

Validated Spectrophotometric Methods for the Estimation of Ceftriaxone in Pharmaceutical Preparations

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Three simple and sensitive visible spectrophotometric methods (I, II and III) have been developed for the quantitative estimation of ceftriaxone in bulk drug and pharmaceutical preparations. Method I is based on the diazotization of ceftriaxone with nitrous acid to form a violet coloured chromogen in alkaline conditions with absorption maximum at 559 nm and obeys Beer's law in the concentration range of 50–150 µg/mL. Method II is based on the diazotization of drug with nitrous acid followed by its coupling with 2-naphthol to form reddish violet coloured chromogen with absorption maximum at 554.5 nm and Beer's law is obeyed in the concentration range of 30–150 µg/mL. Method III is based on the oxidation followed by complex formation reaction of ceftriaxone with potassium ferricyanide in presence of ferric chloride to form greenish blue coloured chromogen with absorption maximum at 797 nm and Beer's law is obeyed in the concentration range of 1–5 µg/mL. The results of analysis for the three methods have been validated statistically and by recovery studies. The results obtained with the proposed methods are in good agreement with the labelled amounts when marketed pharmaceutical preparations are analyzed.

Key Words: Spectrophotometry, Ceftriaxone.

INTRODUCTION

Ceftriaxone¹⁻¹⁵ is chemically (Z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyimino-acetamido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)-thiomethyl]-3-cephem-4-carboxylic acid and available as disodium salt, sesquaterhydrate. It is a third generation cephalosporin antibiotic used for the treatment of susceptible infections which include chancroid, endocarditis, gastroenteritis, gonorrhoea, Lyme disease, meningitis, septicaemia, syphilis, typhoid fever and Whipple's disease. It is also used for surgical infection prophylaxis. It is official in U.S.P. and European Pharmacopoeia. No spectrophotometric methods are found in literature for its quantitative estimation

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in bulk drug and pharmaceutical preparations. The present work deals with the development of three simple and sensitive visible spectrophotometric methods for the quantitative estimation of ceftriaxone in bulk drug and pharmaceutical preparations (injectables).

Method I is based on the diazotization of ceftriaxone with nitrous acid (NaNO_2/HCl) to form a violet coloured diazonium salt in alkaline conditions with absorption maximum at 559 nm and obeys Beer's law in the concentration range of 50–150 $\mu\text{g/mL}$. Method II is based on the diazotization of drug with nitrous acid followed by its coupling with 2-naphthol to form reddish violet coloured chromogen with absorption maximum at 554.5 nm and Beer's law is obeyed in the concentration range of 30–150 $\mu\text{g/mL}$. Method III is based on the oxidation followed by complex formation reaction of ceftriaxone with potassium ferricyanide in presence of ferric chloride to form greenish blue coloured chromogen with absorption maximum at 797 nm and Beer's law is obeyed in the concentration range of 1–5 $\mu\text{g/mL}$. Spectrophotometric parameters are established for standardization of the methods including statistical analysis of data. These methods have been successfully extended to the pharmaceutical preparations (injectables) containing ceftriaxone. The results are compared with those obtained using UV spectrophotometric method in water at 240.4 nm and Beer's law is obeyed in the concentration range of 2–10 $\mu\text{g/mL}$.

EXPERIMENTAL

A Shimadzu UV/Vis double beam spectrophotometer (model 1601) with 1 cm matched quartz cells was used for all spectral measurements. All chemicals used are of AR grade from S.D. Fine Chemicals, Mumbai.

Working standard of drug solution

Ceftriaxone sodium equivalent to 100 mg of ceftriaxone was weighed accurately and dissolved in 40 mL of water in a 100 mL volumetric flask and diluted up to the mark with water (1 mg/mL). The final concentration of ceftriaxone was brought to 100 $\mu\text{g/mL}$ with water.

Sample preparation

Three brands of commercial injectables were analyzed by the proposed methods. Ten vials each containing 500 mg of ceftriaxone were taken and the contents were mixed thoroughly in a beaker and the volume equivalent to 100 mg of ceftriaxone was pipetted out into a 100 mL volumetric flask and the volume was made up to the mark with double distilled water. The final concentration of ceftriaxone was brought to 100 $\mu\text{g/mL}$ with double distilled water.

Assay

Method I: Aliquots of ceftriaxone ranging from 0.5–1.5 mL (1 mL = 1 mg) were transferred into a series of 10 mL volumetric flasks. To each flask, 1 mL of

NaNO₂ (0.1%) (w/v) and 1 mL of HCl (0.5 M) were added at room temperature. After 5 min, 1 mL of NaOH (2%) (w/v) was added. The volumes were made up to the mark with distilled water. The absorbance of the violet coloured chromogen was measured at 559 nm against reagent blank. The colour was stable for more than 1 h. The amount of ceftriaxone present in the sample was computed from the calibration curve.

Method II: Aliquots of ceftriaxone ranging from 0.3–1.5 mL (1 mL = 1 mg) were transferred into a series of 10 mL volumetric flasks. To each flask 1 mL of NaNO₂ (0.1%) (w/v) and 1 mL of HCl (0.5 M) were added at room temperature. After 5 min, 1.5 mL of methanolic 2-naphthol (0.5%) (w/v) and 1 mL of NaOH (2%) (w/v) were added and the volumes were made up to the mark with water. The absorbance of the reddish violet coloured chromogen was measured at 554.5 nm against reagent blank. The colour was stable for more than 2 h. The amount of ceftriaxone present in the sample was computed from the calibration curve.

Method III: Aliquots of ceftriaxone ranging from 0.1–0.5 mL (1 mL = 1 mg) were transferred into a series of 10 mL volumetric flasks. To each flask 1 mL of ferric chloride (0.5%) (w/v) and 1 mL of potassium ferricyanide (0.2%) (w/v) were added. After 20 min, the volumes were made up to the mark with distilled water. The absorbance of the greenish blue coloured chromogen was measured at 797 nm against reagent blank. The colour was stable for more than 2 h. The amount of ceftriaxone present in the sample was computed from the calibration curve.

The results of the above methods are compared with the results obtained with the UV spectrophotometric method. In UV method, ceftriaxone sodium equivalent to 100 mg of ceftriaxone, either pure or formulation, was accurately weighed and a solution of 100 µg/mL was prepared in water. Aliquots of ceftriaxone ranging from 0.2–1.0 mL (1 mL = 100 µg) were transferred into a series of 10 mL volumetric flasks. The volumes were made up to the mark with water. The absorbance of the solutions was measured at 240.4 nm against solvent blank. The amount of ceftriaxone present in the sample was computed from the calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-1. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and the results are summarized in Table-1. The per cent relative standard deviation and per cent range of error (0.05 and 0.01 level of confidence limits) were calculated from the eight measurements; 3/4 of the upper Beer's law limits of ceftriaxone are also given in Table-1. The results showed that these methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for dosage forms (Table-2) confirm the suitability of the methods for pharmaceutical preparations (injectables).

TABLE-1
 OPTICAL CHARACTERISTICS AND PRECISION

| | Method I | Method II | Method III |
|-------------------------------------------------------------------------|----------------------|----------------------|----------------------|
| λ_{\max} (nm) | 559 | 554.5 | 797 |
| Beer's law limits ($\mu\text{g}/\text{mL}$) (C) | 50–150 | 30–150 | 1–5 |
| Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$) | 2.3836×10^3 | 4.5613×10^3 | 1.8639×10^5 |
| Sandell's sensitivity ($\mu\text{g}/\text{mL}$ –0.001 absorbance unit) | 0.0920 | 0.0870 | 0.0180 |
| Regression equation (Y*): | | | |
| Slope (b) | 0.0047 | 0.0075 | 0.2972 |
| Intercept (a) | 0.1010 | 0.0040 | 0.0192 |
| Correlation coefficient (r) | 0.9999 | 0.9996 | 0.9998 |
| %RSD | 0.3578 | 0.2754 | 0.1844 |
| Range of errors†: | | | |
| Confidence limits with 0.05 level | 0.0014 | 0.0204 | 0.0021 |
| Confidence limits with 0.01 level | 0.0021 | 0.0030 | 0.0027 |

* $Y = bC + a$, where, C is the concentration of ceftriaxone in $\mu\text{g}/\text{mL}$ and Y is the absorbance at the respective λ_{\max} .

†For eight measurements.

 TABLE-2
 EVALUATION OF CEFTRIAOXONE IN PHARMACEUTICAL PREPARATIONS

| Samples* | Labelled amount (mg) | Amount obtained† (mg) | | | | Percentage recovery† | | |
|----------------|----------------------|-----------------------|--------|--------|-----------|----------------------|--------|--------|
| | | Proposed methods | | | UV method | I | II | III |
| | | I | II | III | | | | |
| I ₁ | 500 | 498.21 | 498.11 | 498.71 | 499.21 | 98.93 | 98.82 | 99.12 |
| | | ± 0.03 | ± 0.05 | ± 0.03 | ± 0.03 | ± 0.03 | ± 0.04 | ± 0.04 |
| I ₂ | 500 | 498.32 | 498.21 | 498.43 | 499.32 | 98.72 | 98.76 | 99.21 |
| | | ± 0.04 | ± 0.07 | ± 0.02 | ± 0.06 | ± 0.06 | ± 0.06 | ± 0.05 |
| | 500 | 498.41 | 498.61 | 498.55 | 499.46 | 98.85 | 99.63 | 99.34 |
| | | ± 0.06 | ± 0.05 | ± 0.01 | ± 0.02 | ± 0.05 | ± 0.03 | ± 0.04 |

*Injectables from different manufacturers.

†Average \pm S.D. of eight determinations.

The optimum conditions for colour development for Methods I, II and III have been established by varying the parameters one at a time and keeping the other parameters fixed and observing the effects of product on the absorbance of the coloured species and incorporated in the procedures. To evaluate the validity and accuracy of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The per cent recoveries are given in Table-2. Interference studies revealed that the additives like antioxidants, preservatives and solubilizers that were usually present in injectables did not interfere at their regularly added levels.

The proposed visible spectrophotometric methods are found to be simple, sensitive, selective, accurate, precise and economical and can be used in the determination of ceftriaxone in bulk drug and its pharmaceutical preparations in a routine manner.

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