Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2018, 10(3): 151-157
(http://www.derpharmachemica.com/archive.html)

Development and Validation of UV Spectrophotometric Method for the Estimation of Ceftriaxone Sodium in Nanoparticles

Majani Ayushi V, Paradkar Mansi U*

Ramanbhai Patel College of Pharmacy, Charotar University of Science and Technology (CHARUSAT), Changa, Dist. Anand, Ta. Petlad, Gujarat-388421, India

ABSTRACT

The present work describes the simple, accurate and validated UV spectrophotometric method for determination of ceftriaxone sodium loaded in Nanoparticle formulation. The method was validated for different parameters like linearity, precision, specificity, accuracy, Limit of Detection (LOD), Limit of Quantification (LOQ) and Robustness as per ICH guidelines. A wavelength maximum of Ceftriaxone sodium in distilled water was found to be at 241 nm. The method was found to be linear in the range of 2 to 18 µg/ml with a correlation coefficient (r^2) of 0.999. The accuracy of the method was studied by recovery study and % recovery was found in range of 99.27 to 100.06%. The method is simple, accurate and requires relatively inexpensive instrument. The proposed method can be successfully used for determination of Ceftriaxone sodium loaded in to Nanoparticle formulation.

Keywords: UV spectrophotometry, Ceftriaxone sodium, %Assay, Chitosan nanoparticles

INTRODUCTION

Ceftriaxone sodium is a third generation, semi synthetic cephalosporin antibiotic. Cephalosporins are derivatives of 7-aminocephalosporic acid and are closely related to penicillins in structure. Ceftriaxone sodium is a long acting, broad-spectrum cephalosporin antibiotic for parenteral use. It is effective in treatment of infections caused by accurate bacteria, blood poisoning, meningitis etc. The bactericidal activity of ceftriaxone sodium results from inhibition of cell wall synthesis. It exerts *in vitro* activity against a wide range of Gram-negative and Gram-positive microorganisms. It is highly stable to most beta-lactamases, both penicillinases and cephalosporinase of Gram-positive and Gram-negative bacteria [1].

Ceftriaxone sodium is chemically known as, (Z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetylamido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thiamethyl]-3-cephem-4-carboxylic acid, disodium salt. Ceftriaxone contains a highly acidic, heterocyclic system on the 3-thiamethyl group. This unusual dioxotriazine ring system is believed to confer the unique pharmacokinetic properties of this agent [2].

There are very much significant pharmacological activities exhibited by ceftriaxone sodium, several researchers have focused on the development of various analytical methods to determine ceftriaxone sodium in different matrices such as plasma, urine and saliva [3,4]. The commonly used methods are High Performance Liquid Chromatography (HPLC), spectrofluorimetric and chemilumescence has also been described [2,3,5]. As an alternative to HPLC assays, gas chromatography has also been proposed [6].

In this study, the UV spectrophotometric method was developed and validated for the determination of ceftriaxone sodium loaded into the nanoparticles by evaluating its assay.

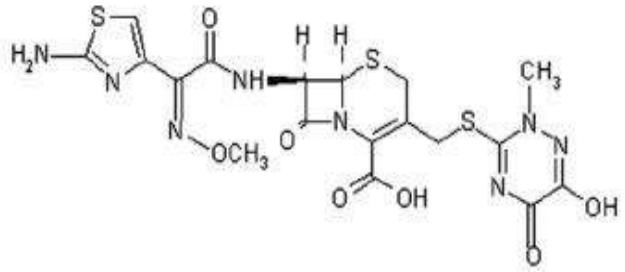

Figure 1: Chemical structure of ceftriaxone sodium

MATERIALS AND METHODS

Equipment

UV- VIS double beam spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Japan).

Materials

Ceftriaxone sodium was procured as a gift sample from JFL Life sciences Ltd, Ahmedabad, India. Chitosan used as the polymeric material was purchased from Sigma Aldrich (USA). Sodium Tripolyphosphate (TPP) and Glacial acetic acid were purchased from Loba Chemicals (Mumbai, India) respectively. All other reagents used were of analytical or equivalent grade.

Selection of wavelength maximum (λ_{max})

In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, a spectrum of 10 µg/ml was recorded using UV-Visible spectrophotometer by scanning in the range of 200 nm to 400 nm against distilled water. The λ_{max} of the drug was noted. The absorption curve showed characteristic absorption maxima at 241 nm for ceftriaxone sodium.

Preparation of stock solution

Accurately weighed (10 mg) of ceftriaxone sodium was transferred to 100 ml amber colored volumetric flask. Small quantity of distilled water was added to ensure complete solubilization of drug and finally volume was made up to the mark with distilled water to produce 100 µg/ml solution.

Preparation of working standard solutions

With the help of pipette, 0.1 ml aliquot was withdrawn from stock solution and transferred to 10 mL amber colored volumetric flask. It was then diluted up to 10 ml with distilled water to produce 1 µg/mL solution. Similarly solutions of concentration 2, 6, 10, 14 and 18 µg/ml were prepared which were used for the construction of calibration curve.

Preparation of ceftriaxone sodium loaded in nanoparticles [7-9]

The formulation was prepared by Ionic crosslinking method. 2 mg/ml chitosan was dissolved in 2% glacial acetic acid solution. 1 mg/ml Sodium Tripolyphosphate (TPP) and drug was added in 10 ml of double distilled water. TPP solution was added to chitosan solution drop by drop at 0.5 ml/min under constant stirring at 800 rpm over magnetic stirrer. After complete addition of TPP solution, chitosan dispersion was allowed to cross linked for 100 min.

Preparation of nanoparticle test solution

Nanoparticle dispersion equivalent to 10 mg ceftriaxone sodium was weighed and transferred to 100 ml amber colored volumetric flask separately and the volume was made up to 100 ml with distilled water to produce 100 µg/ml. 1 ml of test solution was diluted up to 10 ml with distilled water and the absorbance of test solution (10 µg/ml) was recorded against distilled water as a blank at 241 nm.

Method validation [7,8]

Validation can be defined as establishing documented evidence which provides a high degree of assurance that a particular method will consistently produce a product meeting its predetermined specifications. The method was validated for different parameters like linearity, precision, accuracy, specificity, ruggedness, robustness, limit of detection, limit of quantification and robustness as per the ICH guidelines.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. For linearity study, six solutions of different concentration (2, 6, 10, 14 and 18 µg/ml) were prepared from the stock solution by withdrawing aliquots with the help of pipette and transferring to separate 10 ml amber coloured volumetric flasks and making volume up to the mark with distilled water. The absorbance of the solutions was measured at 241 nm. A graph of concentration versus absorbance was plotted and correlation coefficient (r^2) was calculated.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intra-day and inter-day precision. Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability of the method was determined by analyzing six samples of same concentration of drug. For intra-day and inter-day precision studies six solutions of different concentration (2, 6, 10, 14 and 18 µg/ml) were prepared and analyzed three times a day and the same procedure was followed for next two days. The results were reported in terms of % standard deviation, % RSD.

Specificity

The specificity of an analytical method represents its ability to assess unequivocally the analyte in presence of components which are expected to be present. It was checked by comparing the spectra of standard drug solution and nanoparticle test solution.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery study was performed by standard addition method at three levels i.e. 80%, 100% and 120%. At each level, the determination was done in triplicate and the amount of drug recovered was calculated.

Limit of Detection (LOD)

It is the lowest concentration of analyte in sample that can be detected but not necessarily quantified. It was calculated based on standard deviation of response and slope of the curve using following equation:

$$LOD = 3.3\ \sigma/s$$

Where, σ-standard deviation of the response, s-Slope of curve.

Limit of Quantification (LOQ)

It is the minimum concentration of analyte that can be quantified with suitable precision. It was calculated using following equation:

$$LOQ = 10\ \sigma/s$$

Where, σ-is standard deviation of response, s-Slope of curve.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was done by measuring absorbance of a 10 µg/mL solution at detection wavelength 241 ± 2 nm. Each measurement was done in triplicate.

Solution stability

For stability study solution was stored at room temperature for 24 h. The initial absorbance and absorbance at 24 h was measured and the difference was noted. The similarity factor was calculated as follows:

$$\text{Similarity factor} = \frac{\text{Absorbanceofinitialsolution}}{\text{Absorbanceofsolutionafter 24 h}}$$

RESULTS AND DISCUSSION

Selection of wavelength maximum (λ_{max})

The UV spectrum of ceftriaxone sodium in distilled water has maximum absorption (λ_{max}) at 241 nm as per shown in Figure 2. The absorbance of excipients in Nanoparticle solution did not interfere with ceftriaxone sodium. As a result, 241 nm wavelengths was selected for quantitative analysis and validation of ceftriaxone sodium in nanoparticles.

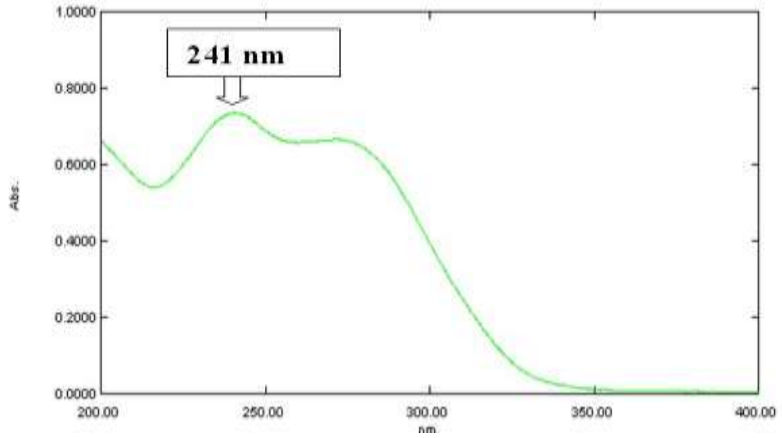

Figure 2: UV spectrum of 10 µg/ml ceftriaxone sodium in distilled water

Linearity

The drug obeyed Beer-Lambert's law in the concentration range of 2-18 µg/ml with regression 0.999 at 241 nm with %RSD < 2% shown in Table 1. Overlay spectra of Ceftriaxone sodium are shown in Figure 3 and calibration curve is shown in Figure 4.

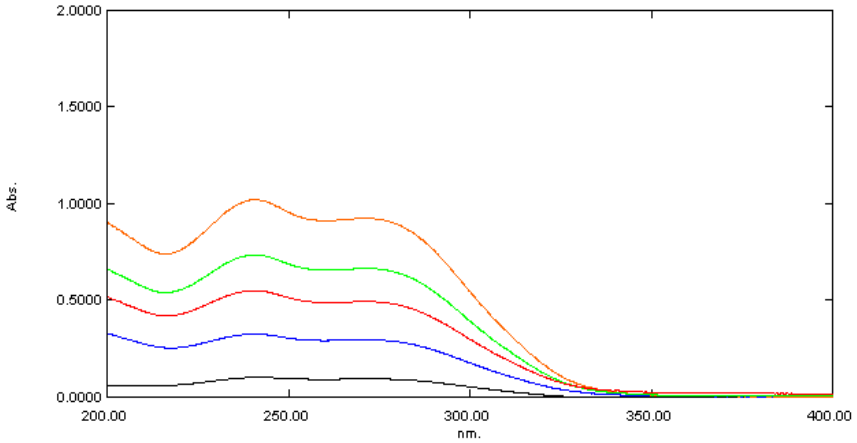

Figure 3: Overlay of the spectra of ceftriaxone sodium (2-18 µg/ml)

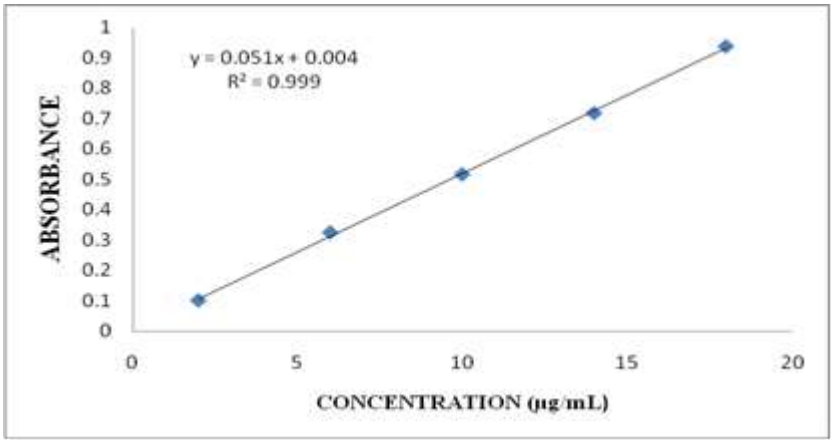

Figure 4: Linearity curve of ceftriaxone sodium (2-18 µg/ml)

Table 1: Linearity of ceftriaxone sodium (2-18 µg/ml) (λ_{max}: 241 nm)

Concentration (µg/ml)	Mean response ± SD (n=6)	%RSD
2	0.1028 ± 0.00097	0.9838
6	0.3263 ± 0.00487	1.5099
10	0.517 ± 0.005	0.58164
14	0.7187 ± 0.00843	1.17901
18	0.9381 ± 0.00236	0.25284
	Linearity Equation	y=0.051x + 0.004
	Correlation Coefficient	0.999
	Slope	0.051
	Intercept	0.004

Precision

The developed method was found to be precise as the %RSD values for intraday (Table 2.1) and interday (Table 2.2) precision were found within limit (< 2%). Repeatability expresses the precision under the same operating conditions over a short interval of time. So, as per Table 3 this method is repeatable.

Table 2: Precision

2.1. Intraday precision					
Conc. (µg/ml)	Absorbance	Concentration (µg/ml)	Mean absorbance ± SD (n=6)	Mean concentration ± SD	%RSD
2	0.1026	1.9333	0.1028 ± 0.0009	1.937 ± 0.0190	0.9838
	0.1039	1.9588			
	0.102	1.9215			
10	0.5169	10.0568	0.5170 ± 0.005	10.124 ± 0.0588	0.5816

	0.5221	10.1588			
	0.5121	10.1588			
18	0.810	18.3333	0.9381 ± 0.0023	18.317 ± 0.0463	0.2528
	0.805	18.3529			
	0.798	18.2647			

2.2. Interday precision					
Conc. (µg/ml)	**Absorbance**	**Conc.(µg/ml)**	**Mean absorbance ± SD**	**Mean concentration ± SD**	**%RSD**
2	0.105	1.9803	0.1050 ± 0.0009	1.9801 ± 0.02	1.0100
	0.106	2			
	0.1042	1.96			
10	0.5123	9.9745	0.5070 ± 0.0048	9.8659 ± 0.0997	1.0107
	0.5061	9.845			
	0.5027	9.7784			
18	0.9402	18.3568	0.9256 ± 0.0039	18.2673 ± 0.0775	0.4247
	0.9335	18.2254			
	0.9332	18.2196			

Table 3: Repeatability

Repeatability			
S. No.	**Conc.(µg/ml)**	**Absorbance**	**Conc.(µg/ml)**
1	10	0.5129	9.9784
2		0.5225	10.1666
3		0.5199	9.9588
4		0.5243	10.2019
5		0.5174	10.0666
6		0.5214	10.1450
	Mean	0.5197	10.0862
	S.D.	0.0040	0.1015
	R.S.D.	0.7860	1.0066

Specificity

The excipients in nanoparticles did not interfere with absorbance of ceftriaxone sodium which indicates that the method is specific as shown in Figure 5.

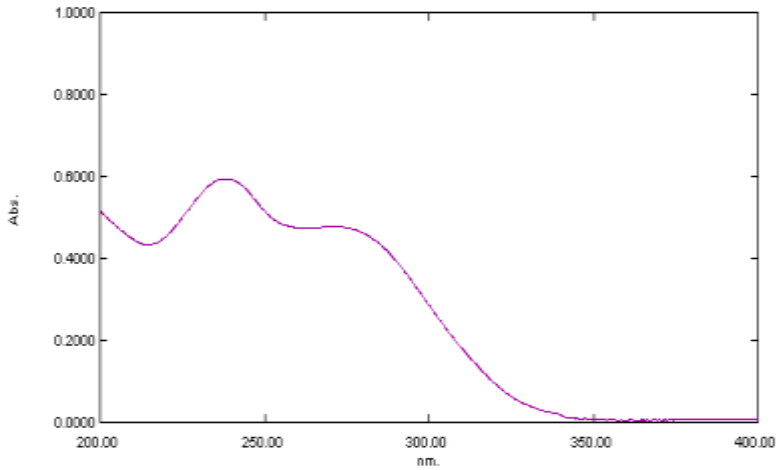

Figure 5: Spectra of ceftriaxone sodium in nanoparticle formulation accuracy

Recovery study shows that the overall % recovery was found to be 99.83% as per in Table 4. So, this method is accurate.

Table 4: Accuracy

Accuracy

Level of recovery	Sample concentration (µg/ml)	Std. Added (µg/ml)	Total amount (µg/ml)	Absorbance	Amount recovered	%Recovery	Mean % recovery
80	5	4	9	0.4592	8.9254	99.17	99.27
	5	4	9	0.4599	8.9392	99.32	
	5	4	9	0.46	8.9411	99.34	
100	5	5	10	0.5122	9.0647	99.64	99.73
	5	5	10	0.513	9.0647	99.8	
	5	5	10	0.5128	9.9803	99.76	
120	5	6	11	0.569	11.078	100.7	100.6
	5	6	11	0.5684	11.0666	100.6	
	5	6	11	0.5639	11.05686	100.51	

LOD

LOD calculated using the equation as mentioned earlier is 0.336805 µg/ml.

LOQ

LOQ calculated using the equation as mentioned earlier is 1.020621 µg/ml.

Robustness

The method was found to be robust when checked for the effect of change in detection wavelength (Table 5).

Table 5: Robustness

Robustness						
Concentration (µg/ml)	Absorbance		Concentration (µg/ml)		%Assay	
	239 nm	243 nm	239 nm	243 nm	239 nm	243 nm
10	0.5094	0.512	9.9098	9.9607	99.09	99.6
	0.5102	0.5108	9.9254	9.9372	99.25	99.37
	0.5099	0.5106	9.9196	9.9333	99.19	99.33
Average	0.5098	0.5111	9.9182	9.9437	9.9437	99.1766
S.D.	0.0004	0.0007	0.0078	0.0148	0.0148	0.0808
RSD	0.0792	0.0792	0.0794	0.1490	0.1490	0.0815

Solution stability

The stability of solution was evaluated by determining similarity factor (0.995) which was found within the acceptance criteria of 0.98-1.02. The developed method was found to be precise, specific and accurate (Table 6).

Table 6: Solution stability

Solution stability							
Concentration (µg/ml)	Initial absorbance	Initial concentration (µg/ml)	Initial assay (%)	Absorbance after 24 h	Concentrate-ion after 24 h	%Assay after 24 h	Difference in % assay
10	0.5067	9.8568	98.56	0.5095	9.9117	99.11	0.55
	0.5059	9.8411	98.41	0.5079	9.8803	98.8	0.39
	0.5053	9.8294	98.29	0.5069	9.8607	98.6	0.31

The overall summary of validation parameters is shown in Table 7.

Table 7: Summary of validation parameters

Summary of validation parameters	
Parameter	**Ceftriaxone sodium loaded nanoparticles**
λ_{max}	241 nm
Linearity	2 to 18 µg/ml
Equation	y=0.051x + 0.004
R^2	0.999
LOD	0.336805
LOQ	1.02062
Repeatability (%RSD, N=6)	0.7860
Intraday precision (%RSD, N=3)	0.6060
Interday precision (%RSD, N=3)	0.8151
% Recovery	99.83
Standard solution stability (Similarity factor)	0.995

CONCLUSION

The proposed UV-Spectrophotometry method for estimation of ceftriaxone sodium in pharmaceutical dosage form that is nanoparticles was successfully developed and validated for its intended purpose. The method was shown to be linear, precise, repeatable, specific, accurate, robust and stable. Therefore it can be used for the determination of ceftriaxone sodium loaded into Nanoparticles.

ACKNOWLEDGEMENT

We are thankful to JFL Life sciences Ltd, (Ahmedabad, India) for providing the gratis sample of Ceftriaxone sodium. We are also thankful toRamanbhai Patel College of Pharmacy, CHARUSAT for providing us the required facilities.

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