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Development And Validation Of UV–Spectrophotometric Methods For Estimation Of Ceftriaxone In Bulk And Tablet Dosage Form.

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Abstract: Ceftriaxone is a third-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad spectrum activity against Gram negative and Gram positive bacteria Various methods for analysis of the same are available but are time consuming and expensive. Here we have developed two new, precise and simple UV spectrophotometric methods for estimation of ceftriaxone from bulk and tablet formulation in phosphate buffer 7.4. The drug obeyed the Beer's law with correlation coefficient 0.996 and 0.998 respectively for Method I and Method II. It showed absorption maxima at 340 nm and 360 nm respectively for method I and Method II; in phosphate buffer 7.4. The linearity was observed between 5 –40 µg/ml. The results of analysis were validated by recovery studies, accuracy, precision, LOD, LOQ and ruggedness. The method was found to be simple, accurate, precise, economical and robust.
Key words: ceftriaxone , Phosphate buffer 7.4, Zero order spectra and second order spectra, validation

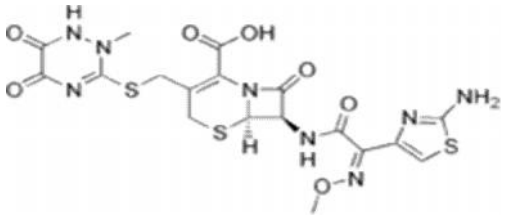
INTRODUCTION

Ceftriaxone is a third-generation cephalosporin antibiotic. Chemically Ceftriaxone (CF) is (6*R*,7*R*,*Z*)-7-(2-(2-aminothiazol-4-yl)-2-(methoxy imino)acetamido)-3-((6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylthio)methyl)-8-oxo-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid.[1] Like other third-generation cephalosporins, it has broad spectrum activity against Gram-positive and Gram-negative bacteria. In most cases, it is considered to be equivalent to cefotaxime in terms of safety and efficacy. Ceftriaxone sodium is marketed by Hoffman-La Roche under the trade name Rocephin. Ceftriaxone sodium is marketed in Bangladesh as "Arixon" by Beximco Pharmaceuticals & Rephco Pharmaceuticals under the trade name Inoxon [1,11] Ceftriaxone is often used (in combination, but not direct, with macrolide and/or aminoglycoside antibiotics) for the treatment of community-acquired or mild to moderate health care-associated pneumonia. It is also a choice drug for treatment of bacterial meningitis. In pediatrics, it is commonly used in febrile infants between 4 and 8 weeks of age who are admitted to the hospital to exclude sepsis. The dosage for acute ear infection in the very young is 50 mg/kg IM, one dose daily up to three days. It has also been used in the treatment of Lyme disease, typhoid fever, and gonorrhea [2] Intravenous dosages may be adjusted for body mass in younger patients and is administered every 12–24 hours, at a dose that depends on the type and severity of the infection.

For the treatment of gonorrhea, a single intramuscular injection is usually given. According to the Journal of Family Practice, Volume 60, NO 12, December 2011; the intramuscular dose of ceftriaxone (Rocephin) has been increased from 125mg IM to 250mg IM due to increasing

resistance of the gonococcal bacteria. It is also recommended that 1000mg of azithromycin be given orally at the same time for dual treatment. This also takes care of treatment of underlying chlamydia since treatment for chlamydia infection is also recommended. It must not be mixed or administered simultaneously (within 48 hours) with calcium-containing solutions or products for patients younger than 28 days old,[3] even via different infusion lines (rare fatal cases of calcium-ceftriaxone precipitates in lung and kidneys in neonates have been described)[4]. To reduce the pain of intramuscular injection, ceftriaxone may be reconstituted with 1% lidocaine[5] Ceftriaxone has also been investigated for efficacy in preventing relapse to cocaine addiction[6]. Ceftriaxone seems to increase EAAT2 pump expression and activity[7] in the central nervous system and has therefore a potential to reduce glutamatergic toxicity[8] Despite earlier negative results in the 1990s, new, large clinical trials are underway to test its effacy in amyotrophic lateral sclerosis (ALS) patients. In August 2012, the Northeastern Amyotrophic Lateral Sclerosis Consortium posted on its website that the trials were stopped because the study was unlikely to reach the positive results it anticipated[9].Hypoprothrombinaemia and bleeding are specific side effects. Haemolysis is reported. Biliary sludging is another known though rare adverse effect which occurs primarily in neonates.[10] It is a third-generation cephalosporin antibiotic. Like otherthird-generation cephalo sporins, it has broad spectrum activity against Gram negative and Gram positivebacteria [11]. CF is often used (in combination withmacrolide and/or aminoglycoside antibiotics) for thetreatment of community-acquired pneumonia. It is also a drug of choice for the treatment of bacterialmeningitis. In pediatrics, it is commonly used in febrile infants. It has also been used in the treatment ofleptospirosis [12], lyme disease and gonorrhea. It is also used as a routine prophylactic antibiotic for the patientsundergoing orthopedic surgery [13]. Several analytical methods have been reported for the analysis of CF,based on spectrophotometric [14-20], derivative spectrophotometric [21], FIA [22], flourimetric [23,24], thin layer chromatographic [25-27], ion selective electrodes [28], ion exchange chromatographic [29],high performance liquid chromatographic [30,31], ionpair liquid chromato graphic [32] and polarographic[33, 34] techniques. For spectrometric analysis, the determination is carried out using suitable reagents suchas metol-chromium(VI) reagent, mixture of Fe(III) and hexacyanoferate(III) ions, leuco crystal violet and 3-methyl-2-benzothiazoline hydrazone hydro chloride and ferric chloride [14-20,35]. This paper reports a study on the development of a new validated UV-spectrophotometric method for the quantitative determination of Ceftriaxone bulk and solid dosage form.

FIG : 1 The structure of ceftriaxone



Systematic (IUPAC**) name of Ceftriaxone is**

(6*R*,7*R*)-7-{[(2*Z*)-2-(2-amino-1,3-thiazol-4-yl)->2-(methoxyimino)acetyl]amino}-3-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio] methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

MATERIALS AND METHODS

Instrumentation, Reagents &Chemicals:
Instruments used were UV-Visible spectrometer, model *JASCO* 1505 Instrument and Shimadzu ELB 300 analytical balance, Ceftriaxone pure drug was obtained as a gift sample from SUN PHARMACEUTICAL S ,HYDERABAD . All chemicals and reagents used were of analytical grade CEFTRIX TABLETS (STELLAR PHARMATECH ,NEW DELHI-110092 INDIA.), and NACEF TABLETS (NASCENT LIFE SCIENCES, AHMEDABAD, GUJARAT , INDIA.) were purchased from the market

Selection of media:
Main criteria for selection of media solubility and stability i.e., drug should be soluble as well as stable for sufficient time in selected media. Ceftriaxone was slightly soluble in distilled water and was soluble in methanol, ethanol, PEG-400/Water and ethanol-water mixture. It was freely soluble in phosphate buffer 7.4 and was considerably stable.

Preparation of standard stock solution:
Standard drug solution of Ceftriaxone was prepared by dissolving 10mg pure Ceftriaxone in phosphate buffer 7.4 and transferred into 100ml volumetric flask to obtain 10µg/ml of stock solution from which desired concentrations 5,10,15,20,25, 30,35,40 µg/ml of solution were prepared.

Preparation of sample solution:
Twenty tablets were weighed; average weight was determined and finely powdered. An accurately weighed quantity of tablet powder equivalent to 10mg of Ceftriaxone was transferred to 100 ml volumetric flask and dissolved by sonication with sufficient quantity of phosphate buffer 7.4, volume was made up to mark. The solution was then filtered through whatman filter paper no.41. A 1 ml portion of the filtrate was further diluted with phosphate buffer 7.4 in a 10 ml volumetric flask up to mark (10µg/ml) on label claim basis. The absorbance of the resulting solution was measured at 340 nm (method I) and 360 nm (method II) against solvent blank. The results of estimation by proposed methods are shown in Table.2.

Determination of λmax:
A 10 µg/ml solution of Ceftriaxone was prepared and scanned in UV range of 200-400nm and spectrum was obtained. The **λ**max was found to be at 340 nm wave length where absorbance was maximum at this wavelength for Method I, and the λmax for Method II was found to be 360 nm. Hence these are considered as absorbance maxima (λmax**)**

Preparation of calibration curve:
Standard stock solution was suitably diluted with phosphate buffer 7.4 to obtain concentrations ranging from 5-40 µg/ml. Absorbance of these solutions was measured at 340nm for Method I and at 360 nm for Method II using UV. The calibration curve was plotted as concentration versus absorbance over the range of 5-40 µg/ml with correlation coefficient of 0.996 and 0.998 for the proposed method I and method II (fig.2).

VALIDATION
Accuracy:
To assess the accuracy of the proposed method, recovery studies were carried out three different levels i.e. 80%, 100% and 120%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded. The % recovery was then calculated as % Recovery = [(A – B) / C] x 100, Where A is total amount of drug estimated; B is amount of drug found on pre analyzed basis; C is amount of pure drug added to formulation (Table No.3).

Precision:
Precision of the method is studied as intra-day and interday precision. Intra-day and Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days. In intermediate precision study, % R.S.D. values were not more than 1.0 % in all the cases (Table No.5).

Limit of detection and Limit of quantitation:
Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by using the formula based on the standard deviation of the response and the slope. Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated by using the equations LOD = 3 × s/S and LOQ = 10 × s/S, where s is standard deviation of intercept, S is the slope of the line (Table No.4).

Ruggedness:
Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions (Table No.5).

Table No.1: Optical Parameters:

S.NO	PARAMETER	Observations Method I	Observations Metho II (Derivative)
1	λ max	340 nm	360nm
2	Beers range	5-40 µg/ml	5-40 µg/ml
3	Correlation coefficient	0.996	0.998
4	Intercept	0.07495	0.0892
5	Slope	24.28	22.36

Table No.2: Assay of CEFTRIAXONE 2.5 mg tablets (NACEF&CEFTRIX):

SR. NO	LABEL CLAIM	% claim found*	
		Method I	Method II
NACEF	2.5 mg	99.77 %	99.12 %
CEFTRIX	2.5 mg	99.84 %	99.35%

**mean of 5 determinations.*

Fig.2-Calibration curve of Ceftriaxone

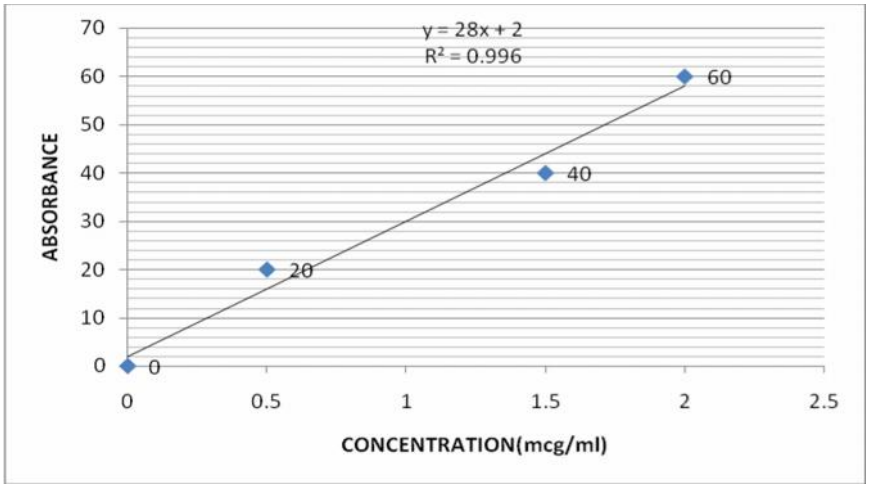


Table No.3: Results of Recovery study of CEFTRIAXONE 2.5 mg (NACEF & CEFTRIX tablets)

Labelled amount	Amount of drug Added (%)	Method I			Method I		
		Amount of drug Recovered (mg)	Percent Recovery (%)*	% RSD	Amount of drug Recovered (mg)	Percent Recovery (%)*	% RSD
2.5 mg	80	1.91	99.56	0.28	1.86	99.09	0.56
2.5 mg	100	2.485	99.39	0.35	2.31	99.59	0.23
2.5 mg	120	3.0156	100.54	0.29	3.0126	100.21	0.31

Table No.4: Validation parameters

Sr. No.	Method I		I Method II (Derivative)	
	LOD	LOQ	LOD	LOQ
1	0.0646	0.189	0.0796	0.176

Table No.5: Validation parameters:

Sr. No.	Parameters	Method I	Method II (Derivative)
1	Intraday precision Amount found + %RSD (n=3)	99.32 + 0.69	98.11 + 0.99
2	Interday precision Amount + %RSD (n=3)	98.67 + 0.99	97.03 + 3.62
3	Ruggedness Amount found + %RSD (n=3)	0.141	0.169

RESULTS AND DISCUSSION

A validated, simple, rapid sensitive and accurate UV-Spectrophotometric methods has been developed for estimation of Ceftriaxone in bulk and pharmaceutical formulation. In phosphate buffer 7.4, Ceftriaxone showed absorbance maxima at 340 nm and 360 nm respectively for Method I and Method II. Linearity was observed in the concentration range 5-40 µg/ml with correlation coefficient value 0.996 and 0.998 respectively for Method I and Method II. The proposed method was applied to pharmaceutical formulation and Percent amount of drug estimated was found in good agreement with the label claim. The recovery experiment was carried out at three different levels

i.e., 80 %, 100 % and 120 %. The percentage recovery was found to be 99.84 % and 99.35 % respectively for Method I and Method II; the low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day and inter-day precision. Ruggedness of the proposed method was studied with the help of two analysts. The Limits of Detection and Quantitation for Ceftriaxone with a lower concentration were 0.0646 and 0.189 for Method I and for Method II 0.0796 and 0.176 respectively, values which are under the lowest expected concentrations in the sample.

CONCLUSION

The present study was undertaken with an objective of developing simple, sensitive and reliable analytical method like UV-Visible spectro photometry for estimation of Ceftriaxone in phosphate buffer 7.4 in tablet dosage form. The method has sufficiently good accuracy, precision and permitted as a cost effective as other methods. The analytical method is simple, sensitive, rapid and specific. Further it can be conveniently employed for the routine analysis and the quality control of Ceftriaxone in tablet formulation.

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