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Stability Indicating Assay For Ceftriaxone Sodium By Rp-Hplc Method

Jaideep Kumar Singh Yadav*[1], Koushlesh Kumar Mishra[1], Naveen Gupta[1], Dharmendra Singh Rajput[1]
[1]Department of Pharmacy, Madhyanchal Professional University, Bhopal, M. P.

Abstract: *Stability indicating methods (SIMs) are critical tools in the pharmaceutical industry for ensuring the quality, safety, and efficacy of drug products. These methods are designed to detect any changes in the chemical, physical, or microbiological properties of a product, especially those that occur due to degradation or interactions with environmental factors. A stability-indicating method must be able to accurately measure the active pharmaceutical ingredient (API) and any degradation products, without interference from the excipients, packaging, or environmental conditions. Stability indicating methods are essential for determining the shelf life of products, supporting regulatory approvals, and ensuring that drugs maintain their therapeutic efficacy throughout their lifespan.*
Keywords: *Stability indicating method, Validation of method, Ceftriaxone sodium, RP-HPLC method*

INTRODUCTION: High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques. More than 85% of general pharmaceuticals are analyzed by HPLC [1]. HPLC is the separation module which contain mainly stationary phase and mobile phase having opposite polarity equipped with high pressure pumps and the separation is achieved by the interaction of stationary phase and the mobile phase. A proper choice of stationary phase and mobile phase is essential to reach desired separation [2]. pH of mobile phase, different types of buffers, column temperature, sample diluents, detection wavelength and many more are the variables which play a major role in method development [3-5]. During the preliminary method development stage, all individual components should be investigated before the final method optimization. This gives us a chance to critically evaluate the method performance in each component and to streamline the final method optimization [6]. A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally, method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling. The goals of HPLC separation need to be specified clearly [7]. Column is the heart of HPLC system. Good silica and bonding process will provide the reproducible and symmetrical peak necessary for accurate qualification. Commonly used RP columns include C18 (USP L1), C8 (USPL8), Phenyl (USP L11) and Cyno (USP L18). There is no good or bad column. They are chemically different boned phases and demonstrate significant changes in selectivity using same mobile phase. Column vary from manufacturer to manufacturer relative to their pore volumes, pore size, surface area, particle size, carbon load and whether they are end capped or not. Column length also plays a vital role in the separation resolution [8-9]. There is no absolute end to the method development process. The question is which is the "acceptable method performance"? The acceptable method performance is determined by the objectives set in this step. This is one of the most important considerations often overlooked by scientists. In this section, the different end points (i.e., expectations) will be discussed in descending order of significance [10]. A stability indicating method must resolve all significant degradation products from each other. Typically the minimum requirement for baseline resolution is 1.5. This limit is valid only for 2 Gaussian-shape peaks of equal size. In actual method development, Rs = 2.0 should be used as a minimum to account for day-to-day variability, non-ideal peak shapes and differences in peak sizes [11-12]. The desired method LOQ is related to the ICH reporting limits. If the corresponding ICH reporting limit is 0.1%, the method LOQ should be 0.05% or less to ensure the results are accurate up to one decimal place. However, it is of little value to develop a method with an LOQ much below this level in standard practice because when the method is too sensitive, method precision and accuracy are compromised [13].

MATERIAL AND METHODS:
New stability indicating analytical method development and validation for estimation of Ceftriaxone sodium (CFX) using RP-HPLC.

Selection of Mobile Phase: Initially, the calculation of Ceftriaxone sodium (CFX) in the fixed dosage form of the different mobile phase in different proportions was attempted. In Table 1, the observations are reported. The mobile phase found to be most appropriate for analysis was 10mM KH2PO4: methanol in the 20:80 v/v ratio, taking into account the device suitability parameters such as RT, tailing factor, number of theoretical plates and HETP. To remove particulate matter, the mobile phase was filtered through 0.45m filter paper and then degassed by sonication. For study, the flow rate used was 1.0 ml/min.

Table 1: Mobile Phase Selection of ceftriaxone sodium (CFX)

Mobile Phase	Ratio	Remark
Water : Methanol	50 : 50 v/v	Not Found
		-
Water: Acetonitrile	50:50 v/v	Not Found
		-
ACN: Methanol	50 : 50 v/v	Poor Resolution
		3.968
10mM KH2PO4:Methanol	20:80 v/v	Most suitable
		5.658

Chromatograms of mobile phase trial

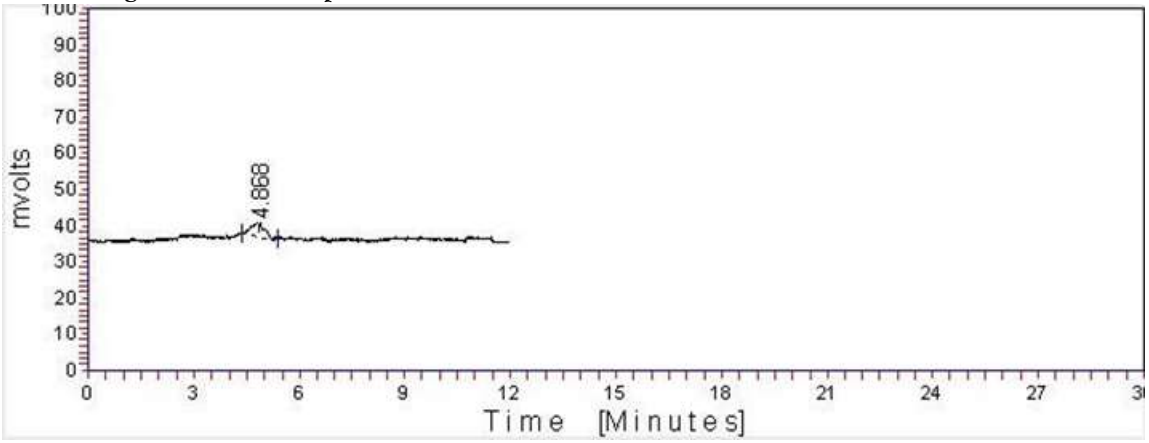

Figure 1: Trail graph of Water: Methanol (50: 50 v/v)

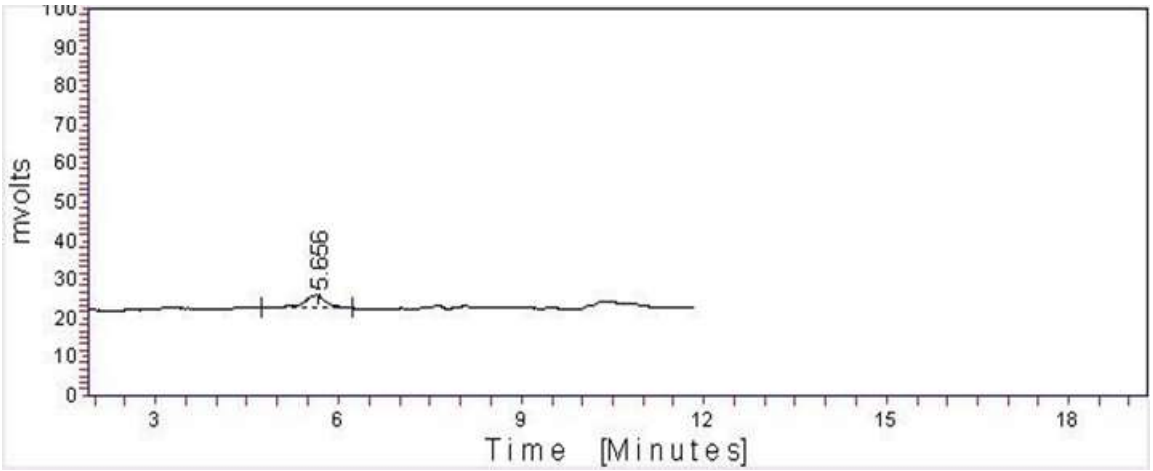

Figure 2: Trail graph of Water: Acetonitrile (50: 50 v/v)

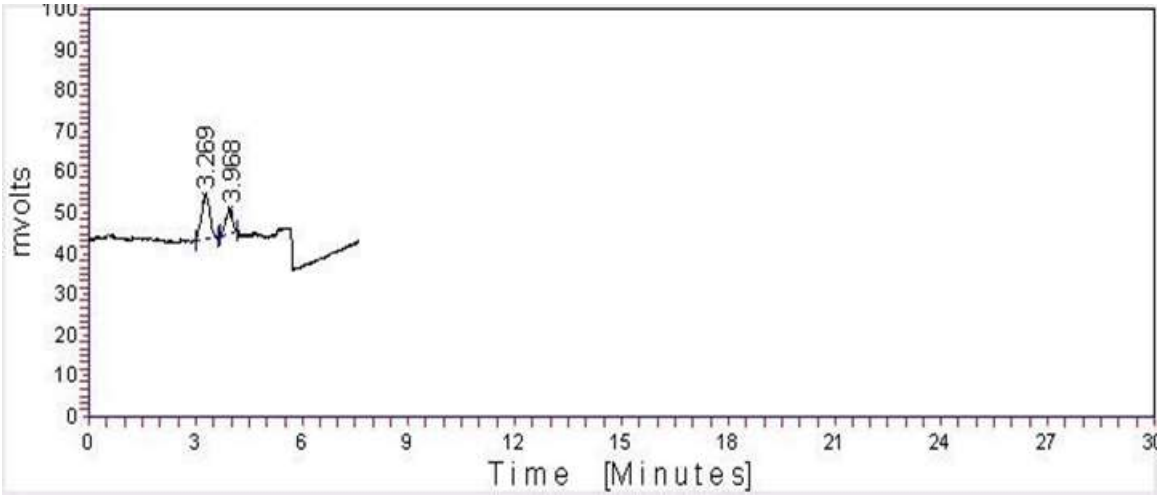

Figure 3: Trail graph of ACN: Methanol (50: 50 v/v)

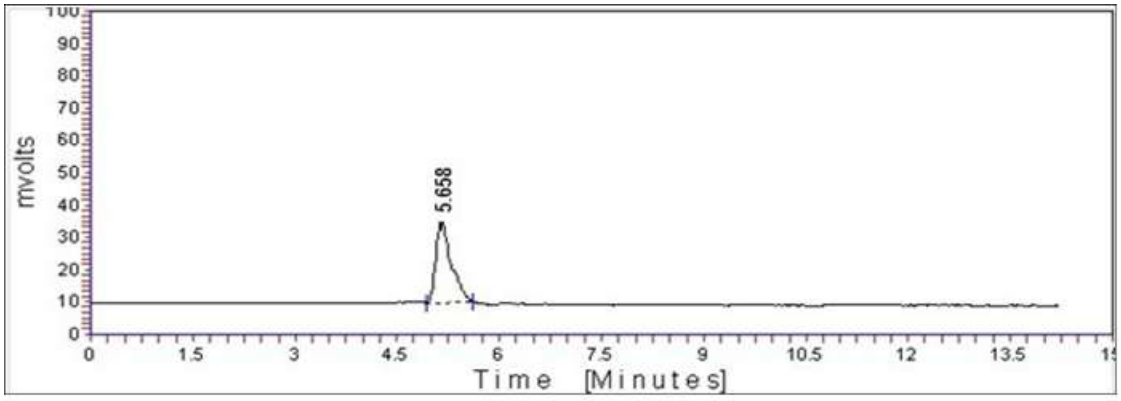

Figure 4: Suitable graph of ceftriaxone sodium (CFX) in 10mM KH2PO4: Methanol (20:80 v/v)

Selection of Diluent: The diluents used for sample preparation were consistent with the mobile process and had no major impact on analyte retention and resolution. Methanol was used as a diluent after several trials.

Table 2: Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5m
Bonded Phase	Octadecylsilane (C18)
Mobile Phase	
Methanol	80
10mM KH2PO4	20
Diluent	Methanol
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 ml
Detection wavelength	234nm
Retention time	
Enoxaparin sodium	5.658± 0.4min

Preparation of Stock Solution: 10 mg of Ceftriaxone sodium (CFX) correctly weighed was transferred to 10 ml volumetric flask and dissolved in methanol and then filtered with by whatmann filter paper (no.41). The concentration of Enoxaparin Sodium was 1000µg/ml. (The stock-A).

Linearity and Calibration Graph: A dilution series ranging from 5-25 µg/ml was prepared to assess the linearity of the analytical process. All the solution was filtered and injected by a 0.2 m membrane filter, chromatograms were reported at 234 nm, and six times were repeated. A calibration graph was diagrammed between the mean peak area and the corresponding equation of concentration and regression was derived.

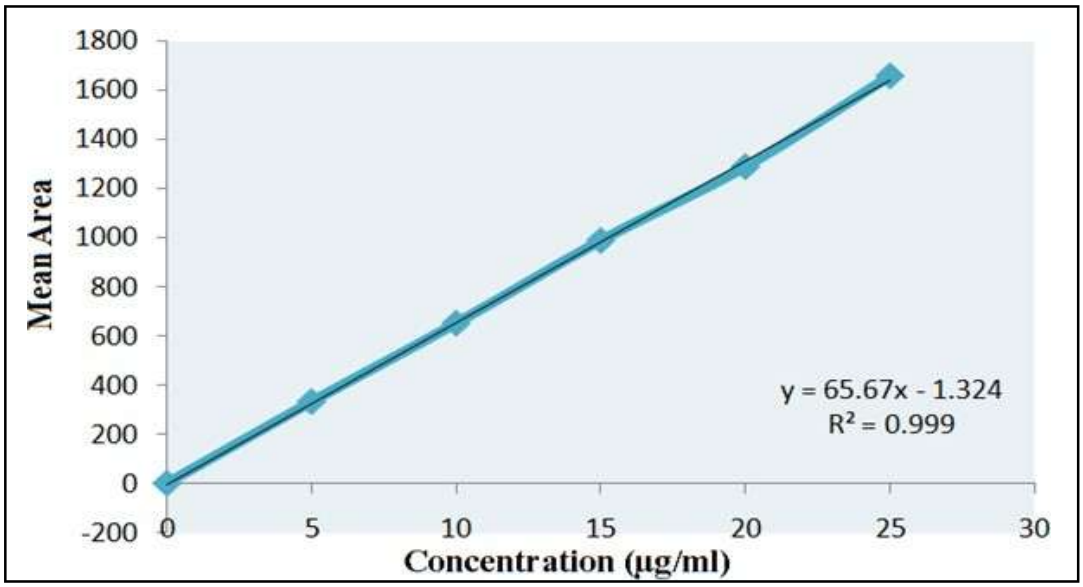

Figure 5: Calibration Curve of Ceftriaxone sodium (CFX)

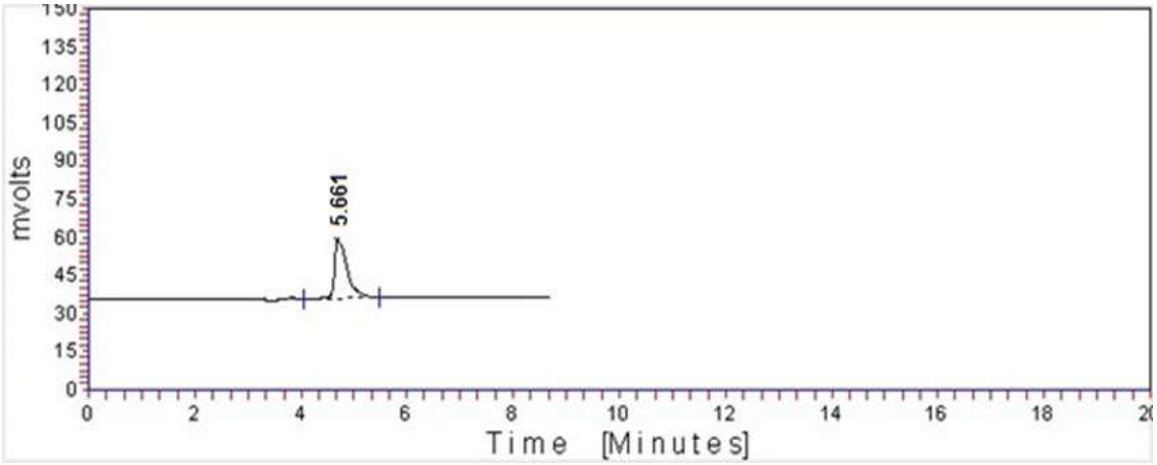

Figure 6: Chromatogram of Curve of Ceftriaxone sodium (CFX) 5 ppm

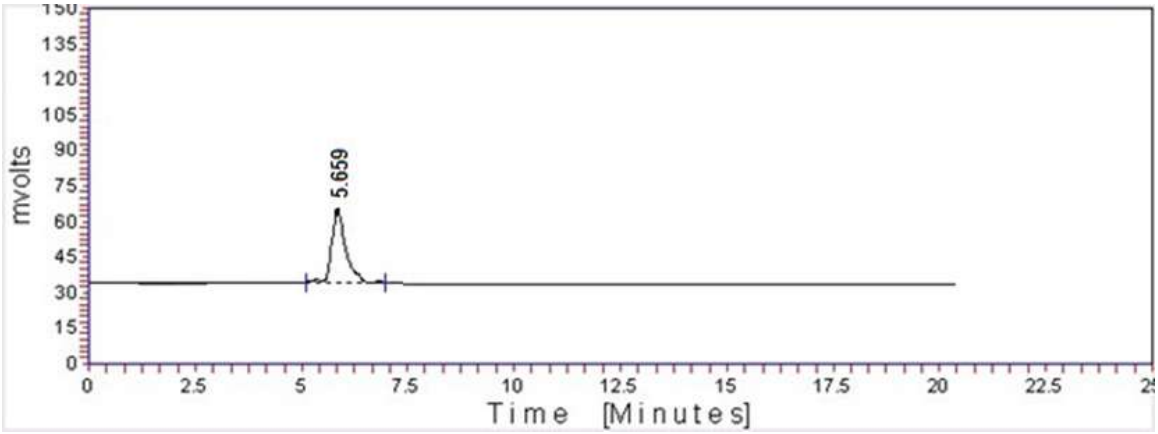

Figure 7: Chromatogram of Ceftriaxone sodium (CFX) 10 ppm

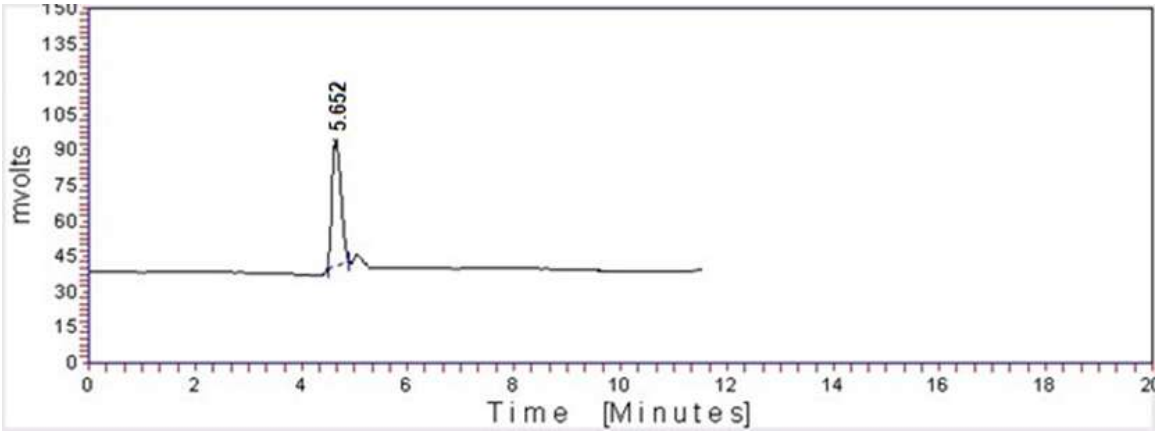

Figure 8: Chromatogram of Ceftriaxone sodium (CFX) 15 ppm

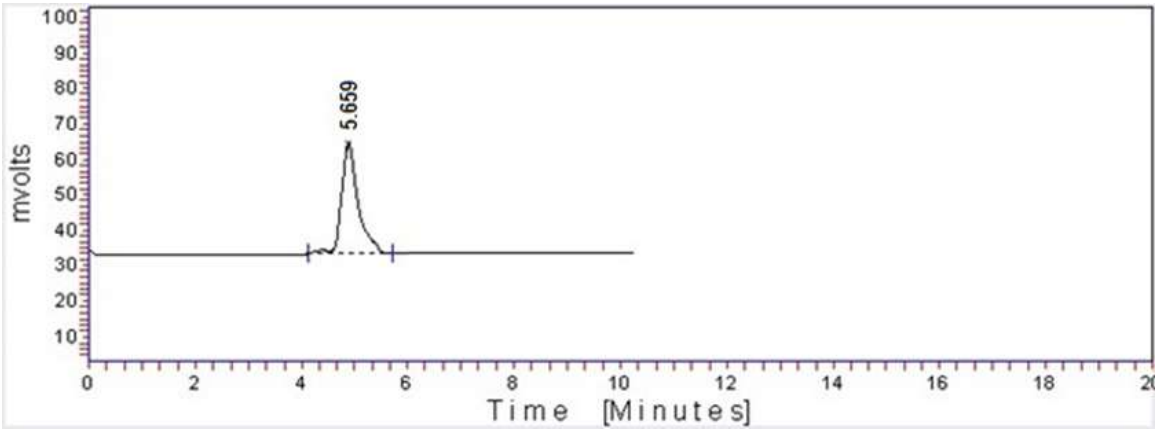

Figure 9: Chromatogram of Ceftriaxone sodium (CFX) 20 ppm

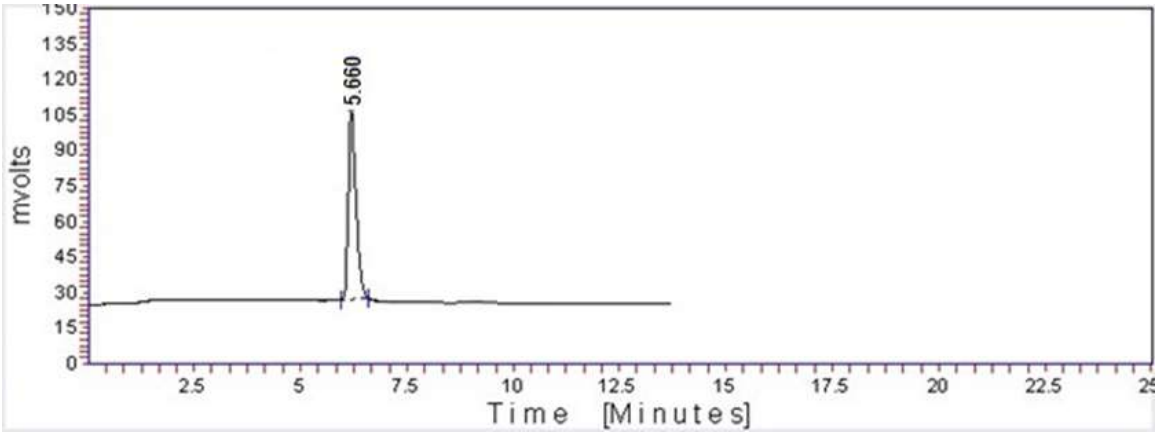

Figure 10: Chromatogram of Ceftriaxone sodium (CFX) 25 ppm

System Suitability Parameters: Variables of separation were developed and the mobile phase allowed the column to saturate at 1.00 ml/min. Six replicates of the working standard of Ceftriaxone sodium (CFX) were injected separately following full column saturation. The peak report and column output report for all chromatograms were reported.

Table 3: System Suitability Parameters of Ceftriaxone sodium (CFX)

System suitability Parameter ®	RT	AUC	No. of theoretical plates	Tailing factor
Rep-1	5.658	645.658	3256	0.98
Rep-2	5.659	660.325	3265	0.95
Rep-3	5.642	652.147	3245	0.96

Rep-4	5.623	653.326	3248	0.97
Rep-5	5.678	655.658	3265	1.05
Rep-6	5.614	640.589	3230	1.09
Mean	5.646	651.284	3251.5	1
S.D.	0.024	7.097	13.428	0.057

Laboratory Sample Analysis: CFX is available in a commercial formulation with strength of 150 mg/ml. In methanol, dilution was made to yield a concentration of 5 to 25 µg/ml.

Table 4: Laboratory Sample Analyses

Serial Number	**Concentration of CFX (mg/ml)**
1	5
2	10
3	15
4	20
5	25

Linearity: The linearity of the analytical method is its ability to achieve a result (within a specified range) that is directly proportional to the analyte region in the sample. After analysis of five different (from 5 to 25 µg/ ml) concentrations, the calibration plot was contracted and areas were reported three times for each concentration, and the mean area was determined. The equation of regression equation and the correlation coefficient are given. Figure 10 displays the normal calibration curve of the drug. The response ratio (response factor) was determined from the mean AUC observed and the respective CFX concentration by dividing the AUC by the respective concentration.

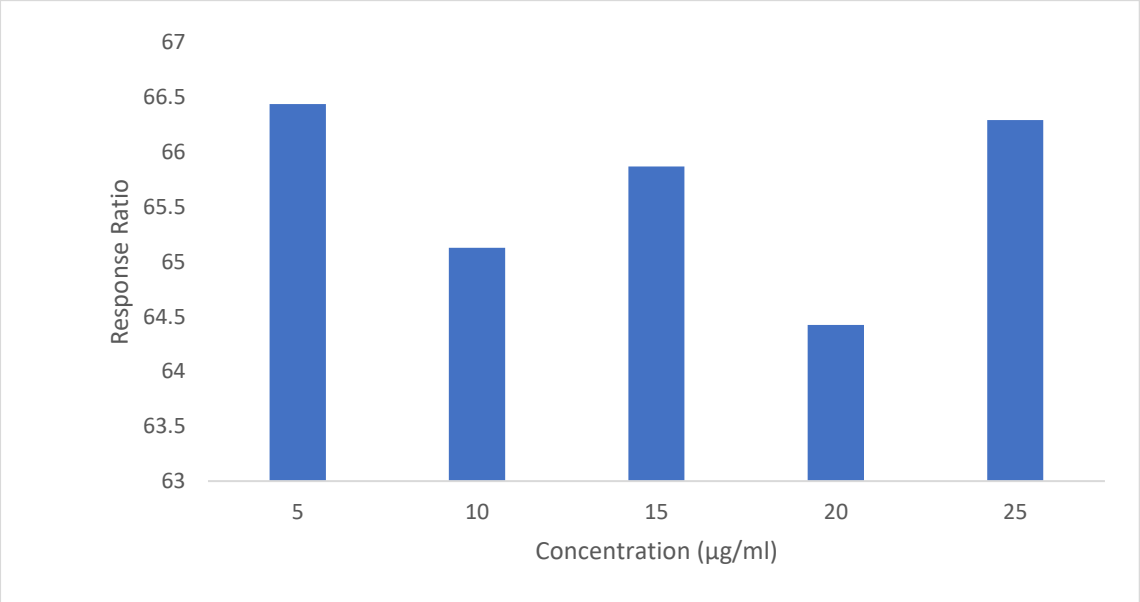

Figure 11: Response Ratio Curve of Ceftriaxone sodium (CFX)

Specificity: "According to the official method validation guideline ICH Q2(R1), specificity is defined as: "Specificity is the ability to unambiguously evaluate the analyte in the presence of components that may be assumed to be present.

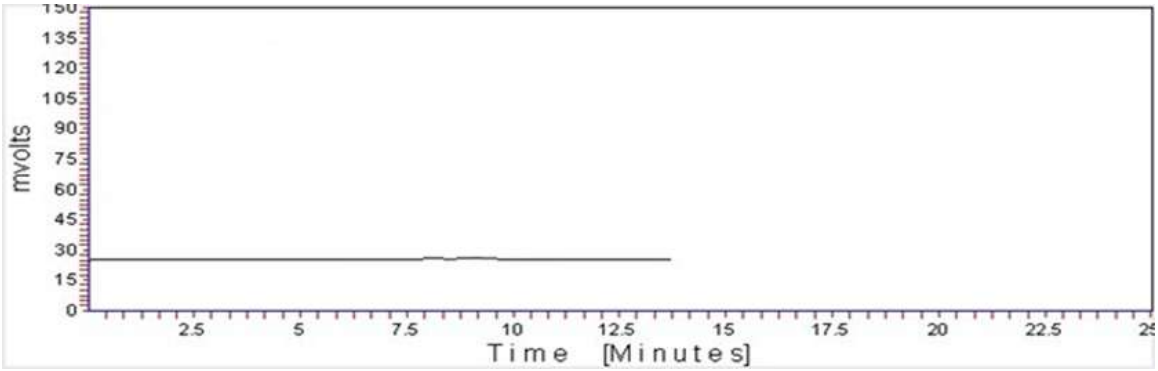

Figure 12: Chromatogram of the blank

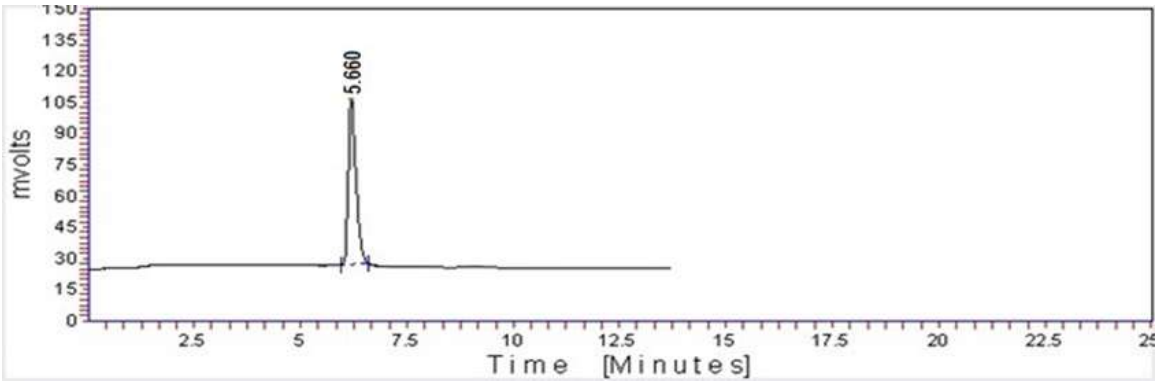

Figure 13: Chromatogram of the standard drug

Accuracy: Recovery experiments were conducted to measure the accuracy of the procedure developed for the pre-analysed sample solution, applying a definite standard drug concentration (80%, 100%, and 120%) and then evaluating its recovery.

Table 5: Recovery Study of CFX (80% Level)

Conc. of sample (mg/ml)	Amt. Added (mg/ml)	Total	Conc. Found. (mg/ml)			% conc. Found			Mean % conc.
			Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	4	9	3.89	3.88	3.95	97.25	97	98.75	**97.667**
10	8	18	7.85	8.01	7.92	98.13	100.13	99	**99.083**
15	12	27	11.98	11.65	12.02	99.83	97.08	100.17	**99.028**
MEAN									98.593
SD									0.802
% RSD									0.814

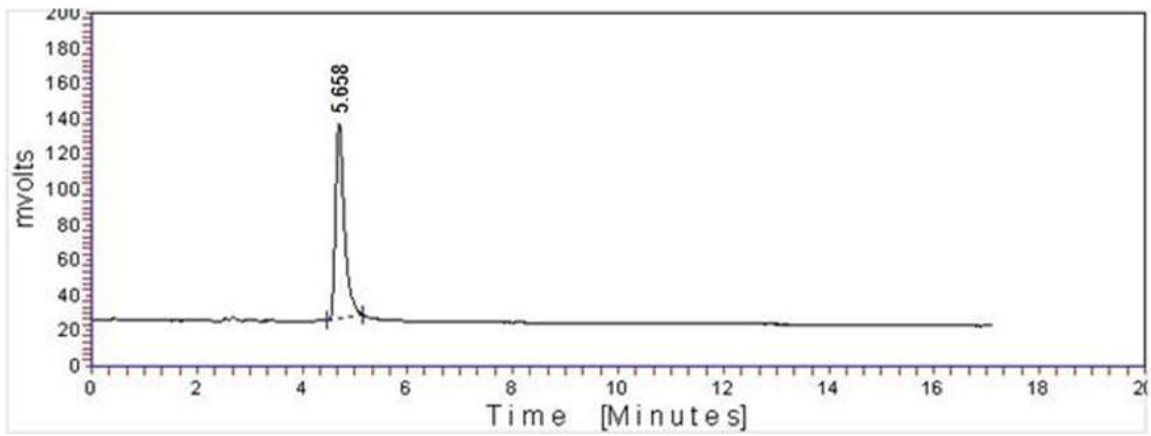

Figure 14: Chromatogram of Recovery Study of CFX (80% Level)

Table 6: Recovery Study of CFX (100% Level)

Conc. of sample (mg/ml)	Amt. Added (mg/ml)	Total	Conc. Found. (mg/ml)			% conc. Found			Mean % conc.
			Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	5	10	4.98	4.95	5.02	99.6	99	100.4	99.667
10	10	20	9.95	9.96	9.85	99.5	99.6	98.5	99.2
15	15	30	14.85	14.92	14.65	99	99.47	97.67	98.711
MEAN									99.193
SD									0.478
% RSD									0.482

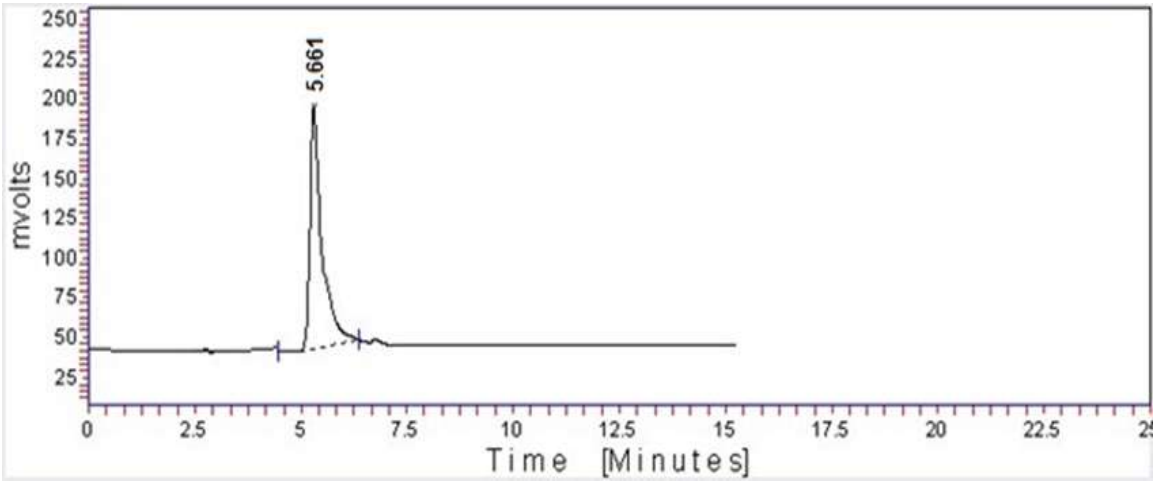

Figure 15: Chromatogram of Recovery Study of CFX (100% Level)

Table 7: Recovery Study of CFX (120% Level)

Conc. of sample (mg/ml)	Amt. Added (mg/ml)	Conc. Found. (mg/ml)			% conc. Found			Mean % conc.
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	
5	6	5.85	5.98	5.91	97.5	99.67	98.5	98.556
10	12	11.45	11.98	12.01	95.42	99.83	100.08	98.444
15	18	17.85	17.65	17.84	99.17	98.06	99.11	98.778
MEAN								98.593
SD								0.17
% RSD								0.172

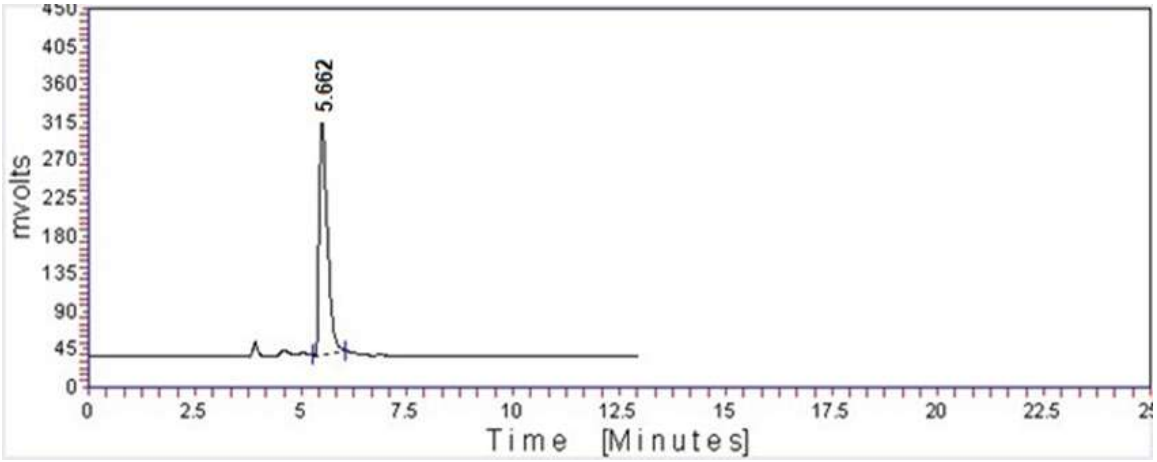

Figure 16: Chromatogram of Recovery Study of CFX (120% Level)

Precision: The stock solution was prepared. The precision are established in three differences: Repeatability was performed for five replicates at five concentrations in the linearity range of 5, 10, 15, 20 and 25 µg/ml for CFX, suggesting consistency over a short time period under the same operating condition. Repeatability findings are listed in table 16.

Table 8: Repeatability of CFX

Conc. Rep.	Concentration Found (mg/ml)					Mean
	5	10	15	20	25	
Replicate-1	4.95	9.98	14.78	19.98	24.65	
Replicate-2	4.98	9.96	14.85	19.65	24.78	
Replicate-3	4.65	9.95	14.65	19.63	24.96	
Replicate-4	4.78	9.63	14.79	19.45	24.85	
Replicate-5	5.02	9.97	14.95	20.01	24.65	
Mean	4.876	9.898	14.804	19.744	24.778	
% Mean	97.52	98.98	98.693	98.72	99.112	**8.605**
SD	0.156	0.15	0.109	0.242	0.133	**0.158**
% RSD	**0.16**	**0.152**	**0.111**	**0.245**	**0.134**	**0.161**

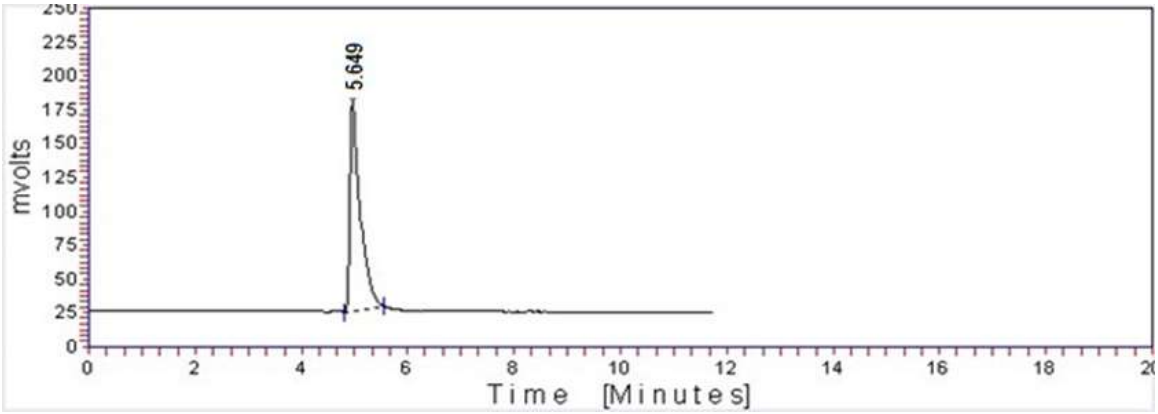

Figure 17: Chromatogram of Repeatability of CFX

Robustness: As per ICH requirements, small but deliberate variations in mobile phase concentration were made to verify the ability of the system to remain unaffected. The mobile phase ratio was changed from 10mM KH2PO4: methanol (20:80% v/v) to (15:85% v/v). Results of robustness are reported in table 17.

Table 9: Robustness of CFX

Conc.	Concentration Found (mg/ml)					Mean
Rep.	5	10	15	20	25	
Replicate-1	4.85	9.95	14.85	19.85	24.65	
Replicate-2	4.92	9.98	14.65	19.65	24.78	
Replicate-3	4.86	9.65	14.78	19.78	24.89	
Replicate-4	4.78	9.86	14.65	19.84	24.65	
Replicate-5	4.65	9.92	14.92	19.98	24.85	
Mean	4.812	9.872	14.77	19.82	24.764	
% Mean	96.24	98.72	98.467	99.1	99.056	**98.317**
SD	0.103	0.132	0.12	0.12	0.111	**0.117**
% RSD	0.107	0.134	0.122	0.121	0.112	**0.119**

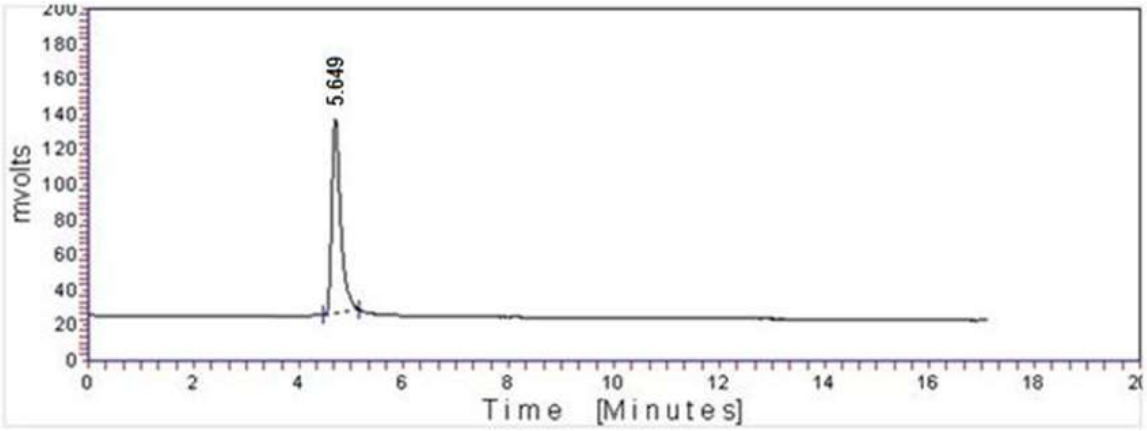

Figure 18: Chromatogram of Robustness of CFX

Detection Limit and Quantitation Limit: Based on the normal standard deviation and slope of the linearity curve, the LOD and LOQ of the established method were determined.

Table 10: LOD and LOQ of TDF and CFX

Name	LOD (mg/ml)	LOQ (mg/ml)
CFX	0.075	0.225

CONCLUSION:

The development and validation of an analytical technique for the estimation of ceftriaxone sodium (CFX) using RP-HPLC, also known as the stability indicating analytical method. The mobile phase consisted of methanol in a ratio of 20:80 v/v, while the stationary phase consisted of a Prontosil C-18 column with dimensions of 4.6 x 250 mm and a particle size of 5µ. The chromatogram was taken at a wavelength of 234 nm. In the next step, the created approach was verified by using a variety of parameters. For the purpose of ensuring that the analytical system was operating appropriately and was able to provide findings that were dependable and accurate, the system suitability parameter was carried out. Following the injection of the six duplicates of the Ceftriaxone sodium (CFX) reference standard at a concentration of 10 mg/ml, chromatography of the samples was recorded. In terms of system appropriateness, the number of theoretical plates discovered was 3251.500±13.428. The Tailing Factor was 1.000±0.057, and the retention duration was 5.646±0.024. A percentage mean and standard deviation was also found. The linearity of the analytical technique was examined in order to determine whether or not it is capable of producing test findings that are proportionate to the concentration of the analyte in the sample within a certain range. The chromatogram was recorded after various levels were generated and injected into the high-performance liquid chromatography (HPLC) system using standard solutions. The linearity concentration range was between 5 and 25 micrograms per milliliter (µg/ml), the correlation coefficient (r2)* was 0.999, the slope (m)* was 65.67, and the intercept (c)* was -1.324.

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