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Research Article

Development and Validation of a Visible Spectrophotometric Method for Ceftriaxone using Ninhydrin

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Abstract

Procedures for the cephalosporine antibiotic, Ceftriaxone, were devised and validated to be simple, accurate, and exact. It is based on a visible spectrophotometric analysis of Ceftriaxone using Ninhydrin, which reacts with Ceftriaxone's aromatic amino group to form a purple chromogen. The reaction at 90˚C for 30 minutes produced a chromogen with a λmax of 568.5 nm. Beer-Lambert's law applies to 10 to 100 µg/ml concentrations. The limit of detection (LOD) and limit of quantification (LOQ) values were 1.54 and 4.67 µg/mL, respectively. This approach was validated in accordance with ICH criteria, and the results indicated that the procedures were appropriate for routine quality control of pharmaceuticals in bulk medications and pharmaceutical formulations.

Keywords: Ceftriaxone; Ninhydrin; Colorimetric Method; Spectrophotometric Validation; LOQ; LOD

Abbreviations

API: Active Pharmaceutical Ingredient; LOD: Limit of Detection; LOQ: Limit of Quantification; HPLC: High Performance Liquid Chromatography; LC-MS/MS: Liquid Chromatography–Mass Spectrometry/Mass Spectrometry

Introduction

Ceftriaxone is an important antibiotic drug that has been used as a prominent drug for a number of bacterial infectious diseases for more than 30 years since it's discovery in 1980s by Hoffmann-La Roche. It's a third-generation cephalosporin antibiotic with a chemical structure as shown in figure 1. It is effective in respiratory, urinary, cutaneous, and soft tissue infections by blocking bacterial cell wall formation [1]. There are some reported analytical methods available in literature and they are based on UV spectrophotometric [2-4], colorimetric [5], HPLC [6-8], UPLC [9,10], and LC-MS/MS techniques [11,12].

Although these methods are accurate, they frequently necessitate advanced instrumentation or difficult procedures where visible spectroscopic methods are a good option. In visible spectroscopic methods a colourless compound is selectively cconverted to a coloured moiety using chromogenic reagents which will react with the specific functional groups on the analyte. This process enhances the specificity and selectivity of the analytical process and hence preferred over UV spectroscopic methods. This method is also called as colorimetry. Since very less visible colorimetric techniques are available for ceftriaxone, we have tried to develop a new

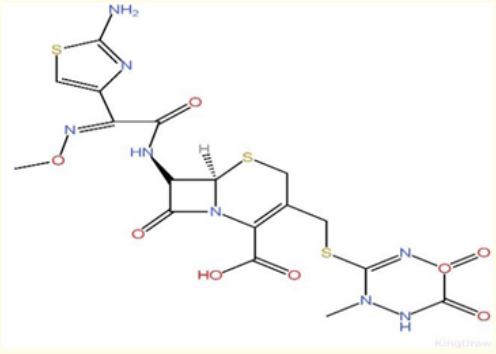

Figure 1: Structure of Ceftriaxone (drawn with chemdraw software).

method based on its reaction with the ninhydrin reagent. The current study, therefore, develops and evaluates a sensitive and cost-effective ninhydrin-based colorimetric technique in compliance with ICH Q2(R1) recommendations [13].

Materials and Methods

Materials

Ceftriaxone is a pure medication (API) obtained as gratis sample from Covalent Laboratories, Hyderabad. Ninhydrin and methanol were procured from s d fine-chem Ltd, Mumbai.

Instruments

UV – Visible Spectrophotometer Shimadzu UV-1800 is used for the spectral recordings.

Experimental method

Preparation of 5% ninhydrin reagent solution

Accurately weighed 1.25 g of Ninhydrin and dissolved in sufficient methanol to produce 25 mL.

Preparation of standard solution

To create the standard solution (1000 µg/mL), 10 mg of Ceftriaxone was dissolved in 10 mL of water.

Preparation of standard solution

The normal stock solution of Ceftriaxone was made by dissolving 10 mg in 10 ml of water.

Optimization method

Impact of the ninhydrin reagent's concentration

A series of 10 mL volumetric flasks was filled with 0.1 mL of the standard stock solution of Ceftriaxone. Different volumes of 5% ninhydrin reagent (0.5, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5%) were added to each flask and stirred. After 30 minutes of immersion at 90^0C in a water bath, the flasks were allowed to cool to room temperature, and their water volumes were adjusted to 10 mL each. At 568.5 nm, the absorbance of the resultant solutions was measured in comparison to a blank.

The impact of temperature

A series of 10 mL volumetric flasks was filled with the standard stock solution of Ceftriaxone (0.1 mL). 1 mL of 5% Ninhydrin reagent was added to each flask, which was then heated for 30 minutes at various temperatures (70, 80, and 90 °C). After cooling to room temperature, the volume in each flask was adjusted to 10 mL using water. Each solution's absorbance was measured at 568.5 nm in relation to a reagent blank.

Heating time's impact on the chromogenic response

A series of 10 mL volumetric flasks was filled with the standard stock solution of Ceftriaxone (0.1 mL). One milliliter of the 5% Ninhydrin reagent was added to each flask and stirred. For varying durations (5, 10, 15, 20, 25, 30 minutes), the flasks were sub-

merged in a water bath at 90^{0}C. After cooling to room temperature, the volume of water in each flask was adjusted to 10 mL. At 568.5 nm, each solution's absorbance was measured in comparison to a blank. After 30 minutes, the absorbance reached its maximum and stayed there for three hours.

Validation

Linearity

Stock solutions ranging from 10 to 100 µg/mL were accurately transferred into a 10mL volumetric flask. To each flask, add 1 mL of 5% w/v Ninhydrin reagent and 1 mL of acetate buffer, pH 5. Mix thoroughly. The flasks were immersed in a water bath at 900 degrees Celsius for 30 minutes, then cooled to room temperature and filled with water to a volume of 10 milliliters. The absorbance of the colored solution was measured in the 400-800 nm range against a reagent blank. The maximum absorbance was measured at 568.5 nm.

Accuracy

Calculating Ceftriaxone recoveries using the usual addition approach allowed for the determination of the method's accuracy. Ceftriaxone standard solutions at 80, 100, and 120% levels were added to pre-quantified sample solutions. The spiked solutions were measured at 568.5 nm to estimate the quantity of Ceftriaxone. Drug estimation in triplicate preparations at each designated concentration level was used to confirm the recovery.

Precision

The proposed colorimetric method's intra-day precision was assessed by estimating the response three times on the same day for three different Ceftriaxone concentrations (20, 60, and 100 µg/mL). Similarly, the suggested colorimetric method's inter-day precision was tested using the identical Ceftriaxone concentrations, and the resulting responses were recorded three times on three distinct days over one week. The intra-day and inter-day precision results were presented as a percentage of relative standard deviation.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Ceftriaxone's limit of detection (LOD) and limit of quantification (LOQ) were determined by calculating the signal-to-noise ratio (S/N), which was 3.3 for LOD and 10 for LOQ, using the following formulae in accordance with International Conference on Harmonization (ICH) criteria.

$LOD = 3.3\ \sigma/S$

The quantitation limit (LOQ) may be expressed as:

$LOQ = 10\ \sigma/S$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Results and Discussion

Optimization of method

Effect of concentration and volume of ninhydrin reagent

The presence of 1 mL of 5% w/v Ninhydrin reagent resulted in the highest absorbance as shown in figure 2.

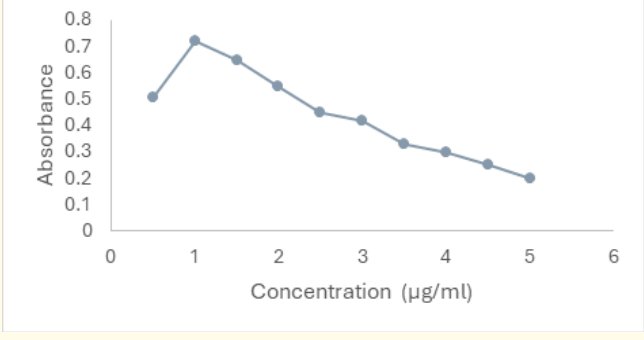

Figure 2: Effect of concentration of Ninhydrin.

Effect of temperature

The ideal temperature for maximum absorption was found to be 90˚C and is shown in figure 3.

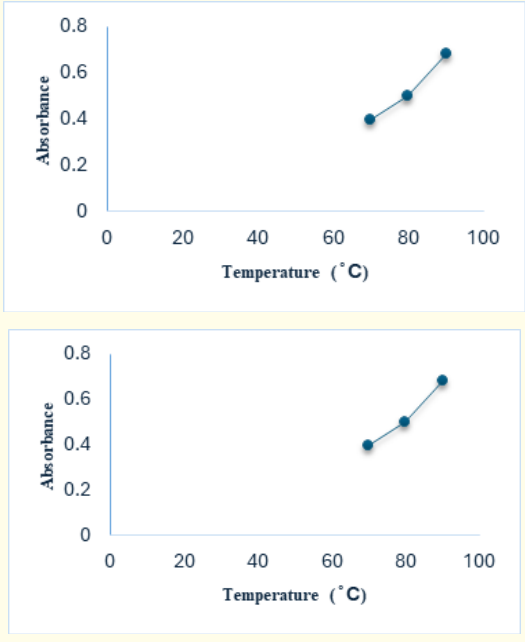

Figure 3: Effect of Temperature.

Effect of heating time on chromogenic reaction

The maximal absorbance was reached after 30 minutes and remained steady for 3 hours. Figure 4 illustrates this.

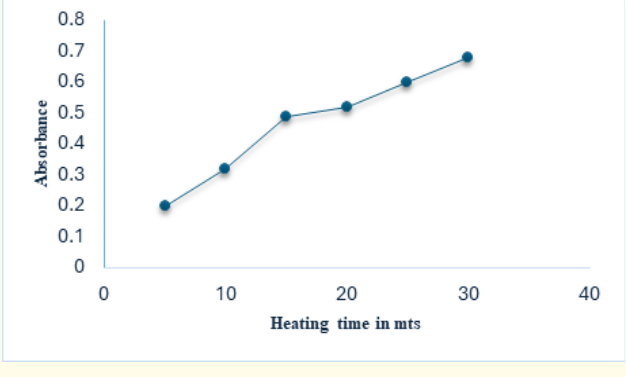

Figure 4: Effect of heating time on chromogenic reaction.

Selection of suitable wavelength

Figure 5 displays the spectra of a standard solution of Ceftriaxone derivative that was scanned in the UV-visible range (400–800 nm). The color derivative of Ceftriaxone was discovered to have a λmax of 568.5 nm based on the spectra.

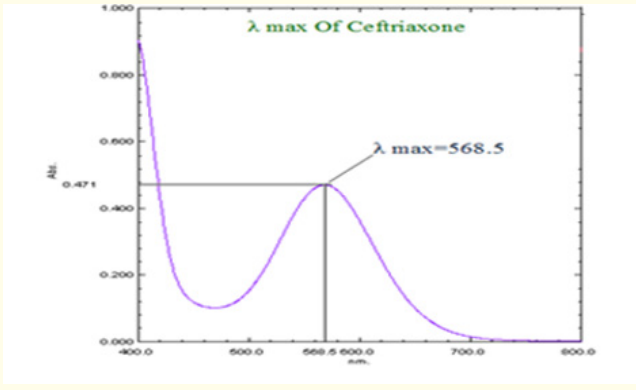

Figure 5: Visible spectrum of Ceftriaxone using Ninhydrin reagent.

Analytical method validation

Calibration Plot for cceftriaxone

For the Ceftriaxone derivative, the linearity of the calibration curve (absorbance vs. concentration) was examined over the concentration range of 10–100 µg/mL. Figure 6 shows the Ceftriaxone derivative's overlay spectra in the linearity range. As determined by the linear regression study, Ceftriaxone's correlation coefficient value (R2) was 0.999, and the regression equation was y = 0.006x + 0.007. Table 1 provides Ceftriaxone's linearity statistics. Figure 7 shows the calibration graph for the Ceftriaxone derivative, which shows that the absorbance increased in par with the drug concentration. The absorbance data was collected at 568.5 nm where the absorbance is maximum.

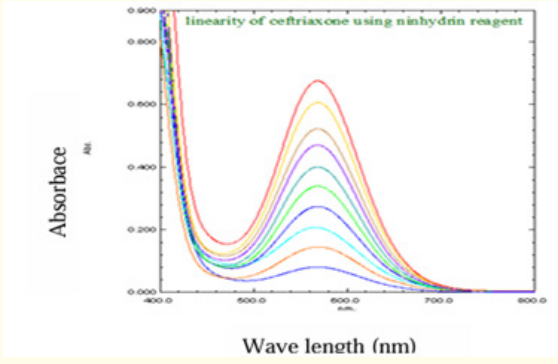

Figure 6: Overlay spectra of ceftriaxone derivative with ninhydrin reagent (10 - 100 µg/mL).

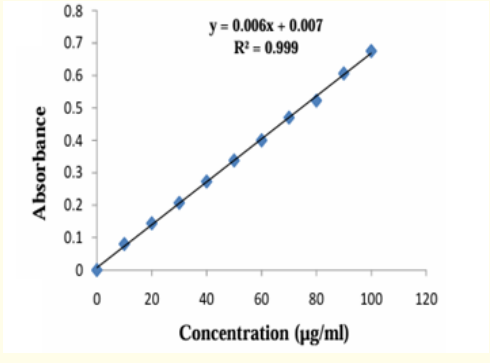

Figure 7: Standard graph of Ceftriaxone using ninhydrin reagent.

Table 1: Linearity Data of Ceftriaxone.

Sl. No	Con. (µg/ml)	Absorbance (AM ± SD) (n = 3)
1	0	0.000
2	10	0.081 ± 0.030
3	20	0.144 ± 0.003
4	30	0.207 ± 0.001
5	40	0.273 ± 0.002
6	50	0.338 ± 0.001
7	60	0.400 ± 0.02
8	70	0.470 ± 0.02
9	80	0.522 ± 0.022
10	90	0.606 ± 0.002
11	100	0.675 ± 0.005

Precision

Three standard solutions of Ceftriaxone with concentrations of 20, 60, and 100 µg/mL were analyzed intra-day (n = 3) to assess the method's repeatability (intra-day precision). The inter-day (n = 3) examination of three standard Ceftriaxone solutions at the afore-mentioned concentrations yielded intermediate precision. Table 2 presents the information gathered from precision studies. The method's precision was confirmed by the intra-day and inter-day precision study's % RSD values, which were less than 2.0.

Table 2: Precision data of Ceftriaxone.

Theoretical amount (µg/mL)	Intra-day Precision (n = 3)		Inter-day Precision (n = 3)	
	Amount Obtained ± SD (µg/mL) % RSD	%RSD	Amount found ± SD (µg/mL) % RSD	% RSD
20	19.83 ± 0.001	1.0589	21.05 ± 0.0020	1.39
60	58.92 ± 0.0020	0.518	59.08 ± 0.0041	0.995
100	99.23 ± 0.0076	1.021	100.1 ± 0.0026	0.385

Acceptance Criteria: % RSD should not exceed 2.

Accuracy (Recovery Studies)

The conventional addition method was used to determine the accuracy. In triplicate, three distinct standard amounts (80%, 100%, and 120%) were added to the commercial powder. Table 3 shows the mean of the percentage recoveries and the percentage RSD values. Ceftriaxone recovery was determined to be within a reasonable range of 99.29 to 99.85%.

Acceptance Criteria: % RSD should not be more than 2.

Table 3: Recovery research results illustrate the accuracy of the proposed strategy.

Drug	Level of accuracy	Theoretical content (µg/ml)	Conc. found ± SD (µg/ml)	%Recovery (n = 3)	%RSD
Ceftriaxone	80%	72	71.49 ± 0.013	99.29	0.02875
	100%	80	79.88 ± 0.040	99.85	0.0765
	120%	78	77.73 ± 0.004	99.65	0.084

Table 4: Assay of Monocef (Powder for injection)

Drug Name	Brand Name	Label Claim (mg)	Amount found (mg) ± SD (n = 3)	%Assay	%RSD
Ceftriaxone	Monocef*1g	1000 mg	1000.16 mg ± 0.0020	100.016	0.616

Limit of Detection (Lod) and Limit of Quantification (Loq)

For Ceftriaxone, the LOD was determined to be 1.54 µg/mL and the LOQ to be 4.66 µg/mL, respectively.

Analysis of marketed formulations (Assay)

The assay of a commercially available injectable (Monocef*1g) containing 1000 mg of Ceftriaxone was used to assess the accuracy of the suggested approach. Table 4 presents the findings of a comparison between the Ceftriaxone results and the corresponding labeled quantities. The percentage label claim was 100.016, while the amount of Ceftriaxone detected was 1000.16 mg. These sums fell within the permitted range. The correctness of the suggested approach was demonstrated by the assay results' % RSD being less than 2.

Conclusion

On comparing with chromatographic techniques for pharmaceutical analysis, visible spectrophotometry has maintained its competitiveness due to its ease of use, sensitivity, and selectivity. The current study relies on ceftriaxone and ninhydrin reagent reaction at 90°C for 30 minutes, which absorbs at λ max 568.5 nm in the linearity range of 10-100 µg/mL. The optimized method is successfully validated on par with ICH specifications for various parameters such as linearity, accuracy, precision, LOD, LOQ etc. The validation study results demonstrated the developed colorimetry approach's simplicity, selectivity, accuracy, precision, and linearity. Therefore, the established colorimetric approach can achieve Ceftriaxone quality control in API and pharmaceutical dosage forms.

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Conflict of Interest

The authors declare there is no conflict of interest exists.

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Author Contributions

All the authors involved contributed in the development of the research work.

Contributions

Ceema Mathew designed, planned and executed the study, analysed the data, wrote the final draft of the work. Shashikala Metri – reviewed the work and assisted in the drafting and editing; Bejjanki Jahnavi did the literature review and assisted in planning and executing the work, Bukya Rekha- assisted in planning and executing the study, Shakapuram Neha- analysed the data and assisted the manuscript drafting. Battula Prasanna- - analysed the data and assisted the manuscript drafting, all authors reviewed, edited and approved the final manuscript.

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