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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF AZELASTINE HYDROCHLORIDE AS BULK DRUG AND IN PHARMACEUTICAL LIQUID DOSAGE FORM

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Abstract:

The current work describes development and validation of simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Azelastine hydrochloride as bulk drug and in pharmaceutical liquid dosage form. The chromatographic separation was carried out using precoated silica gel 60F_{254} aluminium plates as stationary phase and mixture composed of Tolune: Methanol: Glacial acetic acid in the ratio of (6.5: 3: 0.5, v/v/v) as mobile phase. The retention factor of Azelastine hydrochloride was found to be 0.33 ± 0.012. The wavelength selected for detection was 220 nm. Drug sample was subjected to different stress conditions like hydrolysis, oxidation, photolysis and thermal degradation.The developed method has been validated for linearity, accuracy, precision, limit of detection and limit of quantification and robustness, as per ICH guidelines. Results were found to be linear in the concentration range of 50-500 ng band^{-1} with high correlation coefficient. The method has been applied successfully for determination of drug in liquid dosage form. The percentage drug content obtained for Azelastine was 99.84±0.95. The developed method can be adopted for routine analysis of drug in and in pharmaceutical dosage form.

Keywords: *Azelastine hydrochloride, HPTLC, Stability Studies, Validation*

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INTRODUCTION:

Azelastine (AZE), chemically, (±)-1-(2H)-phthalazinone, 4-[(4-chlorophenyl) methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-monohydrochloride is selective histamine antagonist which is used for first line therapy of mild intermittent, moderate/severe intermittent and mild persistent rhinitis [1]. Extensive literature review reveals High Performance Liquid Chromatographic (HPLC) [2-6], Spectrophotometric [7-10] and High Performance Thin Layer Chromatographic (HPTLC) [11] methods for determination of ALGP in human plasma and pharmaceutical formulations either as single or in combination with other drugs. To best of our knowledge, no reports were found for stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of ALGP in tablet dosage form. This paper describes development and validation of simple, precise, accurate stability indicating HPTLC method for determination of ALGP in accordance with International Conference on Harmonisation Guidelines [12, 13].

MATERIALS AND METHODS:

Chemicals and reagents:

Pharmaceutical grade working standard AZE was obtained as gift sample from Emcure Pharma Pvt. Ltd. (Pune, India). The pharmaceutical dosage form used in this study was OPTIVAR® eye drops labeled to contain 0.5 mg of AZE per mL was procured from the local market. Toluene and Methanol (AR grade) were obtained from Thomas Baker Pvt. Ltd. (Mumbai, India). Glacial acetic acid was obtained from Loba Chemical Pvt Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions:

Chromatographic separation of drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) from E. MERCK, (Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Sample was applied on the plate as a band with 5 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Tolune: Methanol: Glacial acetic acid in the ratio of (6.5: 3: 0.5, v/v/v) as mobile phase. The mobile phase was saturated in chamber for 15 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner III at 220 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solutions:

Standard stock solutionwas prepared by dissolving 10 mg of drug in 10 mL methanol to get working standard stock solution of 1 mg mL^{-1} from which 0.5 mL was further diluted to 10 mL to get working standard stock solution of 50 ng μL^{-1}.

Selection of Detection Wavelength:

Densitometry scanning of the developed plate was carried out on CAMAG TLC scanner 3 in the reflectance-absorbance mode operated by WINCATS software version 1.4.2. The slit dimension was kept at 6 mm × 0.45 mm and 20 mm/spot scanning speed was employed. Source of radiation utilized was deuterium lamp emitting a continuous UV spectrum over the range of 200- 400 nm. It was observed that drug showed considerable absorbance at 220 nm as shown in **Fig. 1**.

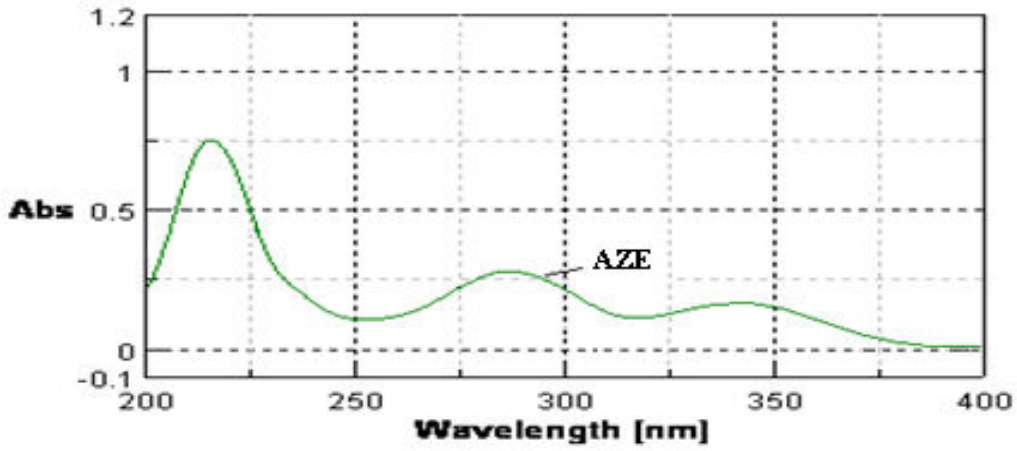

Fig. 1: Spectra of AZE measured from 200 to 400 nm

Analysis of marketed formulation:
For the analysis of marketed formulation, 1ml of eye drop which is equivalent to 0.5 mg of AZE was pipetted out using a volumetric pipette and transferred to a 10 mL of volumetric flask and diluted with methanol to get the concentration of 50 ng μL^{-1}. Two microlitre volume of this solution was applied on the plate. After chromatographic development, peak areas of the bands were measured at 220 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies:
The stability studies were performed by subjecting the bulk drug to the physical stress (acid, base, peroxide, heat and light) and stability was accessed. The stress degradation studies were carried out at initial drug concentration of 1000 µg $mL^{-1}$of AZE in methanol. The hydrolytic studies were carried out by mixing the drug solution of AZE separately with 0.1 N HCl and 0.1 N NaOH and were kept separately at room temperature for 2 h and 30 min to achieve degradation within the acceptable limit. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 200 ng $band^{-1}$ of AZE. Neutral hydrolysis study was performed by treating the drug with water and the resulting solution was kept at room temperature for 2 h. The oxidative degradation was carried out in 6 % H_2O_2 and the sample was diluted with methanol to obtain solution having concentration 200 ng $band^{-1}$. Thermal stress degradation was performed by keeping the solid drug in oven at 60ºC for a period of 48 h. Photolytic degradation was carried out by exposing drug to UV light up to 200-watt h square $meter^{-1}$for 1 d. Thermal and photolytic samples were diluted separately with methanol to get the concentration of 200 ng $band^{-1}$.

RESULTS AND DISCUSSION:

Method optimization:
The prime aim in developing this stability indicating HPTLC method is to achieve the satisfactory resolution of drug from its degradation products. Initially, many method trials were performed using different mobile phases in order to obtain better separation. Finally the mobile phase comprising toluene: ethyl acetate: methanol (6.5: 3: 0.5, v/v/v) was selected as optimal for obtaining well defined and resolved peak for the drug. Densitometric evaluation was carried out at 220 nm. The retention factor of AZE was found to be 0.33 ± 0.012. Representative densitogram of standard solution of AZE is shown in Fig. 2.

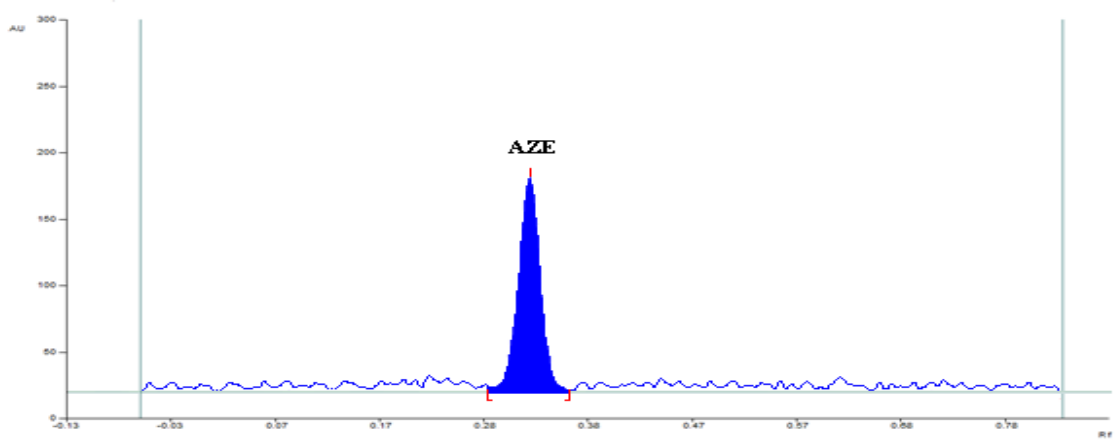

Fig. 2: Representative densitogram of standard solution of AZE (200ng/band, R_f = 0.33± 0.012)

Stress degradation studies:
The stress degradation results indicated that drug was found to be susceptible to acid and base catalysed hydrolysis, oxidation but found to be stable under thermal stress as well as photolysis. Figs. 3-5 show the densitograms of acid, alkali and neutral hydrolytic degradation, while Figs. 6-8 show the densitograms of oxidative degradation, thermal degradation and photolytic degradation, respectively. Marked degradation in the densitograms was observed but the degraded products were well resolved from the drug indicating specificity of the method. The findings of degradation studies are represented in Table 1.

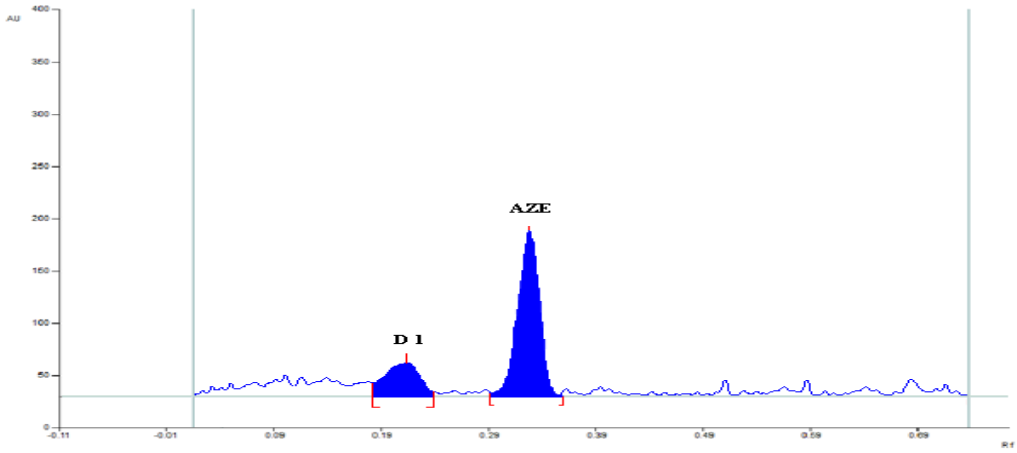

Fig. 3: Representative densitogram after acid treatment with degradation product D1 (Rf= 0.22)

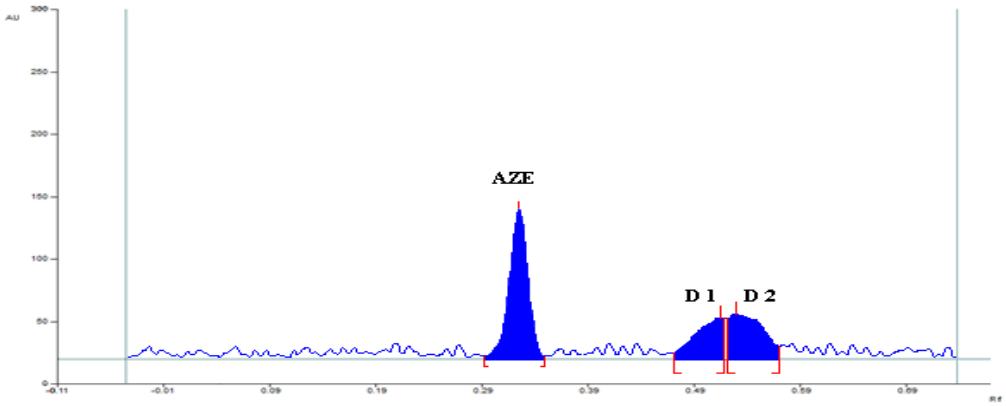

Fig. 4: Representative densitogram after alkali treatment withdegradation product D1 (Rf = 0.54) and D2 (Rf = 0.58)

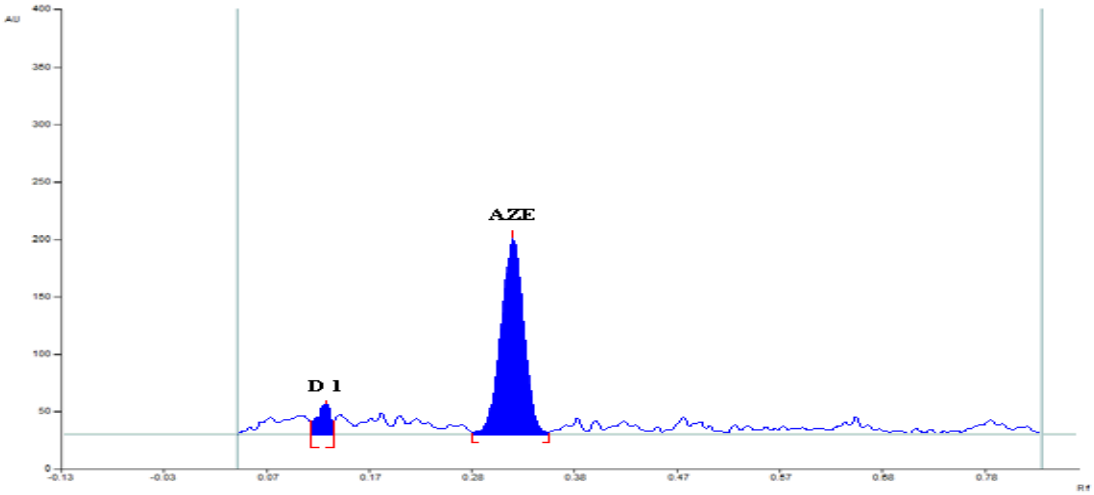

Fig. 5: Neutral degradation densitogramwithdegradation peak D1 (Rf = 0.12)

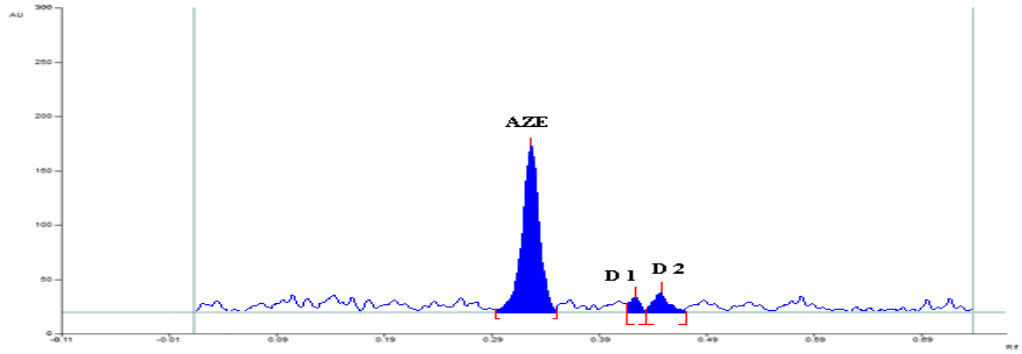

Fig. 6: Oxidative degradation densitogram with degradation peaks D1 and D2 (Rf = 0.42, 0.46)

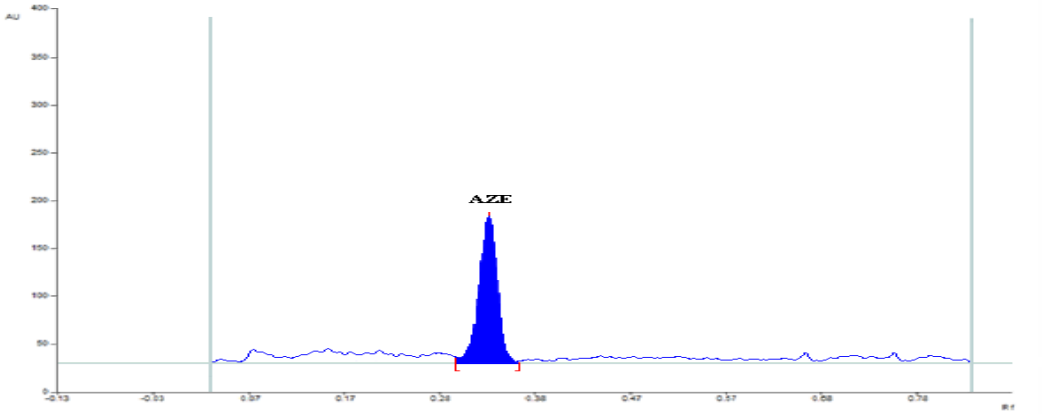

Fig. 7: Densitogram obtained after dry heat degradation

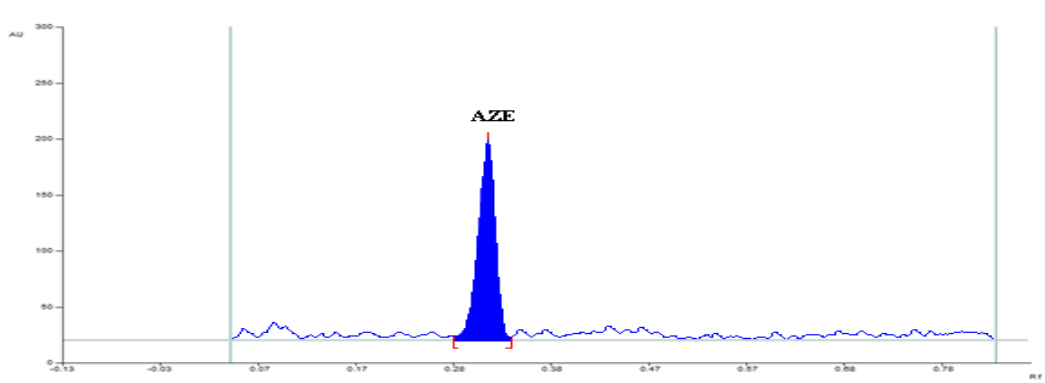

Fig. 8: Densitogram obtained after exposure to UV light

Table 1: Summary of stress degradation studies of AZE

Stress condition/ duration	% assay of active drug	% degradation	Rf values of degraded samples
Acidic / 1 N HCl/ Kept at RT for2hrs	78.47	21.52	0.22
Alkaline /0.5 N NaOH/ Kept at RT for30 min	69.72	30.28	0.49, 0.56
Oxidative /6 % H2O2 / Kept at RT for1hr	82.01	17.98	0.42, 0.46
Neutral/ H_2O/ Kept at RT for2hrs	87.36	12.64	0.12
Dry heat/ 60ºC/ 48 hrs	Stable	Stable	---
Photolysis	Stable	Stable	---

Method Validation

The optimized method was validated in accordance with ICH guidelines with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.

Linearity:

Standard stock solution of AZE (50 ng μL^{-1}) were applied on HPTLC plate in range of 1, 2, 3, 4 and 5 µL with the help of CAMAG 100 µl sample syringe, using Linomat 5 sample applicator to obtain final concentration 50-250 ng $band^{-1}$. The plate was developed and scanned under above established chromatographic conditions. Each standard in six replicates was analyzed and peak areas were recorded. Calibration curve was plotted of peak area Vs respective concentration. Linear response was observed in the concentration range 50-250 ng $band^{-1}$. Excellent correlation exists between peak area and concentration of drug within the concentration range indicated above. The calibration curve obtained for AZE is represented in Fig. 9.

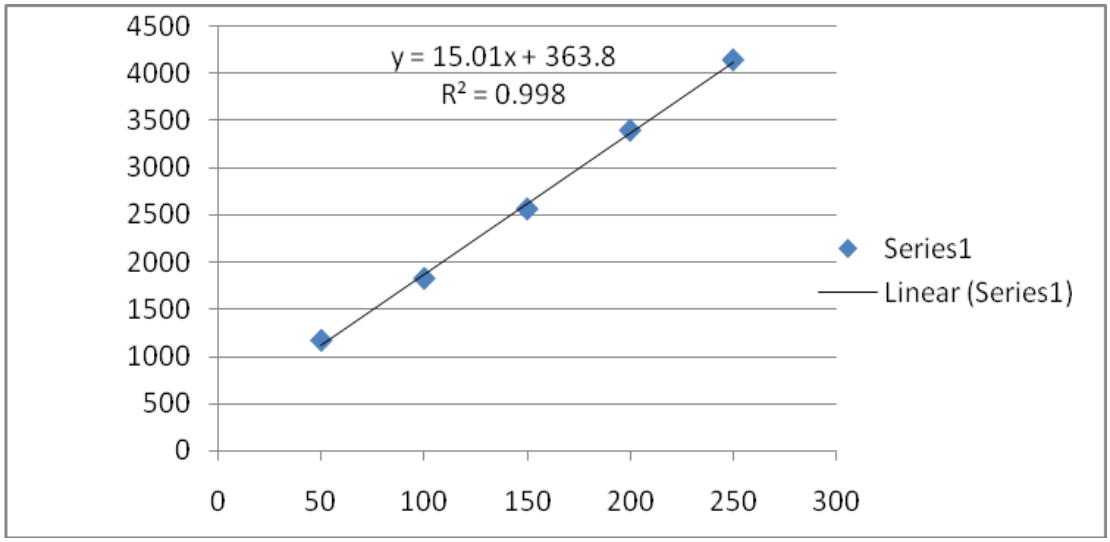

Fig. 9: Calibration curve for AZE

Precision:

Set of three different concentrations of standard solution of AZE were prepared. All the solutions were analyzed thrice, in order to record any intra day variations in the results. The % R.S.D. was found to be in the range of 0.62-1.18 for intraday precision and 1.02-1.48 for interday precision.

Limit of detection (LOD) and Limit of quantitation (LOQ):

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 9.28 ng $band^{-1}$ and 28.13 ng $band^{-1}$, respectively.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 100 ng $band^{-1}$. The areas were noted after development of plate. The drug concentration was calculated by using regression equation. The % mean recovery was found to be 99.55±0.81. The results obtained are shown in Table 2.

Table 2: Recovery Studies of AZE

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% R.S.D.*
AZE	100	80	178.72	99.22	0.81
	100	100	198.41	99.20	0.67
	100	120	220.55	100.24	0.95

*Average of three determinations

Robustness:

The robustness of the method was studied, during method development, by small but deliberate variations in mobile phase composition (± 2 % methanol), chamber saturation time (± 10 min). One factor at a time was changed at a concentration level of 250 ng band^{-1} to study the effect on the peak area of the drug. The areas of peaks of interest remained unaffected by small changes of the operational parameters and % RSD was within the limit (< 2 %) indicating the robustness of the developed method.

CONCLUSION:

Stability indicating HPTLC method has been developed and validated for the determination of AZE as bulk drug and in ophthalmic formulation. The developed method is simple, precise, accurate, and selective and can be used for quantitative analysis of AZE in pharmaceutical dosage form as well as for routine analysis in quality control laboratories.

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