
x-intercept	–0.06530

Table 2: Validation results of ceftriaxone by UV spectroscopy (n = 6)

Parameters	Recommended limits	UV method
Specificity	No interferences	[c]
Precision[a] Inter day, % ([a]) Intraday, % ([a])	NMT[b] 2.00	 99.7(0.2) 99.8(0.3)
Repeatability[a]	NMT[b] 2.00	99.8(0.2)
Accuracy, % ([a])	95–105 % (2.00)	99.6–99.8 (0.2)
Robustness[a], (wave length, nm)	NMT[b] 2.00	0.1 (239.5) 0.3 (243.5)

[a]Percentage relative standard deviation, [b]Not more than, [c]Specific nature of method

Table 3: Validation parameters of ceftriaxone by RP-HPLC (n = 6)

Parameters	Recommended limits	RP-HPLC method
Specificity	No interferences	[c]
Precision[a] Inter day Intraday	NMT[b] 2.00	 0.2[a] 0.3[a]
Repeatability[a]	NMT[b] 2.00	0.29[a]
Ruggedness		0.33[a]
Accuracy, % ([a])	95–105 % (2.00)	100.1 (0.3)[a]
Robustness[a], (Flow rate, mL/min)	NMT[b] 2.00	0.2[a] (1.4) 0.2[a] (1.6)

[a]Percentage relative standard deviation, [b]Not more than, [c]Specific nature of method

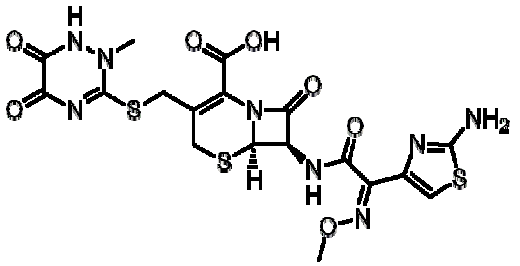

Figure 1: Structure of ceftriaxone

CONCLUSION

The UV and the RP-HPLC method developed were found to be simple, rapid and economical. Moreover both the methods are well validated in terms of accuracy, precision, linearity, and robustness. Thus the two methods can be used for the routine quality control analysis of Ceftriaxone in bulk drugs and pharmaceutical dosage forms both by small and large scale pharmaceutical industries.

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