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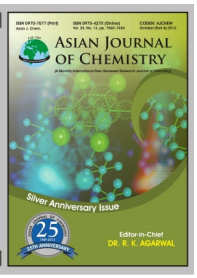

Quantitative Analysis of Ceftriaxone Sodium in Parenteral Dosage Form

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Simple and sensitive kinetic spectrophotometric methods are described for the determination of ceftriaxone sodium in its pure form and parenteral dosage from. The kinetic method is based on spectrophotometric studies of the oxidation of ceftraixone with sodium permanganate in basic medium. Parameters affecting the colour developments are examined and optimized. The developed analytical procedure was validated for its linearity, range, accuracy and precision, limit of detection and limit of quantitation. The calibration curves were linear over the range of 5-45 μg mL^{-1}. The limit of detection and limit of quantitation of the initial rate and fixed time method was found out to be 0.128, 0.558, 0.387 and 1.78, respectively. The possible mechanism of the reaction is also proposed.

Key Words: Ceftriaxone sodium, Kinetic method, Spectrophotometry, Sodium permanganate.

INTRODUCTION

Ceftriaxone is (6R,7R)-3[(acetyl-oxy)methyl]-7-[[2Z) -(2-amino-4-thiazolyl)(methoxyamino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0.]oct-2-ene-2-carboxylic acid[1]. Ceftriaxone, a semi-synthetic antibiotic from the third generation cephalosporin group, for intramuscular or intravenous parenteral administration. It is administered through parenteral route because it is not significantly absorbed from the gastrointestinal tract. Their bactericidal activities result from the inhibition of the bacterial cell wall synthesis, this inhibition is mediated through the drug binding to penicillin binding proteins (PBPs). Similar to that of other cephalosporin of its generation ceftriaxone also has a broad activity against many gram positive and gram negative bacteria[2]. In addition to its use in the treatment in the lower respiratory tract infection, it can also be administered for the treatment of skin, bone, joint, intra-abdominal and urinary tract infection. It can also be used for the perisurgery prophylaxis. Several methods such as flow injection[3,4], fluorimetry[5], thin layer chromatography[6-8] have been reported for the estimation of ceftriaxone. Ceftriaxone alone was determined by HPLC[9-11] with other drugs by HPLC[12-14], polarography[15], spectrophotometry[16-21]. The survey of literature suggest that the potassium permanganate is one of the most widely used reagent for the oxidation of drugs[22-24] whereas, literature on sodium permanganate in drug determination still lacking. There are few advantages of $NaMnO_4$ which includes about 15 fold more solubility than $KMnO_4$ and main advantage is that it is applied where high concentration of MnO_4^- are required, can be used instead of $KMnO_4$ whenever the potassium ion cannot be tolerated or if dusting is a critical issue. The current paper put forward $NaMnO_4$ as an alternate to $KMnO_4$ for the oxidation of variety of drug leading to its determination in pure and dosage form.

EXPERIMENTAL

Evolution 300, thermo UV-visible spectrophotometer, with matched quartz cells was employed to measure the change in absorbance with respect to time.

All the reagents used during the experiment were of analytical or pharmaceutical grade and the solutions were prepared in Milli Q water. The ceftriaxone was obtained from Sigma, USA and was used as received. Aqueous solutions of 1.0×10^{-1} M sodium hydroxide and 1.83×10^{-4} M sodium permanganate (BDH, Poole England) were prepared in Milli Q water. Sodium permanganate (BDH, Poole England) solution should be freshly prepared before conducting experiment. Parenteral dosage form of ceftriaxone namely mesporin (MephaLtd., Aesch-Basel, Switzerland) and rochephin (Roche) were purchased from the local market.

Standard drug solution: The standard test solution of 0.05 % ceftriaxone (Sigma, USA) was prepared by dissolving 50 mg of ceftriaxone in 100 mLMilli Q water.

Procedure for kinetic measurements

Initial-rate method: Aliquots of sodium permanganate 1.83×10^{-4} M (2 mL) and NaOH 1×10^{-1} M (2.2 mL) were transferred into a series of 10 mL volumetric flasks. Accurate

volumes of the working solution of ceftriaxone (0.1- 0.9 mL) were added to each flask and diluted up to the mark with Milli Q water. The content of each flask were mixed and were transferred instantly to the spectrophotometric cell. The absorbance as a function of time was recorded at λ_{max} - 612 nm. The initial rate of reaction (n) at various ceftriaxone concentrations were derived from the slope of the tangent of the absorbance-time plot. The calibration scheme was prepared by plotting the log of initial rate of reaction (log ν) against the log of molar concentrationof ceftriaxone (log C). The ceftriaxone amount was calculated either from the calibration plot or the regression equation.

Fixed-time method: The absorbance of each ceftriaxone sample solution was measured at λ_{max}-612 nm against a reagent blank prepared similarly at a preselected fixed time of 12 min. The calibration curve was prepared by plotting the absorbance against the final concentration of the drug. The amount of the drug was computed either from calibration plot or linear regression equation.

Procedure for determination of studied compounds in parenteral dosage forms: The content of mesporin and rochephin equivalent to 1000 mg was reconstituted in 100 mL and then 5 mL of the reconstituted solution was dilute up to the mark in 100 mL standard volumetric flask to prepare the required solution. An aliquot of the cited solutions was taken and the procedure defined for the estimation of ceftriaxone was applied. The nominal content was calculated either from a previously plotted calibration graph or using the regression equation.

Method validation: Validity of the proposed method based on the sodium permanganate oxidation has been checked by validating the method for its specificity, linearity, accuracy and precision, recovery, limit of detection and limit of quantitation.

Specificity: Stress condition such as acid, base and UV light were applied to the samples of ceftriaxone. All the stress samples were analyzed for the ceftriaxone content and compared to unstressed reference samples and interference due to the common excipients (sodium stearyl fumarate, magnesium stearate, starchand lactose) were checked using the proposed methods.

Linearity: Linearity of the proposed initial rate and fixed time methods were assessed in the concentration range of 5-45 μg/mL.

Accuracy and precision: Accuracy and precision of the methods were determined on the basis of quality control samples of ceftriaxone. The quality control samples were analyzed at concentration levels of 5, 25 and 40 μg/mL. The quality control samples at each concentration level were analyzed for run to run and day to day precision. Standard deviation (S.D), relative standard deviation (RSD, standard analytical errors (SAE) and confidence limit were calculated using the standard methods.

Recovery studies: The recovery studies were carried out using the standard addition procedures. In this experiment known amount of pure ceftriaxone was added to pre-analyze parenteral dosage form of ceftriaxone at three concentration levels.

Limit of detection and limit of quantitation: As per the guidelines of the international conference on harmonization (ICH)[25], the following expression used to calculate limit of detection (LOD) and limit of quantification (LOQ).

$$LOD = 3.3\times\frac{S_o}{b} \text{ and } LOQ = 10\times\frac{S_o}{b}$$

where, S_o and b are the standard deviation and the slope of the calibration curve, respectively.

Evaluation of bias: The evaluation of bias has been done by point and interval hypothesis tests[26,27]. In the interval hypothesis test the proposed method (method 2) is acceptable when the true mean is within ± 2 % of that of reference method (method 1) *i.e.* $-0.02\mu_1 < (\mu_2 - \mu_1) < 0.02\ \mu_1$ the above equation can also be written as

$$\frac{0.98 < \mu_2}{\mu_1 < 1.02}$$

This can be generalized to

$$\frac{\theta_L < \mu_2}{\mu_1 < \theta_U}$$

where, θ_L and θ_U are lower and upper acceptance limits, respectively. The limits of this confidence interval can be calculated as the two roots of the following quadratic equation

$$\theta^2\left(\overline{x_1^2} - \frac{S_p^2 t_{tab}^2}{n_1}\right) - 2\theta\overline{x_1 x_2} + \theta^2\left(\overline{x_2^2} - \frac{S_p^2 t_{tab}^2}{n_2}\right) = 0$$

where,

$$a = \overline{x_1^2} - \frac{S_p^2 t_{tab}^2}{n_1}$$

$$b = -2\overline{x_1 x_2}$$

$$c = \overline{x_2^2} - \frac{S_p^2 t_{tab}^2}{n_2}$$

The values of θ_L and θ_U of the confidence interval can be obtained as:

$$\theta_L = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$$

$$\theta_U = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

RESULTS AND DISCUSSION

For spectral studies, all the solutions were prepared in Milli Q water. During the studies it was observed that ceftriaxone absorbs maximally at 240 nm while, the reagent blank has a λ_{max} at 526 nm. The reaction initiates with the addition of aqueous alkaline sodium permanganate solution to the ceftriaxone solution resulting in shifting of absorption band to 612 nm (Fig. 1). The appearance of band at 612 nm could be attributed to the formation of manganate ion in presence of ceftriaxone. It was also observed that the absorbance of the coloured product varied with change in time due

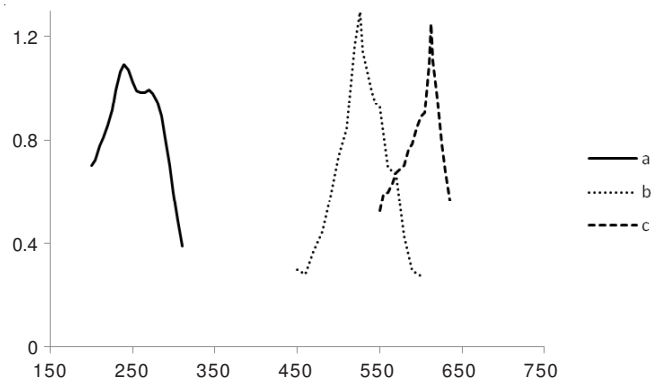
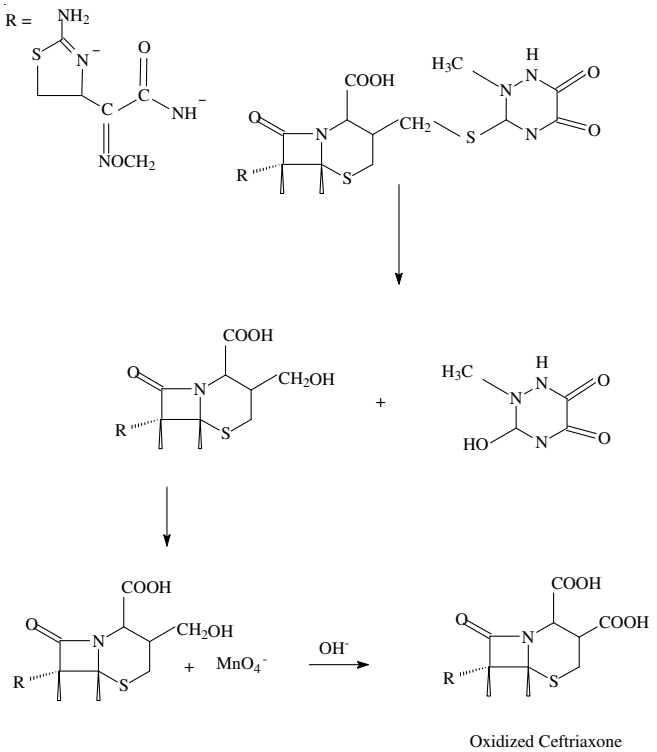


Fig. 1. Absorption spectra of (a) 4.18×10^{-5} M ceftriaxone sodium (b) 1.83×10^{-5} M $NaMnO_4$ + 1.5×10^{-2} M NaOH (c) 7.52×10^{-5} M ceftriaxone sodium + 3.66×10^{-5} M $NaMnO_4$ + 2.2 $\times 10^{-2}$ M NaOH

to the oxidation of drug and this property has been exploited to determine the drug kinetically.

Mechanism of colour reaction: Zajac and muszalska[28] in their study have reported that hydrolysis of ceftriaxone in basic medium. In the first step of hydrolysis a -CH_2-S bond split product leading to formation of-CH_2OH at C-3. Further it has been reported[23] that CH_2OH undergoes oxidation to form carboxylic acid derivative of the hydrolyzed product. The reaction proceeds in the same way and MnO_4^- oxidizes the ceftriaxone in basic medium. On the basis of previous studies a probable mechanism can be proposed which is shown in **Scheme-I**.



Scheme-I: Mechanism of ceftriaxone oxidation with $NaMnO_4$

Optimization of variables: The optimum condition for the proposed methods which leads to the formation of green coloured MnO_4^- ion were studied and the same conditions were maintained during the whole experimental process.

The effect of the sodium permanganate concentration on the initial rate of reaction (v) was studied in the concentration range 1.82×10^{-6} to 4.03×10^{-5} M. The initial rate of reaction increased with increase in $NaMnO_4$ concentration and became constant at 2.93×10^{-5} M concentration. Further increase in concentration (up to 4.03×10^{-5} M) showed no appreciable change in reaction rate. Therefore, 3.6×10^{-5} M $NaMnO_4$ concentration was used as an optimum value for the assay procedure. The results are shown in Fig. 2. In the same way to optimized the reaction condition and maintain the basic medium the concentration of NaOH also needs to be optimized. Fig. 3 shows the effect of NaOH on colour development. It was observed that the on varying NaOH concentration (up to 0.016 M), the absorbance varied while, it became constant from 0.018 to 0.025 M. Thus, 0.022 M NaOH concentration was found to be most appropriate for oxidation reaction.

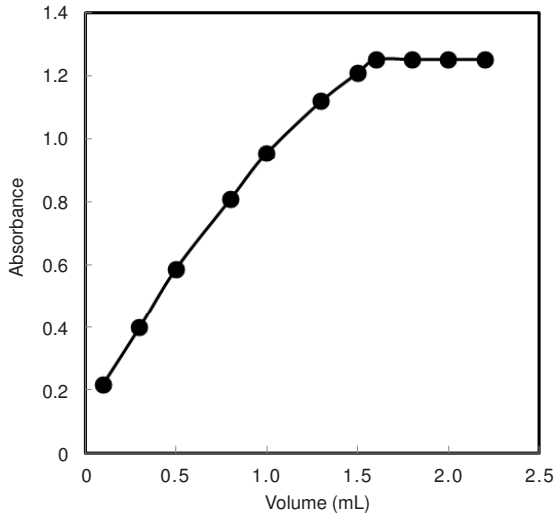


Fig. 2. Effect of volume of $NaMnO_4$ (1.83×10^{-5} M) on color development

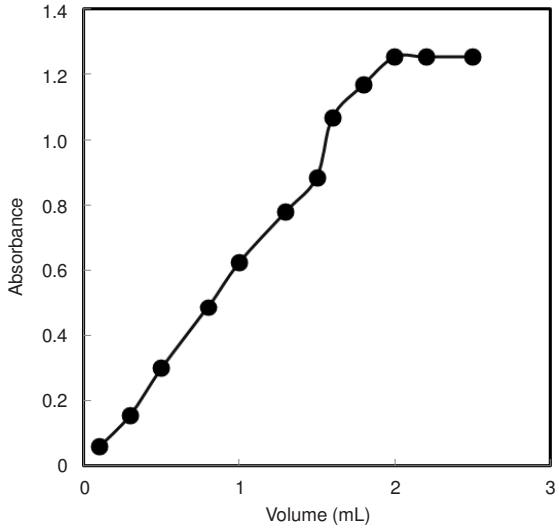


Fig. 3. Effect of volume of NaOH (1.0×10^{-1} M) on color development

Analytical parameters and method validation: Under the optimized and validated experimental conditions, both the initial rate and fixed time method were found to be linear over the concentration range of 5 to 45 µg/mL.The statistical treatment of calibration data (n = 9) yield intercept (a), slope (b),

correlation coefficient (r), confidence limit of intercept ($\pm tS_a$) and slope ($\pm tS_b$) at 95 % confidence level. The results are summarized in Table-1. The higher value of correlation coefficient refers excellent linearity and lower variance indicates higher reproducibility of the newly developed methods.

TABLE-1
OPTICAL CHARACTERISTICS AND ANALYTICAL DATA

Parameters	Values	
	Initial rate	Fixed time
Linear dynamic range (μg mL^{-1})	5-45	5-45
Regression equation	$\nu = 2.64 + 0.933C$	$A = - 0.02711 + 0.02833C$
Correlation coefficient (r)	0.9932	0.9993
Sa	1.84×10^{-1}	3.67×10^{-3}
Sb	4.11×10^{-2}	1.30×10^{-4}
Standard deviation	0.0363	0.0050
Variance	1.13×10^{-3}	2.55×10^{-5}
Limit of detection	0.128	0.588
Limit of quantitation	0.3871	1.78

Kinetic parameters were performed under the pseudo order reaction at 25 ± 1 ºC. The initial rates of the reaction were estimated from absorbance-time plot (Fig. 4) by measuring the slopes of the initial tangent to the absorbance-time plot and are summarized in Table-2. The kinetic calculation for the oxidation of ceftriaxone by $NaMnO_4$ in alkaline medium is written as:

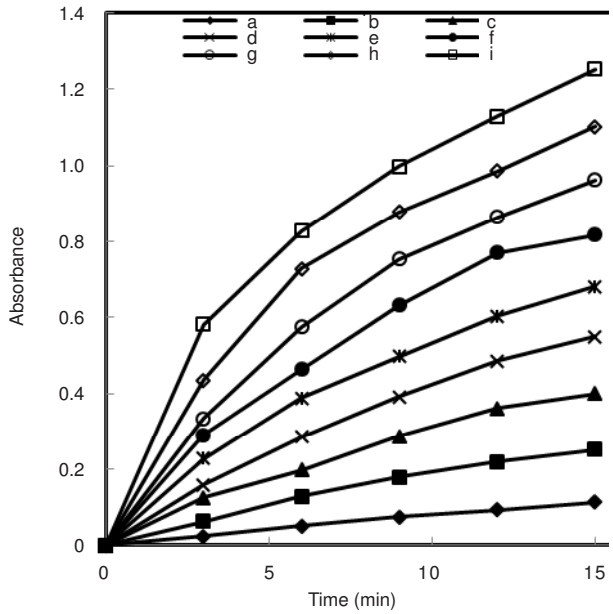


Fig. 4. Absorbance-time plot for oxidation of ceftriaxone with $NaMnO_4$ in basic medium: 3.66×10^{-5} M [$NaMnO_4$] and (a) 8.35338×10^{-6}, (b) 1.67068×10^{-5}, (c) 2.50601×10^{-5}, (d) 3.34135×10^{-5}, (e) 4.17669×10^{-5}, (f) 5.01203×10^{-5}, (g) 5.84737×10^{-5}, (h) 6.68271×10^{-5}, (i) 7.51804×10^{-5} M ceftriaxone sodium concentrations

$$\nu = \frac{d_x}{d_t} = k[C]^n[NaMnO_4]^m[NaOH]^l \quad (1)$$

For $[NaMnO_4] \geq 2.93 \times 10^{-5}$ M and $[NaOH] \geq 2.0 \times 10^{-2}$ M

TABLE-2
INITIAL RATE OF REACTION AT DIFFERENT CONCENTRATIONS OF CEFTRIAXONE KEEPING [$NaMnO_4$] AND [NaOH] CONSTANT

C [ceftriaxone] (mol/L × 10^{-5})	log C	Initial rate of reaction (ν)	log ν × 10^{-2}
0.83	-5.078	-2.1314	0.74
1.67	-4.777	-1.8085	1.55
2.50	-4.601	-1.6256	2.36
3.34	-4.476	-1.4856	3.26
4.17	-4.379	-1.4273	3.73
5.01	-4.299	-1.3423	4.54
5.84	-4.233	-1.2880	5.15
6.68	-4.175	-1.2747	5.31
7.51	-4.123	-1.2619	5.47

Eqn. 1 thus reduced to

$$Rate = k_\infty [C]^n \quad (2)$$

where, ∞ is pseudo order rate constant. C is ceftriaxone concentration and n is the order of reaction.

The equation in the logarithmic form can be written as:

$$\log(rate) = \log k_\infty + n \log C \quad (3)$$

Under the optimum experimental conditions the plot of initial rate *versus* concentration was observed to follow the linearity in the range 5-45 μg/mL. The linear regression equation thus obtained from the plot of initial rate *versus* concentration can be written as:

$$\log(rate) = 2.649 + 0.9338 \log C \quad (4)$$

with coefficient of correlation (r) - 0.9932.

Accuracy and precision of proposed methods: Three different concentration levels *i.e.*, lower, medium and higher were selected to determine the accuracy and precision of the methods proposed. These assays were investigated by taking five independent analyses at 5, 25 and 40 μg/mL concentration levels for 5 days while, intraday assay samples were analyzed within a day. The standard deviation, relative standard deviation, analytical errors obtained using the proposed methods are well within the acceptable range and thus can be considered satisfactory. The results are summarized in Table-3.

Recovery studies: Standard addition method was used to evaluate drug recovery by proposed methods. In standard addition method, known amount of the pure ceftriaxone was added to pre-analyze formulated formof ceftriaxone at three concentration levels by measuring five replicate analyses following the recommended procedures for the determination of active drug. The results are summarized in Table-4. Excellent recoveries (99.05-100.49 %) with low values of relative standard deviation were observed.

Robustness: The reaction conditions for ceftriaxone oxidation and determination thereafter are very robust. Each working parameter was investigated and tested for robustness. The parameters investigated are as follows.

Volume of 4.03×10^{-5} M $NaMnO_4$ (± 0.2 mL)

Volume of 1.02×10^{-2} M NaOH (± 0.3 mL)

Under the above experimental conditions sample solutions from three dosage forms containing 45 μg/mL of active ceftriaxone was assayed by performing five independent analyses by the initial rate and fixed time methods. The results of mean recovery, standard deviation and relative standard deviation indicated good sensitivity and appreciable recovery.

TABLE-3
EVALUATION OF ACCURACY AND PRECISION OF THE PROPOSED METHODS BY INTRADAY AND INTERDAY ASSAYS

Proposed methods	Amount taken (μg/mL)	Found ± SD[a]	RSD (%)	SAE[b]	CL[c]
		Initial rate			
Intraday assay	5	5.00 ± 0.03	0.695	0.016	0.04
	25	25.21 ± 0.17	0.679	0.897	0.21
	40	39.99 ± 0.07	0.432	0.078	0.21
Interday assay	5	5.01 ± 0.04	0.854	0.019	0.05
	25	25.32 ± 0.21	0.837	0.094	0.26
	40	40.35 ± 0.53	1.306	0.235	0.65
		Fixed time			
Intraday assay	5	5.00 ± 0.04	0.745	0.017	0.04
	25	25.36 ± 0.21	0.836	0.095	0.26
	40	40.01 ± 0.12	0.299	0.054	0.15
Interday assay	5	5.07 ± 0.05	0.975	0.022	0.06
	25	25.09 ± 0.26	1.049	0.117	0.31
	40	40.40 ± 0.29	0.720	0.129	0.36

[a]Mean of five independent analyses; [b]SAE, standard analytical error; [c]CL, confidence limit at 95 % confidence level and 4 degree of freedom (t =2.776).

TABLE-4
STANDARD ADDITION METHOD FOR DETERMINATION OF CEFTRIAXONE IN PARENTERAL DOSAGE FORMS

Methods	Formulation	Taken	Added	Found ± SD[a]	Recovery (%)	SAE	CL
Initial rate	1	5	5	9.91 ± 0.01	99.11	0.058	0.16
		20	5	25.03 ± 0.10	100.10	0.045	0.12
		35	5	40.03 ± 0.03	100.08	0.050	0.15
	2	5	5	10.01 ± 0.01	100.08	0.050	0.14
		20	5	24.98 ± 0.06	99.90	0.029	0.08
		35	5	39.93 ± 0.40	99.82	0.179	0.50
Fixed time	1	5	5	9.91 ± 0.08	99.05	0.035	0.10
		20	5	25.06 ± 0.27	100.24	0.124	0.33
		35	5	40.14 ± 0.17	100.35	0.780	0.22
	2	5	5	10.04 ± 0.13	100.49	0.059	0.16
		20	5	25.00 ± 0.28	100.01	0.124	0.35
		35	5	40.08 ± 0.58	100.19	0.268	0.73

[a]Mean of five independent analyses.

Thus, the conditions of the proposed initial rate and fixed time methods are very robust.

Stability of solution: The stability of the test solution of ceftriaxone in Milli Q water was studied by recording UV absorption spectra of the drug for three consecutive days. The ceftriaxone solution, having λ_{max} at 240 nm showed no change in the absorption spectra of the test and sample solutions of drug for the assigned time period, when the solutions were stored at room temperature in the dark.

Applicability of proposed methods: The proposed methods were successfully applied to the determination of ceftriaxone in formulated product. The results of the methods proposed were compared with those of the reference method[18] using point and interval hypothesis tests and are summarized in Table-5. The calculated t- (paired) and F- values at 95 % confidence level do not exceed the theoretical ones[26] which indicates that there are no significant differences between the performance of the proposed methods and the reference method. A bias of ± 2 %, which is based on recovery experiments, is permissible by the Canadian Health Protection Branch[27]. Therefore, the acceptable limit lies within θ_L = 0.98 and θ_U = 1.02. It is evident from Table-5 that the true bias of all samples of drug is smaller than ± 2 % thus, confirming that the proposed methods are reliable with acceptable recovery.

TABLE-5
POINT AND INTERVAL HYPOTHESIS TEST: EVALUATION OF APPLICABILITY OF THE PROPOSED METHOD WITH THE REFERENCE METHOD AT 95 % CONFIDENCE LEVEL

Formulations	Parameters	Methods		
		Initial rate	Fixed time	Reference
Mesporin	Recovery (%)	99.99	99.62	100.08
	RSD	1.79	1.02	0.561
	θ_L	0.991	0.999	–
	θ_U	1.006	1.003	–
	t	0.337	1.69	–
	F	0.614	0.304	–
Rochepin	Recovery (%)	100	99.54	99.50
	RSD	1.03	0.935	0.228
	θ_L	0.994	0.998	–
	θ_U	1.003	1.002	–
	t	0.541	0.698	–
	F	1.85	0.369	–

Conclusion

Initial rate and fixed time methods were applied for the routine quality control analysis of ceftriaxone in parenteral dosage forms. The proposed method does not require any laborious clean up procedure prior to analysis and therefore, can be frequently used in research laboratories, hospitals and pharmaceutical industries. The proposed method is sensitive, selective and having low limit of detection.

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