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Development And Validation Of HPTLC Method For The Simultaneous Estimation Of Ceftazidime Sodium And Tazobactam Sodium In Marketed Formulation.

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Abstract: This paper describes a method developed and validated using high performance thin layer chromatography (HPTLC) for the simultaneous estimation of Ceftazidime Sodium (CEFTA) and Tazobactam Sodium (TAZO) in a combined dosage form. Procedure does not require prior separation of components from the sample.The method was carried out in TLC Precoated silica gel on aluminum plate 60 F_{254} (0.2 mm thickness, 10 cm ×10 cm, prewashed by methanol and activated at 60° C for 5 min prior to chromatography). The solvent system was Chloroform: Ethyl acetate: Glacial acetic acid:Water in the proportion of 4:4:4:1.8, (v/v/v/v) with Rf value for CEFTA and TAZO was 0.16 and 0.45 respectively. Calibration curves were established showing the dependence of response (peak area) on the amount chromatographed. The validated linearity ranges were 500–2500 ng /spot (r^2 = 0.999) and 10–62.5 µg/spot (r^2 = 0.998) for CEFTA and TAZO respectively. The spots were scanned at λ =254 nm. The suitability of this HPTLC method for quantitative determination of the compounds was proved by validation in accordance with the requirements of the ICH guidelines. The method was used for determination of the compounds in commercial pharmaceutical dosage forms. The method is simple, reproducible, accurate and can be used as a more economical alternative to other chromatographic techniques for routine quality control.

Key words: Ceftazidime Sodium, Tazobactam Sodium, High Performance Thin Layer Chromatography, Quantitative Analysis.

INTRODUCTION

Ceftazidime Sodium is (1-{[(6R,7R)-7-[(2Z)-2-(2amino-1,3-thiazol-4-yl)-2-[(1-carboxy methyl lethoxy)imino]acetamido]-2-carboxylato-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl} pyridin-1-ium).[1,2,3] It is official in IP and BP. It is approved semisynthetic, broad-spectrum antibacterial derived from cephaloridine and it is widely used especially for Pseudomonas and other gram-negative infections in debiliated patients [4]. It is used in the treatment of Biliary tract infection, Bone & joint infection, Endometriosis, GI infections, Intra-abdominal infection, Lower respiratory tract infection and Urinary tract infection. [5,6] Ceftazidime Sodium alone or in combination with other drugs has been reported by spectrophotometric method. [7,8,9,10] Analysis has been carried out using RP-HPLC methods for single as well as in combination with other drugs. [11,12,13] Tazobactam Sodium is (2S,3S,5R)-3-methyl-4,4,7-trioxo-3-(1H-1,2,3-triazol-1-ylmethyl)-4S/l{6}-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid [14]. TAZO is not official

in any pharmacopoeia. Some RP-HPLC methods have been reported for Tazobactam as single as well as in combination with other drugs. [15,16] It is an antibacterial penicillin derivative which inhibits the action of bacterial beta-lactamases. Cephalosporins are destroyed by a family of enzymes called beta-lactamases, which hydrolyze the four member beta-lactam ring. Tazobactam inhibits these enzymes and shows synergistic antimicrobial effect. Various combinations of Ceftazidime & Tazobactam are available in the market. To our knowledge, no article related to HPTLC determination of CEFTA and TAZO in fixed dose combination has been reported in literature. This present study reports for the first time simultaneous estimation of Ceftazidime sodium and Tazobactam Sodium by HPTLC in bulk drug and in pharmaceutical dosage forms. The proposed method is optimized and validated as per the international conference on harmonization (ICH) guidelines (Q2B) .[17]

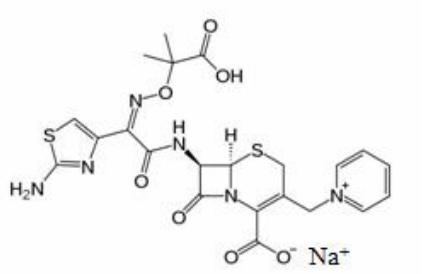

Fig.1(a): Chemical structure of Ceftazidime Sodium

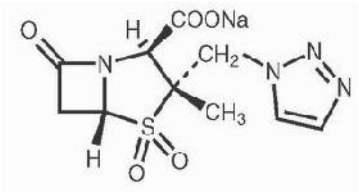

Fig.1 (b) : Chemical structure of Tazobactam Sodium

INSTRUMENTATION

HPTLC was performed with a Camag (Muttenz, Switzerland) Linomat V Sample applicator, a Camag twin trough TLC chamber, a Camag TLC scanner 3, Camag Wincats software (V 4.06, Camag) and a Hamilton (Reno, Nevada, USA) Syringe (100 µL).

MATERIALS AND METHODS

Analytically pure samples of CEFTA (Hindustan Antibiotic Limited, Pimpri, Pune, India) and TAZO (Gensen Laboratories, Mumbai) were used in the study. The pharmaceutical Fixed dose combination dry powder injection vial containing 1000 mg CEFTA and 125mg TAZO (8:1) were procured from Abbott Healthcare Pvt. Ltd. Mumbai. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

HPTLC METHOD OPTIMIZATION AND CHROMATOGRAPHIC CONDITIONS

The samples were spotted in the form of bands of width 6mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60F–254 plates, [10cm × 10cm with 250 µm thickness; E. Merck, Dermstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. A constant application rate of 2 µL/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5mm × 0.45mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of chloroform:ethyl acetate:glacial acetic acid:water (4: 4: 4: 1.8 (v/v/v/v). Linear ascending development was carried out in a 20cm × 10cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30min at room temperature (25°C ±2) at relative humidity of 60% ±5. The length of each chromatogram run was 8cm. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 254nm and operated by Win CATS software (V 4.06, Camag).

STANDARD STOCK SOLUTIONS

Standard Stock solutions of CEFTA and TAZO were prepared by accurate weighing of 5 mg of CEFTA in 10 ml methanol and 5 mg of TAZO in 1 ml of methanol having concentration of 500 µg/mL for CEFTA and 5000 µg/mL for TAZO. From the above stock solutions transfer suitable aliquots and prepare standard mixture solution having concentration of 2.2 µg/band of CEFTA and TAZO 0.275µg/band for simultaneous quantitative studies. Because of low sensitivity for detection of TAZO it has to be externally added 20mg in same mixture which gives extra 10µg/band of TAZO (B) in the mixture fig. (2), Table I.

ASSAY OF MARKETED FORMULATION

To determine the content of CEFTA and TAZO simultaneously in conventional pharmaceutical

dosage form (label claim: 1000mg CEFTA and 125 mg CEFTA per vial), The powder equivalent to 2.2 mg of Ceftazidime & 0.275 mg of Tazobactam weighed and transferred to 10 ml volumetric flask and dissolved in methanol. Because of low detection sensitivity problem of Tazobactam 20 mg of tazo added externally to the same 10 ml volumetric flask. The solution was ultrasonicated for 20 min. and filtered through Whatman filter paper No. 42. Then an aliquots of sample solution 5µL containing 2200ng/band of CEFTA and 10275 ng/band of TAZO were applied on HPTLC plates fig. (3), Table II.

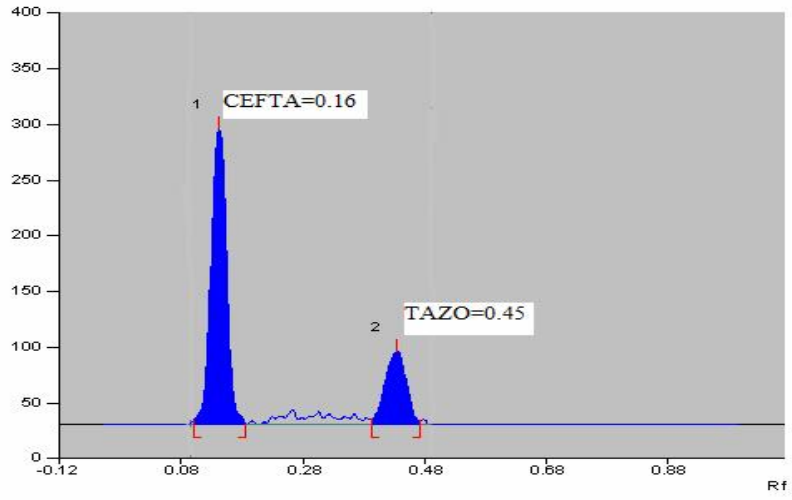

Fig 2: Densitogram of lab mixture

Table I: Analysis of lab mixture

Sr. No.	Amount present (ng/band)			Area under curve*(AUC)			% of drug found*	
	CEFTA	TAZO		CEFTA	TAZO		CEFTA	TAZO
		A	A-B		A	A-B		
1.	2200	10275	275	7566.1	4618.6	209.81	99.65	99.17

* denotes average of six determination
Where, A= Total concentration of tazo (10275ng)
B= Externally added tazo 10000 ng for purpose of increased detection.
A-B= Concentration of tazo in the linearity range (275ng) in the ratio (8:1)

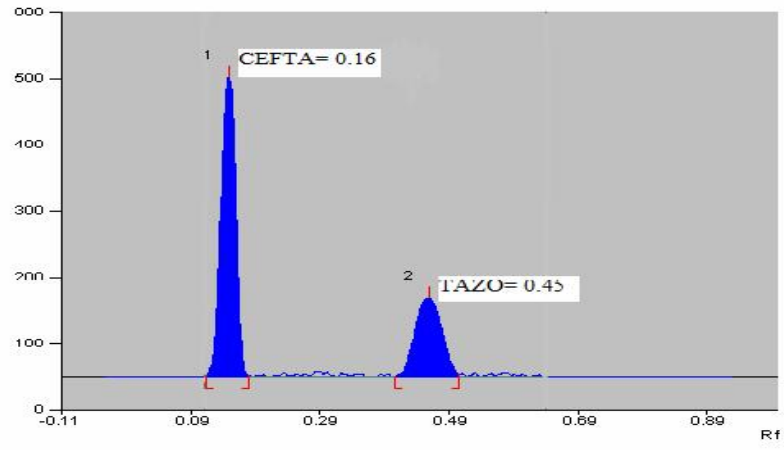

Fig. 3: Densitogram of Injection mixture

Table II: Assay results for the combined dosage form using the proposed HPTLC method.

Sr. No.	Amount present (ng/band)			Area under curve*(AUC)			%of drug found*	
	CEFTA	TAZO		CEFTA	TAZO		CEFTA	TAZO
		A**	A-B**		A	A-B		
1.	2200	10275	275	7569.8	4659.1	204.31	99.86	100.59

* denotes average of six determination
** refer Table I for A and B

Table III: System suitability parameters

System suitability parameters	Proposed method	
	CEFTA	TAZO
Rf	0.16	0.45

Number of samples analyzed is six

RESULTS AND DISCUSSION

HPTLC Method development for HPTLC analysis, initially various mobile phases and stationary phases were tried in attempts to obtain the best separation and resolution between CEFTA and TAZO. The mobile phase consisting of Chloroform: Ethyl acetate: Glacial acetic acid:water in the proportion of (4:4:4:1.8, v/v/v/v) was selected that gave satisfactory separation and gave two well resolved peaks for CEFTA and TAZO which is shown in Fig.2. As CEFTA and TAZO exhibit significant absorbance at wavelength 254 nm was selected as detection wavelength for the simultaneous determination. The R*f* value for CEFTA and TAZO was 0.16 and 0.45 respectively. Various system suitability test parameters were calculated and are shown in Table III.

METHOD VALIDATION

The developed method was validated for the simultaneous assay determination of CEFTA and TAZO using following parameters.

LINEARITY (CALIBRATION CURVE)

Linearity was checked by preparing standard solutions of both CEFTA and TAZO at five different concentration levels in the same volumetric flasks using their respective stock solutions. The calibration curves for CEFTA and TAZO were drawn in the concentration range of 500 ng/band-2500 ng/band and 10µg/band-62.5 µg/band respectively as shown in fig.(4). The calibration curves were constructed by plotting peak areas versus concentrations with the help of win-CATS software which are shown in fig.4(a) and fig 4 (b). Each reading was the average of three determinations. The regression coefficient (R^2) for calibration curve of CEFTA and TAZO was 0.999 and 0.998 respectively (Table IV).

ACCURACY (% RECOVERY)

Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The results of the accuracy study are reported in Table V. From the recovery study, it was clear that the method is very accurate for quantitative estimation of CEFTA and TAZO in Dry Injection dosage form because all the statistical results were within the acceptance range (i.e., % RSD <2.0).

METHOD PRECISION (REPEATABILITY):

The repeatability of sample application and measurement of peak area were expressed in terms of %R.S.D. and were found to be 0.964 and 1.430 for CEFTA and TAZO respectively (Table VI).

INTERMEDIATE PRECISION (REPRODUCIBILITY):

Intermediate precision was carried out by doing intra- and interday precision studies.In the intraday study, the concentrations of two drugs were calculated on the same day at an interval of 1 h. In the interday study, the concentrations of drug contents were calculated on three differentdays, and the study expresses within-laboratory variation in different days (Table VII). Intra-day and Inter-day precision were expressed in terms of %R.S.D. and were found to be 0.606, 0.518 and 1.291, 1.382 for CEFTA and TAZO respectively. The developed method was precise for quantitative study because the precision study was found statistically significant (% RSD <2.0 for intra- and interday studies).

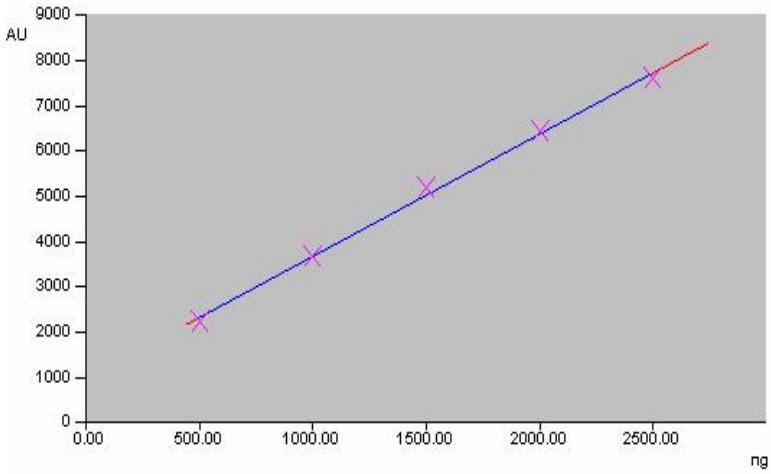

Fig 4(a):Calibration curve of CEFTA 500ng-2500ng/band

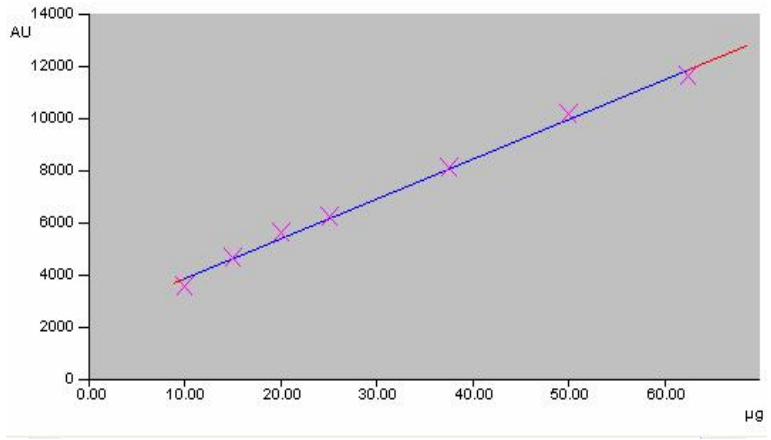

Fig 4(b): Calibration curve of TAZO 10µg-62.5µg/band

Table IV :Regression analysis of the calibration curves for CEFTA and TAZO for the proposed HPTLC method

Parameters	CEFTA	TAZO
Linearity Range	500ng/band – 2500 ng/band	10µg/band – 62.5µg/band
Slope	2.652	152.4
Intercept	707.2	2355
Regression Co-efficient (R^2)	0.999	0.998
Regression Equation	Y= 707.2+2.652X	Y=2355+152.4X
LOD	1.18	4.22
LOQ	3.70	12.79

Table V : Recovery studies

Drug	Level of % recovery *	Amount present (ng/band)*	Amount added (ng) *	Total amount recovered (ng)	% Recovery ± % RSD
CEFTA	80%	2200	1760	3952	99.81±0.969
	100%	2200	2200	4399	99.98±0.217
	120%	2200	2640	4839	99.78±0.045
TAZO	80%	275	220	493	99.61±0.602
	100%	275	275	549	99.81±0.318
	120%	275	330	604	99.88±0.578

*denotes average of three determination

Table VI: Repeatability study:

Drugs	Mean Content* (%)	S.D.*	% R.S.D.*
CEFTA	99.78	0.963	0.964
TAZO	100.07	1.431	1.430

* denotes average of three determination

Table VII: Intermediate precision

Drug	Intra-day Precision*			Inter-day Precision*		
	Mean % content	S. D.	%R.S.D	Mean % content	S.D.	%R.S.D.
CEFTA	99.84	0.605	0.606	99.95	0.518	0.518
TAZO	100.35	1.296	1.291	100.13	1.384	1.382

*denotes average of six determination

Table VIII: Robustness Study

Parameters	Drug	Mean % content*	S.D.*	% R.S.D.*
Mobile Phase Composition (±0.1 ml)	CEFTA	99.18	0.861	0.868
	TAZO	98.72	0.856	0.867
Amount of Mobile Phase (± 5 %)	CEFTA	99.65	0.857	0.201
	TAZO	98.64	0.388	0.393
Time from spotting to chromatography (± 10 min)	CEFTA	98.52	1.339	1.359
	TAZO	98.96	1.193	1.205

* denotes average of six determination

LOD AND LOQ:

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times [\sigma]/S$

$LOQ = 10 \times [\sigma]/S$

Where σ = the standard deviation of the response and S = the standard deviation of y- intercept of regression lines.

LOD for CEFTA and TAZO was found to be 1.18 ng/spot and 4.22 ng/spot, and LOQ was found to be 3.70 µg/spot and 12.79 µg/spot respectively (Table IV).

ROBUSTNESS:

By introducing small deliberate changes in the mobile phase composition (±0.1 ml), Amount of Mobile Phase (± 5 %), Time from spotting to chromatography (± 10 min) the effects on the results were examined. % RSD found in the ranges of 0.201-1.359 and 0.393-1.205 for CEFTA and TAZO respectively. The low values of %RSD obtained after introducing small changes in mobile phase composition indicated robustness of the method. There was no significant variation in the slope values**. (**Table VIII).

CONCLUSION

The developed HPTLC technique is simple, accurate and reproducible for simultaneous determination of CEFTA and TAZO in pharmaceutical dosage forms. Statistical analysis proves that the method is applicable for the analysis of CEFTA and TAZO as bulk drug and in pharmaceutical formulations without any interference from the excipients. The method was validated in accordance with ICH guidelines. The method reduces analysis time compared with other methods mentioned in literature survey and seems to be suitable for routine analysis of pharmaceutical formulations in quality-control laboratories, where economy and speed are essential.

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