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DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF CEFTRIAXONE SODIUM IN DIFFERENT BRANDS OF PHARMACEUTICAL PREPARATION INVOLVING DIAZOTIZATION

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ABSTRACT: A simple, precise, and robust spectrophotometric method for the determination of Ceftriaxone sodium has been investigated and validated. The proposed chemical reaction involves the reaction primary amine of Ceftriaxone sodium with nitrite solution in acidic medium to form a diazotized product followed by the reaction of the diazotized product with suitable coupling reagent to obtain a colored product. Ceftriaxone sodium has shown λ_{max} 510nm with absorbance of 0.126. The method obeys Beer Lambert law at concentration range of 1-20ppm by showing linearity curve between absorbance and concentration. In the method detection limits was observed 0.726ppm with quantification limit of 2.42ppm and standard deviation of 0.242. In the present work an attempt was made to develop a simple, less expensive and more reliable method for the determination of Ceftriaxone sodium in pure and pharmaceutical preparations.

INTRODUCTION: Ceftriaxone sodium is a third generation cephalosporin antibiotic used in the treatment of bacterial infection caused by susceptible, usually gram-positive organisms. It is also used for the treatment of infections (respiratory, skin, soft tissue, UTI, ENT) caused by *S. pneumonia*, *H. influenza*, *staphylococci*, *S. pyogenes*. Ceftriaxone sodium has the longest half life of 6-8 hours.^{1,4}

Chemical formula: C₁₈H₁₆N₈Na₂O₇S₃, 3½H₂O

Molecular formula: 662

The structural formula of Ceftriaxone sodium is given in **Fig.1**.

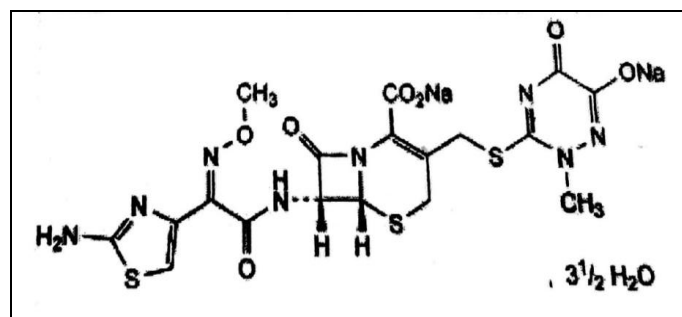


FIG.1: STRUCTURE OF CEFTRIAXONE SODIUM

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	Article can be accessed online on: www.ijpsr.com
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Characteristics:

Almost white or yellowish, crystalline powder, slightly hygroscopic freely soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol.

Experimental:

Method development strategy for the spectrophotometric determination of Ceftriaxone sodium: Ceftriaxone sodium has an amine group in the molecule as shown in **Fig.1** the proposed method for determination of Ceftriaxone

sodium is based on direct exploitation of the amine group for the formation of azo dye.

The proposed chemical reaction involved has two steps as shown in **Fig.2** the first step involves the reaction of Ceftriaxone sodium with nitrite solution in acidic medium to form a diazotized product. The second step is based on the reaction of the diazotized product with suitable coupling reagent to obtain a colored product. In the investigation the final product that is the colored azo dye was used for the determination of Ceftriaxone sodium.

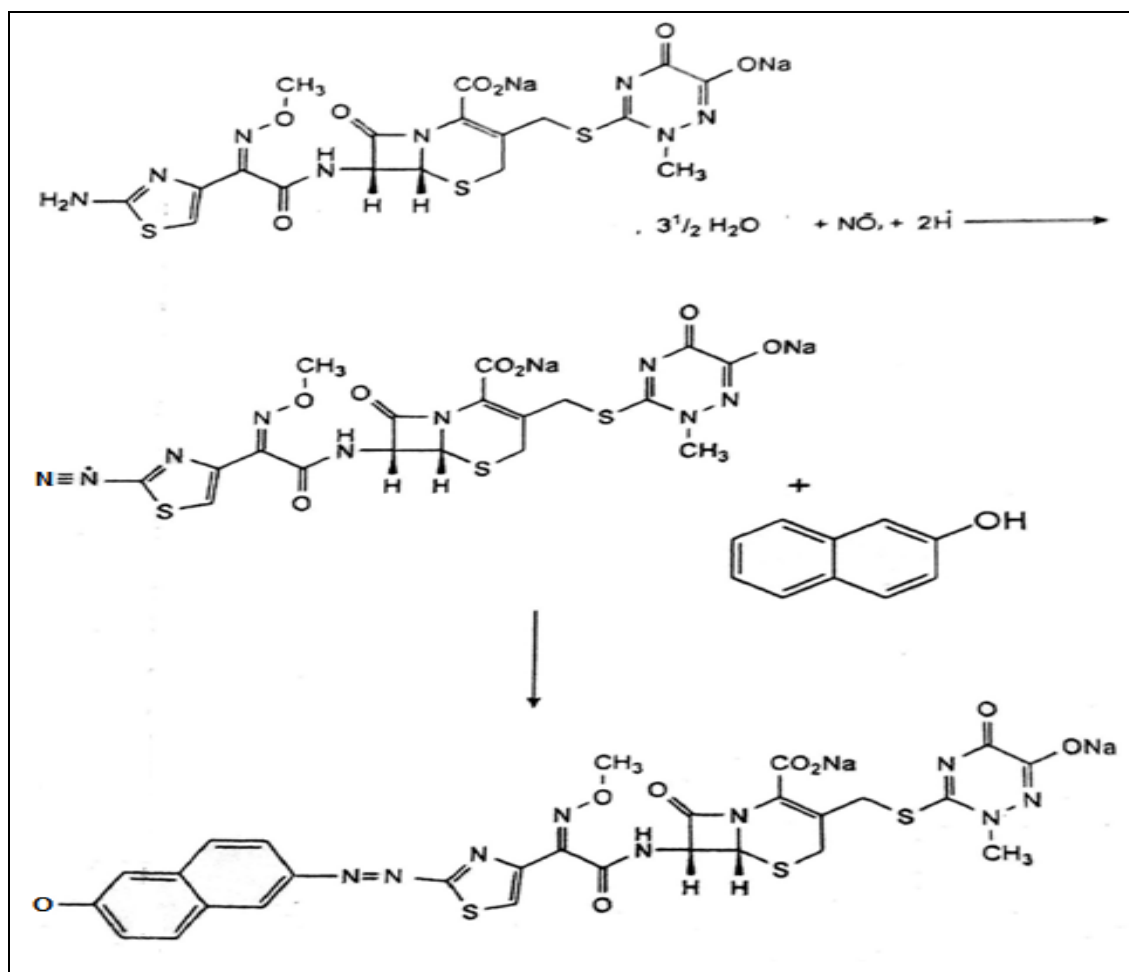


FIG.2: PROPOSED CHEMICAL REACTION FOR THE FORMATION OF AZO DYE FROM CEFTRIAOXONE SODIUM

Preliminary investigation of the possibility of diazotization and coupling for spectrophotometric determination of Ceftriaxone sodium:

Preliminary studies were conducted to investigate the possibility of the formation of expected azo dye. Initially high concentration of Ceftriaxone sodium (1000 ppm), relatively large volume of concentrated hydrochloric acid, sodium nitrite and

various coupling reagents like aniline and β -Naphthol were tried to check formation of the expected azo dye. The formation of colored azo dye indicated the possibility of the reaction and the subsequent spectrophotometric determination on Ceftriaxone sodium by this method. Further studies were focused on optimization of various parameters and are given below.

Investigation of suitable wavelength for the determination of Ceftriaxone sodium.

Instruments:

UV/Visible spectrophotometer and digital analytical balance were used during this investigation.

Reagents:

Analytical reagent grade sodium nitrite, β -Naphthol, hydrochloric acid 0.1N and Ceftriaxone sodium were used during this work.

Solution preparation:

- 1. Nitrites solution:** Nitrite solution (1000 ppm) was prepared by dissolving 0.15g of sodium nitrite in distilled water and diluted to 100 mL with distilled water
- 2. β -Naphthol solution:** β -Naphthol (1000 ppm) solution was prepared by dissolving 0.1 g of β -naphthol in 0.1 sodium hydroxide and diluted to 100 mL with 0.1 N sodium hydroxide
- 3. Hydrochloric acid:** Hydrochloric acid (0.1 N) was prepared by dissolving 8.2 mL of

concentrated HCL in distilled water and diluted to 1000 mL with distilled water

4. Standard Ceftriaxone sodium solution:

Ceftriaxone sodium (1000 ppm) was prepared by dissolving 0.1g of Ceftriaxone sodium in distilled water and diluted to 100 mL with distilled water.

Procedure:

Ceftriaxone sodium standard solution 1 mL from 1000 ppm stock solution was transferred to a 50 mL volumetric flask, to this 1.0 mL of 0.1 N HCL was added followed by the addition of 2.5 mL of (1000 ppm) sodium nitrite solution this solution kept for 5 minutes for the formation of the diazotized product. Then 1.2 mL of β -Naphthol (1000 mL) was added as coupling reagent. Blank solution was prepared in the same manner without the addition of Ceftriaxone sodium. The absorbance of the resulting color azo dye was measured from 390-590 nm. Using Genesys 5 spectrophotometer for finding out optimum absorption. Each time wavelengths were calibrated with blank solution. The results are given in **Table-1** and are shown in **Fig.3**.

TABLE 1: WAVELENGTH OPTIMIZED FOR SPECTROPHOTOMETRIC DETERMINATION OF CEFTRIAZONE SODIUM

Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
390	0.060	460	0.092	530	0.116
400	0.062	470	0.103	540	0.102
410	0.065	480	0.111	550	0.088
420	0.068	490	0.119	560	0.074
430	0.071	500	0.123	570	0.060
440	0.079	510	0.126	580	0.052
450	0.085	520	0.122	590	0.049

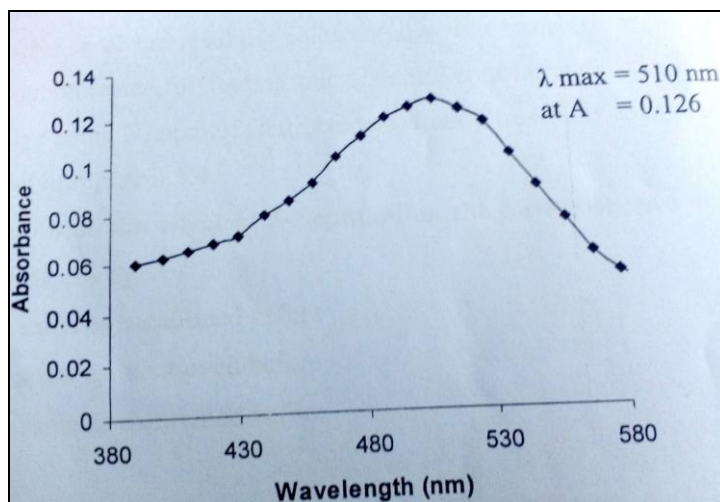


FIG.3: WAVELENGTH OPTIMIZATION FOR SPECTROPHOTOMETRIC DETERMINATION OF CEFTRIAZONE SODIUM

Conditions:

λ_{\max}	varied
Ceftriaxone solution (1000 ppm)	1.0 mL
β -Nepthol (1000 ppm)	1.2 mL
Hydrochloric acid 0.1N	1.0 mL
Sodium nitrate (1000 ppm)	2.5 mL

Investigation of effect of nitrite solution on the absorbance behavior of azo dye:

Instruments: The same as mentioned before

Reagents: The same as mentioned before

Solution: The same as mentioned before

Procedure:

Ceftriaxone sodium standard solution 1 mL from 1000 ppm stock solution was transferred to six separate 50mL volumetric flasks, to each of this 1.0 mL of 0.1 N HCL was added followed by the addition of varied volume of (1000 ppm) sodium nitrite solution. These solutions were kept for some time for the formation of the diazotized product. Then 1.2 mL of β -Nepthol (1000 ppm) was added as coupling reagent. Blank solution was prepared in the same manner without the addition of the Ceftriaxone sodium. The absorbance of the resulting color azo dye measured at 510 nm using Genesys 5 spectrophotometer for finding out optimum concentration of nitrite for the formation of azo dye using 20 ppm of Ceftriaxone sodium the results are given in **Table 2** and are shown in **Fig.4**.

TABLE 2: OPTIMIZATION OF THE VOLUME OF NITRITE SOLUTION FOR THE FORMATION OF AZO DYE

Nitrite (1000 ppm) (mL)	1.0	1.5	2.0	2.5	3.0	3.5
Absorbance	0.084	0.113	0.128	0.135	0.120	0.103

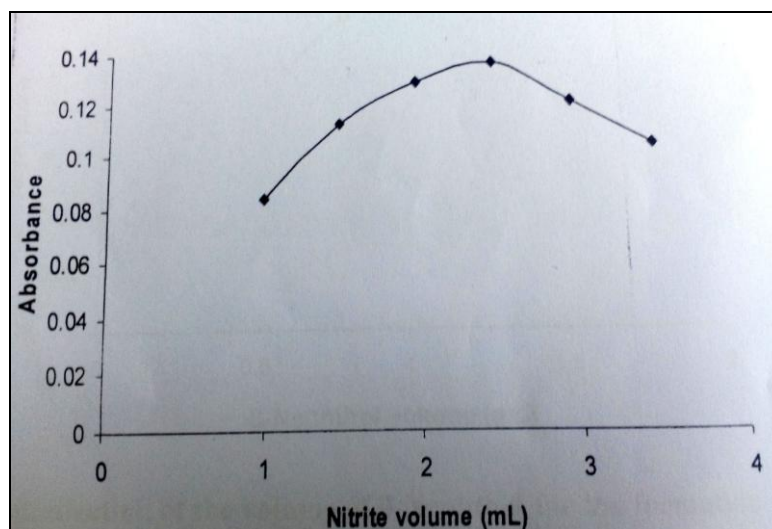


FIG.4: OPTIMIZATION OF THE VOLUME OF NITRITE SOLUTION FOR THE FORMATION OF AZO DYE

Investigation of the effect of β -Nepthol on the formation and absorbance behavior of azo dye:

Instruments: The same as mentioned before

Reagents: The same as mentioned before

Solution: The same as mentioned before

Procedure:

Ceftriaxone sodium standard solution 1 mL from 1000 ppm stock solution was transferred to five

separate 50mL volumetric flasks and to each of these flasks 1.0 mL of 0.1 N HCL was added followed by the addition of 2.5 mL (1000 ppm) sodium nitrite solution. The resulting solutions were kept for 5 minutes for the formation of the diazotized products. Then varied volume of β -Nepthol (1000 ppm) ranging from 0.6-1.4 mL was added as coupling reagent. The blank solution was prepared in the same manner without the addition of the analyte Ceftriaxone sodium. The absorbance

of the resulting radish color azo dye was measured at 510 nm using Genesys 5 spectrophotometer, for finding out optimum concentration of β -Nephthol.

The results are given in **Table 3** and are shown in **Fig.5**.

TABLE 3: OPTIMIZATION OF THE VOLUME OF β -NEPHTHOL FOR THE FORMATION OF AZO DYE

Volume used in (mL)	0.6	0.8	1.0	1.2	1.4
Absorbance	0.092	0.112	0.117	0.139	0.131

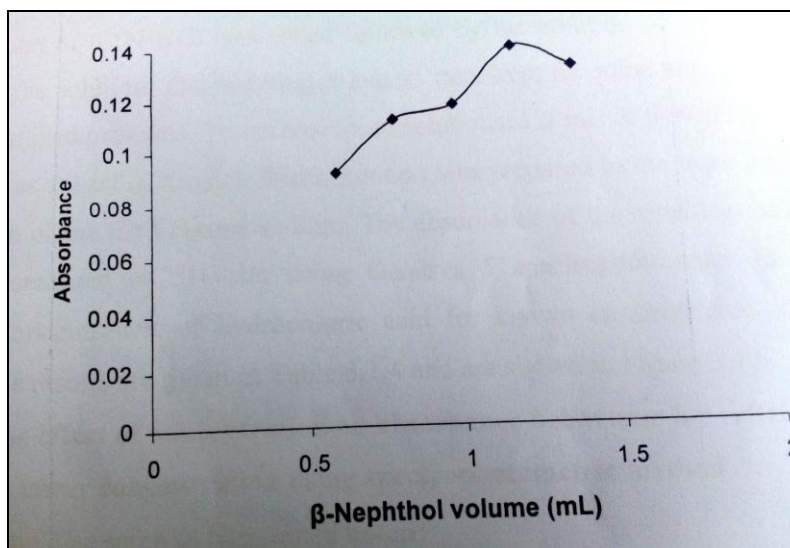


FIG.5: OPTIMIZATION OF THE VOLUME OF β -NEPHTHOL FOR THE FORMATION OF AZO DYE.

Conditions:

λ_{\max}	510 nm
Ceftriaxone solution (1000 ppm)	1.0 mL
β -Nephthol (1000 ppm)	1.2 mL
Hydrochloric acid 0.1N	1.0 mL

Investigation of the effect of hydrochloric acid (0.1N) on the formation and absorbance behavior of azo dye:

Instruments: The same as mentioned before

Reagents: The same as mentioned before

Solutions: The same as mentioned before

Procedure: Ceftriaxone sodium standard solution 1 mL from 1000 ppm stock solution was transferred to five separate 50 mL volumetric flasks, to each of these flask varied concentration of 0.1N HCL was added followed by the addition of 2.5 mL (1000 ppm) sodium nitrite solution. The resulting solutions were kept for the sometime for the formation of the diazotized products. To each of these solutions, 1.2 mL of β -Nephthol (1000 ppm) was added as coupling reagent. Blank solution was prepared in the same manner without the addition as Ceftriaxone sodium.

The absorbance of the resulting radish color azo dye was measured at 510 nm using Genesys-5 spectrophotometer for the finding out optimum concentration of hydrochloric acid for known concentration of Ceftriaxone sodium. The results are given in **Table 4** and are shown in **Fig.6**.

TABLE 4: OPTIMIZATION OF THE VOLUME OF HYDROCHLORIC ACID FOR THE FORMATION OF AZO DYE

Volume used in (mL)	0.5	1.0	1.5	2.0	2.5
Absorbance	0.094	0.124	0.106	0.098	0.081

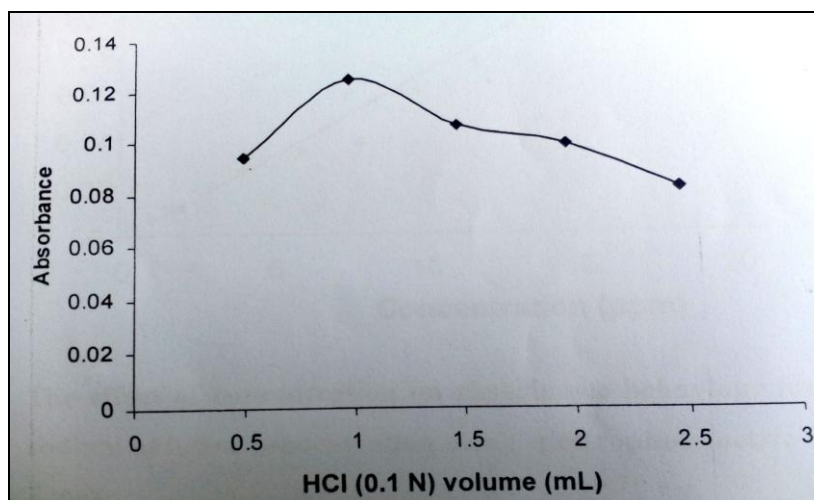


FIG.6: OPTIMIZATION OF THE VOLUME OF HYDROCHLORIC ACID FOR THE FORMATION OF AZO DYE.

The effect of concentration on absorbance behavior for the Ceftriaxone sodium at lower concentration using spectrophotometer method:

Instruments: The same as mentioned before

Reagents: The same as mentioned before

Solutions: The same as mentioned before

Procedure:

Varied amount of standard Ceftriaxone sodium solution with final concentration after dilution ranging from 1-20 ppm were taken in eleven separate 50 mL volumetric flasks. To each of these

flasks 1.0 mL of 0.1N HCL was added followed by the addition of 2.5 mL (1000 ppm) Sodium nitrite solution. The resulting solutions were kept for 5 minutes for the formation of the diazotized product, followed by the addition of 1.2 mL of β -Nephthol (1000 ppm) as coupling reagent. Blank solution was prepared in the same manner without the addition of the Ceftriaxone sodium. The absorbance of the resulting color azo dye was measured at 510 nm using Genesys-5 spectrophotometer to find out absorbance behavior near detection limit. The results are given in **Table-5** and are shown in **Fig.7**.

TABLE 5: THE EFFECT OF CONCENTRATION ON THE ABSORBANCE BEHAVIOR FOR CEFTRIAXONE SODIUM AT LOWER CONCENTRATION USING SPECTROPHOTOMETER METHOD

Ceftriaxone	1.0	2.0	4.0	6.0	8.0	10	12	14	16	18	20
Absorbance	0.006	0.011	0.026	0.034	0.037	0.059	0.070	0.092	0.108	0.117	0.132

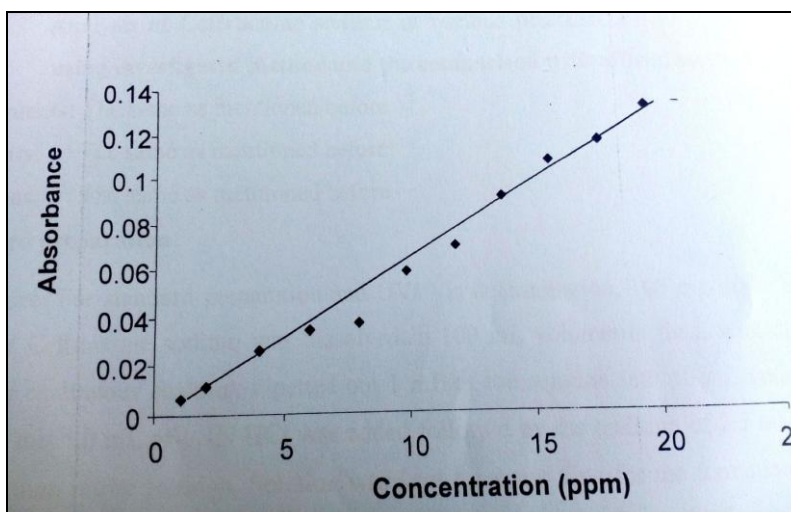


FIG.7: THE EFFECT OF CONCENTRATION ON ABSORBANCE BEHAVIOR FOR CEFTRIAXONE SODIUM AT LOWER CONCENTRATION USING SPECTROPHOTOMETRIC METHOD.

Conditions:

λ max	510 nm
Ceftriaxone solution (1000 ppm)	1.0 mL
β -Nepthol (1000 ppm)	1.2 mL
Sodium nitrite (1000 ppm)	2.5 mL

Analysis of Ceftriaxone sodium in various pharmaceutical preparations using investigated method and the comparison with official method.**Conditions:**

λ max	510 nm
Ceftriaxone solution (1000 ppm)	1.0 mL
β -Nepthol (1000 ppm)	1.2 mL
Hydrochloric acid 0.1N	1.0 mL
Sodium nitrite (1000 ppm)	2.5 mL

Instruments: The same as mentioned before**Reagents:** The same as mentioned before**Solutions:** The same as mentioned before**Standard preparation:**

Procedure: For standard preparation and UV/Vis determination, 100 mg of standard Ceftriaxone sodium was dissolved in 100 mL volumetric flask with distilled water by continuous shaking. Pipette out 1 mL of the solution into 50 mL volumetric flask, to this 1.0 mL of 0.1N HCL was added followed by the addition of 2.5 mL (1000 ppm) sodium nitrite solution. Solution was kept for 5 minutes for the formation of the diazotized product. Then 1.2 mL of β -Nepthol (1000 ppm) was added as coupling reagent. Blank solution was prepared in the same manner without the addition of the Ceftriaxone sodium. The absorbance of the resulting reddish color azo dye was measured at 510 nm using Genesys 5 Spectrophotometer.

Sample preparation:

Weigh the contents of 10 vials and take average weight of 10 vials. Weigh out 100 mg of powder in 100 mL volumetric flask and dissolved it with distilled water. Shake vigorously make up the volume with distilled water. Pipette out 1 mL of the

solution into 50 mL volumetric flask to this 1.0 mL of 1.0N HCL was added followed by the addition of 2.5 mL (1000 ppm) sodium nitrite solution. Solution was kept for some time for the formation of the diazotized product. Then 1.2 mL of β -Nepthol (1000 ppm) was added as coupling reagent. The blank was prepared in the same manner without the addition of the Ceftriaxone sodium. The absorbance of the resulting color azo dye was measured at 510 nm using Genesys 5 spectrophotometer.

Calculations:

$$\% \text{ age} = \frac{\text{Au}}{\text{As}} \times \frac{\text{std wt}}{100} \times \frac{1}{50} \times \frac{100}{\text{sp wt}} \times \frac{50}{1} \times \frac{\text{potency of std}}{100} \times \text{avg wt}$$

Au = Absorbance of standard solution

As = absorbance of sample solution

Std wt = weight of standard taken

Sp wt = weight of sample taken

Potency of std = potency of standard

Avg wt = average wt of 10 samples

Determination of Ceftriaxone sodium by official method (HPLC method):

Buffer pH 7.0 – Dissolved 13.6g of dibasic potassium phosphate and 4.0g of monobasic potassium phosphate in water to obtained 1000 mL of solution adjusted the solution with phosphoric acid or 10N potassium hydroxide to pH 7.0 ± 0.1 .^{5,6}

Buffer pH 5.0 - Dissolved 25.8 g sodium citrate in 500 mL of water, adjusted pH of the solution with citric acid solution (1 in 5) to a pH of 5.0 ± 0.1 and diluted with water to volume of 1000 mL.^{5,6}

Mobile phase- Dissolved 3.2 g of tetraheptyl ammonium bromide in 4 mL of pH 5.0 buffer, and made up volume with water up to 1000 mL, filtered through a membrane filter of 0.5 μm .⁷

Standard preparation:

Dissolved an accurately weighed quantity of USP Ceftriaxone sodium in mobile phase, to obtain a solution having a known concentration of 0.2 mg/mL. Resolution- Dissolved a quantity of USP Ceftriaxone sodium E-isomer in standard preparation and diluted with mobile phase to obtain

a solution containing about 100 µg of Ceftriaxone USP E-isomer per mL and 160 µg of USP Ceftriaxone sodium.

Assay preparation: Transferred about 40 mg of Ceftriaxone sodium to a 200 mL volumetric flask, dissolved in and diluted with mobile phase to volume.

Chromatographic system:

Wavelength: 270 nm detector

Flow rate: 2 mL per minute

Column: 4.0 mm x 15 cm L1 packing

Procedure:

Separately injected equal volumes of (20 µL) of the standard preparation and the assay preparation into chromatograph, record the chromatograms, and measured the response for major peaks.⁷

Calculation: calculated in µg of Ceftriaxone per mg of Ceftriaxone sodium taken by formula:

$$200(C_p/W) (r_u/r_s)$$

C= concentration in mg/mL

P= potency of standard

W= weight in mg

R_u and r_s = Ceftriaxone sodium peak response obtained from assay and standard preparation, respectively.

RESULTS AND DISCUSSION: The proposed chemical reaction involved in this procedure is shown in Fig.2 Ceftriaxone have amine group in molecule. In this procedure the amine was directly exploited for the nitrite solution to form the azo dye. In the first step Ceftriaxone was reacted in acidic medium with nitrite solution to form the diazotized product, which was subsequently coupled with β-Nepthol. A colored azo dye was the final product. Various optimization studies for the parameters like wavelength, hydrochloric acid volume, sodium nitrite concentration and β-Nepthol concentration were investigated for the formation of maximum azo dye. Calibration curve, limit of detection, limit of quantification, RSD, correlation coefficient, molar absorptivity were also investigated for the proposed method.

After preliminary experiments, the azo dye formed was investigated for optimum wavelength. The results are given in Table 1 and are shown in Fig.3 The resulting azo dye has maximum absorbance at 510 nm and was used as optimum wavelength for further investigation of Ceftriaxone sodium determination.

Sodium nitrite solution (1000 ppm) was used for the diazotization of Ceftriaxone sodium. Various volumes of nitrite solution (1000 ppm) in the range of 1 - 3.5 mL were tried for reaction with 20 ppm of Ceftriaxone sodium concentration to form the maximum azo dye. The results are given in Table 2 and are shown in Fig.4 As can be seen in Fig.2 that 2.5 mL of nitrite solution was found to be optimum volume for the maximum azo dye formation with 20 ppm of Ceftriaxone sodium, this optimum volume of nitrite solution was used for the further investigation of Ceftriaxone determination.

The volume of 0.1N hydrochloric acid was also optimized for the formation of maximum azo dye for 20 ppm Ceftriaxone sodium. The results are given in table-4 and are shown in figure-6 as can be seen from table-4, 0.1N hydrochloric acid 1.0 mL was found to be optimum volume and was used in further studies.

After optimization of the diazotizing agent the volume of the coupling reagent β-Nepthol (1000 ppm) was optimized for the 20 ppm of Ceftriaxone sodium to form the maximum dye. The results are given in Table 3 and are shown in Fig.5 As can be seen from Table 3 That 1.2 mL of β-Nepthol solution was found to be the optimum volume of the coupling reagent and was used further for the studies of Ceftriaxone sodium.

At optimum condition the effect of concentration at lowers level on the absorbance behavior of Ceftriaxone sodium was investigated to calculate the limit of detection (LOD) and limit of quantification (LOQ). Ceftriaxone sodium 2 ppm was selected for investigation of detection limit as this was the minimum concentration for which the absorbance could be noted. Six replicate readings were taken for this concentration. The results are given in Table 6 molar absorptivity, LOD, LOQ were calculated and are given in Table 7. The

following formulas were used for calculation of LOD, LOQ, S.D and R.S.D.

TABLE 6: REPLICATE READING FOR 2ppm CONCENTRATION OF CEFTRIAXONE SODIUM

Absorbance	Concentration (ppm) found (X)
0.011	2.0
0.010	1.8
0.009	1.6
0.012	2.2
0.009	1.6
0.011	2.0

TABLE 7: PARAMETERS DETERMINED & CALCULATED

λ max	510 nm
Celebration range	1-20 μ g
Standard deviation	0.242
R.S.D	12.96
Slope	0.0060
Correlation coefficient	0.999
M	2.719×10^{-5}
Σ	$4.045 \times 10^{+3}$
L.O.D	0.726
L.O.Q	2.42

TABLE 8: QUANTATIVE ANALYSIS OF CEFTRIAXONE SODIUM IN PHARMACEUTICAL INJECTIONS BY SPECTROPHOTOMETRIC METHOD AND COMPARISON WITH HPLC METHOD (OFFICIAL)

s.#	Name of drug	Label claim	Investigated method	Official method
1	Cefcin	250 mg/ vial	252.24 mg/vial \pm 0.873	251.21 mg/vial \pm 0.242
2	Efxone	250 mg/ vial	250.76 mg/vial \pm 1.296	253.04 mg/vial \pm 1.640
3	Axone	250 mg/ vial	251.8 mg/vial \pm 1.389	251.62 mg/vial \pm 0.779

CONCLUSION: The method for spectrophotometric determination of Ceftriaxone sodium is based on direct exploitation of the primary aromatic amine group for diazotization followed by formation of azo dye. Various analytical conditions like wavelength, nitrite solution hydrochloric acid volume and coupling reagents were optimized and were found to be 510nm, 2.5mL (1000 ppm), 1.0 mL (0.1 N) and 1.2 mL (1000 ppm), respectively for 20 ppm of Ceftriaxone sodium. The method was validated and compared with literature method for the analysis of Ceftriaxone sodium in pharmaceutical preparations. The method was found linear in range of 1-20 ppm. The limit of detection and limit of quantification for investigated method were found to be 0.726 ppm and 2.42 ppm respectively, while standard deviation was found to be 0.242. The method is very sensitive with comparable in simplicity and reproducibility.

Limits of detection (for concentration) = $3 \times S$

Limits of quantifications (for concentration) = $10 \times S$

Standard deviation $S = \sqrt{n/n-1}$

Relative standard deviation R.S.D = $S/X^- \times 100$

Where as

$n = X - X^-$

X= Concentration in (ppm) found

X^- = Average founded concentration (ppm) of six samples

Application of the investigated method for the analysis of Ceftriaxone sodium in various pharmaceutical preparation and comparison with official method:

The investigated method was applied for determination of Ceftriaxone sodium in different injections. The method used for determination and calculation has been shown above and was compared with official method, While the results of comparison have been shown in **Table 8**.

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