



Original article

Simultaneous determination of Ceftriaxone and Tazobactam in injectables by UHPLC method

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ABSTRACT

Aim: A stability indicating UHPLC method was developed and validated for the determination of Ceftriaxone and Tazobactam in injectable dosage form.

Methods: Separation was performed in a Dionex Ultimate 3000 UHPLC system equipped with Chromeleon software using Acclaim 120 C₁₈ (250 mm × 4.6 mm, 5 μm particle size) column with mobile phase (pH 7.0) containing methanol, potassium dihydrogen phosphate and triethylamine in the ratio of 14:86:0.2 v/v/v with a flow rate of 1.55 mL/min and detection wavelength of 220 nm. Stress studies were performed using HCl, NaOH, H₂O₂ and UV radiation.

Results: The method was found to be linear in the concentration range of 280–480 μg/mL ($R^2 = 0.997$) and 35–60 μg/mL ($R^2 = 0.997$) with the regression equation $y = 24060x + 200$ and $y = 9880x - 9461$ for Ceftriaxone and Tazobactam, respectively. The %RSD of 0.56 and 0.62 for intra-day and 1.08 and 1.62 for inter-day precision, respectively for Ceftriaxone and Tazobactam suggest the precision of the method as all these values are less than 2%. It was found from the stress studies that both the drugs are very susceptible to alkaline condition. The method has shown good, consistent recoveries for Ceftriaxone (98.88–101.24%) and Tazobactam (98.42–100.94%) which are close to 100%.

Conclusion: The method was found to be accurate, precise, specific, robust, linear and stability indicating for the determination of Ceftriaxone and Tazobactam in injectable dosage form.

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1. Introduction

Ceftriaxone (Fig. 1) chemically known as, (Z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl amido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thiamethyl]-3-cephem-4-carboxylic acid^{1,2} is a third-generation cephalosporin with a broad-spectrum of bactericidal activity *in vivo* and *in vitro* against aerobic gram-negative and gram-positive micro-organisms, including penicillin resistant *Pneumococci*, and some anaerobic bacteria.³ It is having comparatively longer half-life of 8–10 h than other third-generation cephalosporins which allows once a daily administration.⁴ Tazobactam (Fig. 2) chemically known as (2S,3S,5R)- (9CI);4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-ylmethyl)-, 4,4-dioxide,¹ is a derivative of the penicillin nucleus and is a penicillanic acid sulfone. It does not have antibacterial activity when used alone but

broadens the spectrum of penicillins and cephalosporins by making it effective against organisms that express β-lactamase and would normally degrade penicillins and cephalosporins.

Various analytical methods such as spectrophotometry^{5–7} HPLC^{8,9} TLC¹⁰ exist for the analysis of Ceftriaxone (CTX) and similarly various HPLC methods are available for the analysis of Tazobactam (TZB) in pharmaceutical preparations^{11,12} and in plasma^{13,14} either alone or in combination with other drugs. A detailed review of the analytical methods available to analyze these drugs is already published by the authors.¹⁵ The HPLC method¹² which have attempted to develop a simultaneous method for the estimation of these drugs together is suffering from the drawbacks such as very narrow linearity range, scrupulous control of experimental variables and use of ion-pairing reagent which is having the disadvantage of long equilibration time, short column life, high cost and problems with stability in retention time. As pharmacopoeias also do not describe a suitable method for the simultaneous estimation of CTX and TZB in the pharmaceutical preparations, we have developed a simple, precise, accurate, stability indicating liquid chromatographic method for their determination in injectable formulation.

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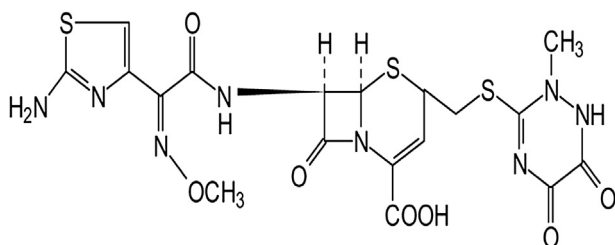


Fig. 1. Chemical structure of Ceftriaxone.

2. Material and methods

2.1. Reagents and chemicals

Qualified standard of CTX and TZB were obtained as gift samples from Alkem Laboratories Ltd, Sikkim, India. Methanol (HPLC grade), potassium dihydrogen phosphate and triethylamine were obtained from S.D. Fine Chem Ltd., Mumbai, India. HPLC grade water was obtained from Millipore direct Q3 (India). Commercially available sterile powder for injection vials (Monotax XP, CTX 1 g and TZB 0.125 g) and (Montaz, CTX 1 g and TZB 0.125 g) were procured from local market.

2.2. Chromatography instruments and conditions

The chromatograph consisted of a Dionex Ultimate 3000 UHPLC system with quaternary pump, auto injector, vacuum degasser and Ultimate 3000 diode array detector. The data were evaluated by Dionex Chromeleon software. The separation was accomplished using an Acclaim 120 C₁₈ (250 × 4.6 mm i.d, 5 μm particle size) column and a mobile phase (pH 7) consisting of methanol, potassium dihydrogen phosphate and triethylamine in the ratio of 14/86/0.2 v/v/v in the isocratic mode with a flow rate of 1.55 mL/min. The column eluents were monitored at 220 nm with the overall run time of 6 min and the injection volume of 20 μL. All the solutions were filtered and degassed before use.

2.3. Preparation of standard solution

Weighed accurately and transferred 100 mg of CTX and 12.5 mg of TZB in a 50 mL volumetric flask, dissolved with 30 mL of mobile phase and sonicated for 10 min and finally made up the volume with the mobile phase. From this solution 5 mL was transferred in a 25 mL volumetric flask and the volume was made up with the mobile phase. This solution was filtered through a 0.45 μm filter before use.

2.4. Preparation of sample solution

An amount of sample containing 100 mg equivalent of active pharmaceutical ingredient CTX was transferred to a 50 mL volumetric flask, dissolved with 30 mL of mobile phase and sonicated for 10 min and finally made up the volume with the mobile phase. The solution was filtered through a Whatman filter paper and from

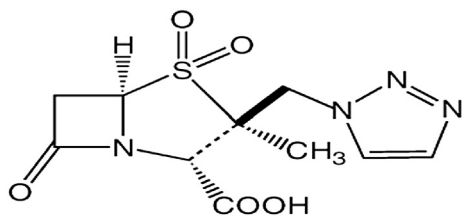


Fig. 2. Chemical structure of Tazobactam.

the filtrate, 5 mL was transferred in a 25 mL volumetric flask and the volume was made up with the mobile phase. This solution was filtered again through a 0.45 μm filter before use.

2.5. System suitability

The various system suitability parameters such as tailing factor, theoretical plates, resolution and %RSD of area of five replicate injections were evaluated to verify that the analytical system is working properly and can give accurate and precise results.

2.6. Analytical method validation

It was performed as per ICH guidelines¹⁶ and other available literatures.¹⁷

2.6.1. Specificity

It is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. The sample was subjected to various stress conditions¹⁸ such as acidic (0.1 M HCl for 30min) and basic (0.1 M NaOH for 15 min) hydrolysis, oxidative (5% H₂O₂ for 30 min) degradation and photolytic degradation (UV light for 24 h). Analysis of these stressed sample solutions were carried out and the %degradation in various conditions were calculated. The chromatographic interferences, if any, due to presence of degraded products were studied. The blank chromatogram was also compared with the standard chromatograms to check the interference due to blank.

2.6.2. Linearity

The linearity of the method was established by constructing calibration curves over a concentration range of 280–480 μg/mL for CTX and 35–60 μg/mL for TZB. After injecting each solution into the HPLC system the peak area of the chromatogram obtained was noted. The peak area against the corresponding analyte concentration was plotted and the slope, intercept and correlation coefficient were determined using linear regression analysis.

2.6.3. Precision

Precision of the method was evaluated in terms of intra-day and inter-day precision. Intra-day precision was reported as %RSD on six separate weights of the sample at 100% test concentration against a qualified reference standard. Inter-day precision was also carried out similarly but in two different days and the %RSD was calculated.

2.6.4. Accuracy

The accuracy of the method was evaluated in triplicate by adding pure drug of CTX and TZB in an already analyzed sample solution. The total amount of drugs present was determined by the proposed method and the percentage recovery of pure drugs were calculated.

2.6.5. Robustness

The robustness of the method was evaluated by introducing small deliberate variations in method parameters such as flow rate (1.45 and 1.65 mL/min), percentage of methanol in the mobile phase (12% and 16%) and pH (6.8 and 7.2). Only one parameter was changed at a time and for all the changes the sample was analyzed in triplicate.

2.6.6. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ for CTX and TZB were determined by signal-to-noise ratio method. The LOD and LOQ were taken as signal-to-noise ratio of 3:1 and 10:1, respectively of the average response of the blank solution.

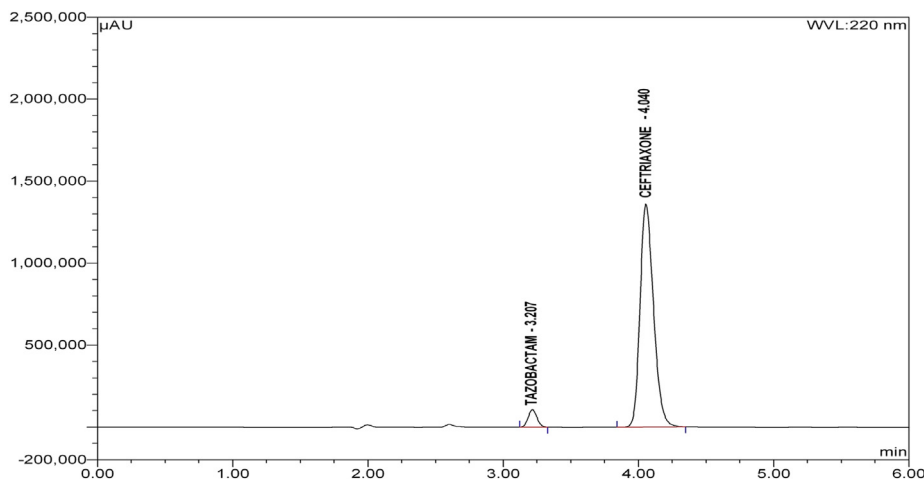


Fig. 3. Representative chromatogram for Ceftriaxone and Tazobactam standard.

2.6.7. Solution stability

The stability of CTX and TZB in the proposed mobile phase solvent was determined at room temperature by keeping the test sample in tightly closed volumetric flask and analyzing at 1 h interval against a freshly prepared standard solution. The %RSD of the peak areas obtained for the test samples were determined in different time intervals.

3. Results and discussion

3.1. Method development

The previous knowledge acquired by the authors through the method development of this class of drugs has been utilized in this method development process.^{19,20} Thus the method development started with methanol and potassium dihydrogen phosphate buffer with a reverse phase C₁₈ (250 × 4.6 mm) column. The methanol percentage was optimized at 14% and the strength of the buffer was kept at 50 mM because at buffer concentration below this level the asymmetry factor of CTX is more than 1.5 which is not acceptable. The pH of the buffer was maintained at 7.0 by using triethylamine as this pH was found to be suitable for the maximum stability of CTX¹⁹ and it also reduces the tailing. Cephalosporins are highly degradable drugs due to their β-lactam ring thus for longer solution

stability it is necessary to develop analytical methods at the pH at which the drug is most stable. The wavelength of detection was set at 220 nm as it was found that in higher wavelengths the absorption of TZB was negligible. By developing method with this approach the use of any ion-pairing reagent was avoided. As both CTX and TZB are polar in nature they tend to produce tailing peaks under ordinary reversed phase conditions. Thus to reduce the tailing, generally, ion-pairing reagents are added which combines with anions of polar drugs such as CTX and form hydrophobic ion-pairs which can be easily retained in reversed phase condition. However, the use of ion-pairing reagents are having its own set of problems such as long equilibration time, short column life due to dissolving of the packing material, high cost and instability of retention time. Moreover, it is known that ion-pair chromatography is on the whole less perfect than the conventional RP-HPLC.²¹ Thus, pH and ionic strength of the buffer was manipulated in this method to fulfill the objective of developing a simple UPLC method without using ion-pairing reagent.

3.2. Method validation

3.2.1. System suitability

A representative chromatogram for system suitability test is shown in Fig. 3 which displays a tailing factor of 1.27 for CTX and

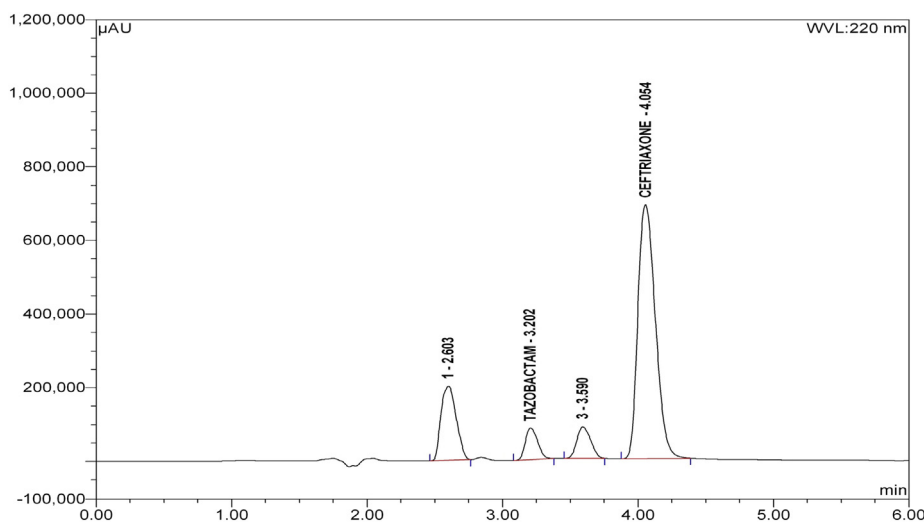


Fig. 4. Acid degradation chromatogram.

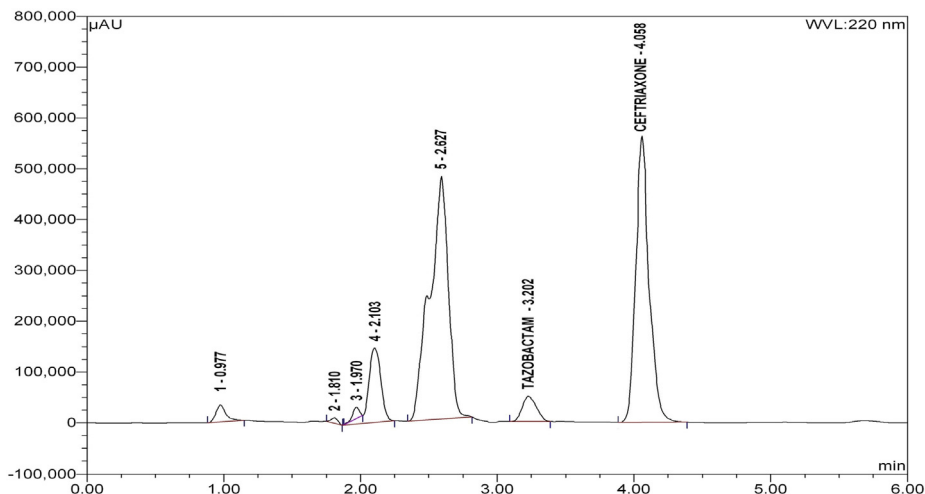


Fig. 5. Base degradation chromatogram.

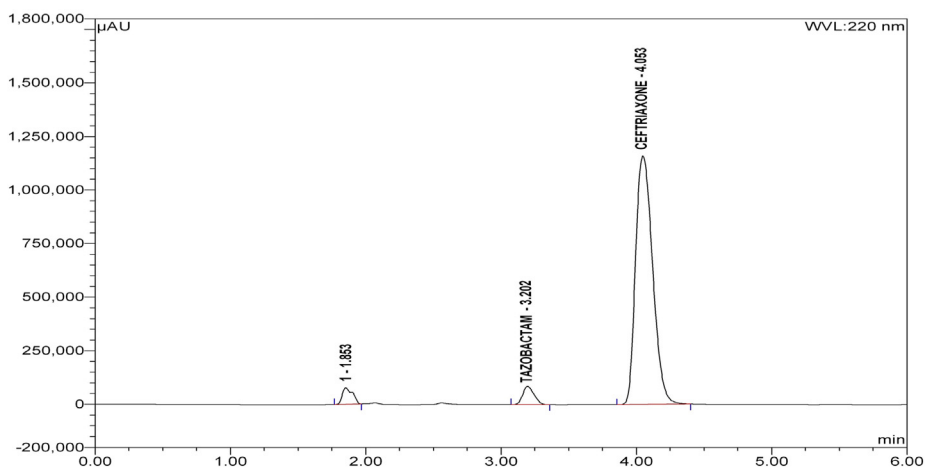


Fig. 6. Oxidative degradation chromatogram.

1.08 for TZB (less than 1.5 for both the peaks) with a resolution of 5.1 (more than 2). The %RSD of five replicate injections was 0.12 and 0.25 (less than 2%) with theoretical plates of 8000 and 11,000 (more than 2000) respectively for CTX and TZB. All the system suitability parameters obtained with the proposed method exceeds the minimum requirements proposed by the various regulatory authorities.

3.2.2. Specificity

The specificity and the stability indicating capability of the method were established from the separation of CTX and TZB peaks from the degraded product peaks. Representative chromatograms for the various stressed samples are shown in Figs. 4–7. The chromatogram of blank (Fig 8) also when compared with the sample

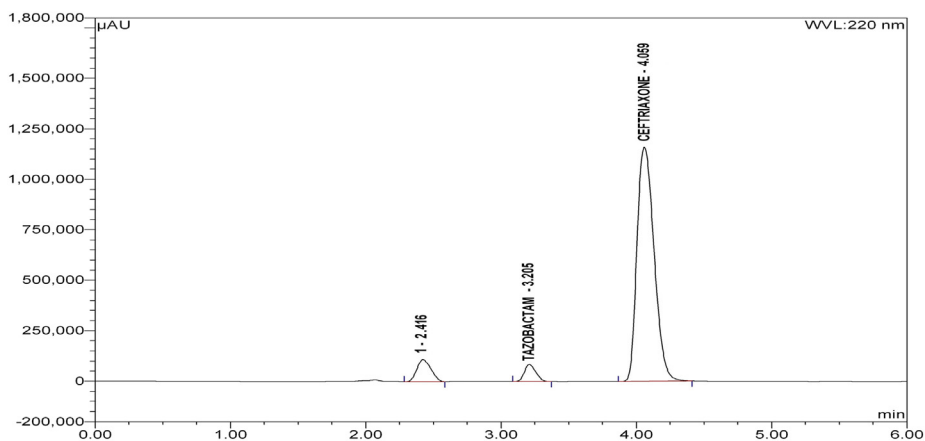


Fig. 7. Photolytic degradation chromatogram.

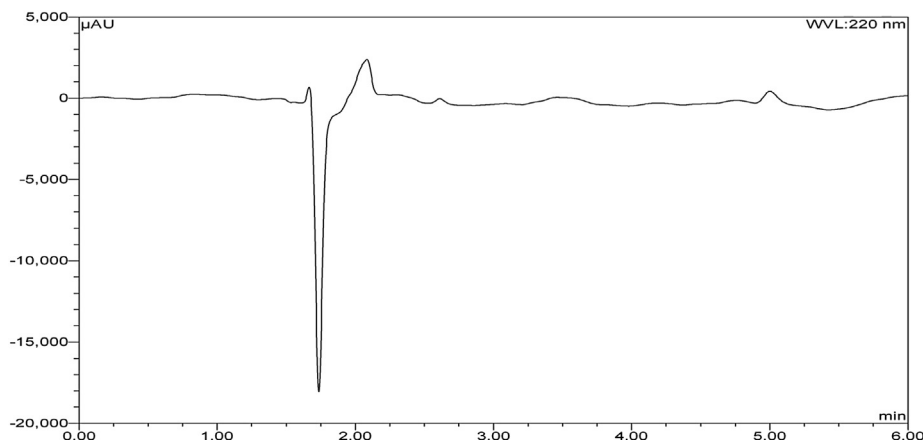


Fig. 8. Blank chromatogram.

Table 1
Forced degradation studies of CTX and TZB.

Stress parameters	Sample treatment	Ceftriaxone		Tazobactam	
		Assay (%)	Degradation (%)	Assay (%)	Degradation (%)
Reference	Fresh solution	99.10	0	99.01	0
Acid Hydrolysis	0.1 M HCl for 30 min	79.63	19.65	87.04	12.09
Base Hydrolysis	0.1 M NaOH for 15 min	45.67	53.92	72.58	26.69
Oxidative degradation	5% H ₂ O ₂ for 30 min	90.38	8.80	91.77	7.31
Light degradation	UV light for 24 h	89.83	9.35	92.16	6.91

chromatogram indicated no interferences as there was no peak in the blank chromatogram at the retention time of either CTX or TZB. The %degradation was also calculated in various stress conditions and presented in Table 1.

3.2.3. Linearity

The calibration curve for CTX and TZB was linear over the concentration range of 280–480 μg/mL and 35–60 μg/mL respectively. The data for the peak area against the concentration were treated by linear regression analysis and the correlation coefficient value obtained was 0.997 and 0.997 with the regression equation $y = 24060x + 200$ and $y = 9880x - 9461$ for CTX and TZB respectively.

3.2.4. Precision

The precision of the method was determined by intra-day and inter-day precision studies at 100% test concentration by taking six separate weights of the sample. Values of %RSD for intra-day were 0.56 and 0.62 and for inter-day 1.08 and 1.62 for CTX and TZB respectively, which is well within the acceptance criteria of 2% as shown in Table 2.

Table 2
Precision study result for CTX and TZB.

Serial no.	Ceftriaxone		Tazobactam	
	Intra-day	Inter-day	Intra-day	Inter-day
	Precision (% assay) ^a	Precision (% assay) ^a	Precision (% assay) ^a	Precision (% assay) ^a
1	99.77	99.67	99.83	99.60
2	99.39	99.65	98.91	101.66
3	100.81	100.97	100.08	102.02
4	99.91	98.27	99.46	98.72
5	100.49	98.03	100.01	98.25
6	99.49	99.57	98.57	101.42
Mean %	99.98	99.36	99.48	100.28
RSD %	0.56	1.08	0.62	1.62

^a Average of three readings.

3.2.5. Accuracy

The accuracy of the method was proven by recovery test. Known amounts of CTX standard (40, 60 and 80 μg/mL) and TZB standard (5, 7.5 and 10 μg/mL) were added to the already analyzed sample solutions and the analysis was carried out. The method has shown good, consistent recoveries for CTX (98.88–101.24%) and TZB (98.42–100.94%) which are close to 100% as shown in Table 3.

3.2.6. Robustness

The robustness of the method was checked by deliberately varying the mobile phase composition, flow rate and pH which shows that the small changes of the method parameters do not affect the performance of the method. All the results obtained were in accordance with the results for original conditions. The %RSD value obtained for the assay in the changed condition was less than 2% which indicates the robustness of the proposed method.

3.2.7. Solution stability

The %RSD of the peak areas of the test samples were less than 1% for 7 h which indicates that the sample was stable under proposed mobile phase condition within this period only.

3.2.8. LOD and LOQ

The LOD and LOQ were found to be 0.17 μg/mL and 0.41 μg/mL for CTX and 0.56 μg/mL and 1.37 μg/mL for TZB, respectively.

Table 3
Accuracy–recovery study of CTX and TZB by standard addition method.

Sample	Amount of standard added (μg/mL)	Total amount found (μg/mL)	Mean ^a recovery %	Mean ^a RSD %
Ceftriaxone	40	40.5	101.25	
	60	60.44	100.74	1.24
	80	79.11	98.88	
Tazobactam	5	5.03	100.6	
	7.5	7.57	100.94	1.37
	10	9.84	98.42	

^a Average of three readings.

Table 4
Analysis of CTX and TZB commercial formulation (Injectables).

Sample no.	Name of the formulation	Label claim (mg)		Amount found ^a (mg)		% Label claim ^a ± SD	
		CTX	TZB	CTX	TZB	CTX	TZB
1	Monotax XP	1000	125	990.24	124.7	99.02 ± 1.65	99.76 ± 1.27
2	Montaz	1000	125	1005.18	125.83	100.52 ± 1.60	100.67 ± 1.23

^a Average of three readings.

3.2.9. Analysis of commercial formulations

The developed method was applied for the determination of CTX and TZB in injectable dosage form (Monotax XP, Biochem and Montaz, Aristo) and the results obtained were presented in Table 4. The assay value of 99.02 and 100.52 for CTX and 99.76 and 100.67 for TZB indicates that the method is selective for the assay of CTX and TZB without interference from the commonly used excipients of injectable formulation.

4. Conclusion

The newly developed method is simple and cost effective as it uses simple mobile phase without ion-pairing reagent which was previously unreported, to effect the separation in 6 min only. The method was validated as per ICH guidelines. The stability indicating nature of the method was established by testing stressed samples successfully. All other parameters such as specificity, linearity, precision, accuracy, robustness passes the criteria set forth by ICH guidelines. There was no interference from any components of the formulation or degradation products. Hence, in this light the method stands validated and can be used for routine quality control and stability sample analysis of CTX and TZB.

Conflicts of interest

All authors have none to declare.

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