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Research Article

# DEVELOPMENT AND VALIDATION OF HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR THE ESTIMATION OF POSACONAZOLE IN FORMULATION

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**ABSTRACT**

**Objective:** The study aims to developed and validate novel high performance thin layer chromatographic method for estimation of posaconazole in tablet dosage form and validate as per the international conference on harmonization (ICH) guidelines.

**Methods:** The method was developed on silica gel 60 $F_{254}$ HPTLC plates and the mobile phase was used as toluene: chloroform: ethanol in ratio (5:4:1 V/V/V). Densitometric analysis of Posaconazole was performed at the wavelength 265 nm.

**Results:** The $R_F$ of Posaconazole was found to be 0.331. The calibration curve was found to be linear in the concentration range of 100–500 ng/band. The correlation coefficient is 0.9991 for Posaconazole. The precision result was found to be satisfactory, which indicate that the method is precise. The recovery value lies in the range of 98–102% indicating the accuracy of the method.

**Conclusion:** A new, simpleaccurate, fast, economic, precise, and robust high performance thin layer chromatographic method was developed for the estimation of Posaconazole. The method is selective, specific it was applied in pharmaceutical analysis of Posaconazole tablet dosage form.

**Keywords:** Posaconazole, High-performance thin layer chromatography, Silica gel 60 $F_{254}$.

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## INTRODUCTION

Posaconazole4-{4-[4-(4-{[(3R,5R)-5-(2,4-difluorophenyl)-5-(1H-1,2,4-triazol-1-ylmethyl)-tetrahydrofuran-3-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-2-[(1S,2S)-1-ethyl-2-hydroxy-propyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (Fig. 1). Is a triazole antifungal drug, approved by FDA in 2006 and characterized for the Broder spectra of action between triazoles, beside the less potential of interactions. It is a first azole agent to demonstrate activity against zygomycetes, a difficult to treat family that includes mucor and Rhizopus species [1]. Posaconazole is also used to prevent invasive fungal infections in patient whose immune system is weakened because of treatment; they are receiving for blood or bone marrow cancers or medicines use in hematopoietic stem cell transplant [2,3].

The selected formulation for the present study is Posaconazole tablet which is useful for the treatment of Aspergillus and Candida infections. It is also used for the treatment of oropharyngeal candidiasis.

In the present study, chromatographic method development and validation for the estimation of Posaconazole by HPTLC, in a tablet dosage form. For the estimation of Posaconazole by HPLC, several methods are available but so far there is only one HPTLC method using acetone and chloroform as a mobile phase for estimation of Posaconazole in suspension dosage form is reported [3]. Hence, the objective of this work is to develop and validate a high-performance thin layer chromatography (HPTLC) method for estimation of Posaconazole in tablet according to official guidelines.

## METHODS

### Reagent and materials

Reference standards of Posaconazole obtained from alkem laboratories and their formulation; Picasa GR tablets were obtained from local chemist shop (Mumbai, India). Analytical grade toluene, methanol, and chloroform used were from Finar Chemicals (Mumbai, India) and Silica gel 60 $F_{254}$ plates from Sigma-Aldrich, Merck (Mumbai, India).

### Preparation of standard solution

10 mg of Posaconazole reference standard were accurately weighed and transferred in to a 100 mL volumetric flask add 50ml methanol sonicate 3-min volume was make up to mark with methanol. To get a final Concentration of 100µg/mL of Posaconazole.

### Instrumentation and chromatographic conditions

Spotting was done using CamagLinomat 5 sample applicator (CAMAG, Switzerland) and Camag Hamilton microlitre syringe (100 µl) on HPTLC aluminum plates pre-coated with silica gel 60 $F_{254}$ (20 cm × 10 cm with 250 µm thickness; Sigma-Aldrich). The plates were prewashed with ethanol for 30 min in a CAMAG twin trough glass chamber closed with lid. The plates were activated at 110°C for 10 min. The samples were spotted in the form of narrow bands having length of 8 mm. The application position X and Y was kept at 8 mm and 20 mm, respectively, to avoid edge effect. The distance between the two bands was 11.4 mm. Bands were applied at a constant rate of 15 nL/s using a nitrogen aspirator. Linear ascending development of chromatogram was carried out in a CAMAG twin trough glass chamber saturated with the mobile phase for 20 min and chromatogram run was kept up to 80 mm. Following the development, the HPTLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. Spectrodensitometric analysis of the separated components was carried out using Camag TLC Scanner 4 in the reflectance–absorbance mode at 265 nm using a deuterium lamp. The

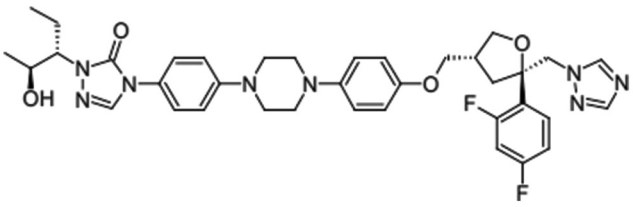


**Fig. 1: Chemical structure of Posaconazole**

slit dimension used was 6.0 mm × 0.45 mm and sensitivity was kept at auto mode. Scanning speed was 100 nm/s. Evaluation was achieved by linear regression of the peak area response against amount of drug by using vision CATS (CAMAG) software for peak area measurement and data processing.

**Optimization of the HPTLC method**

The HPTLC procedure was optimized with a view to develop an assay method for Posaconazole. Considering the chemical nature and polarity of the molecules to be separated, Silica gel $F_{254}$ TLC plates were used. Initial trails were made using different solvent system with different ratios including toluene, ethyl acetate, chloroform, methanol, ethanol, and acetone. After trails toluene: chloroform: ethanol (5:4:1 V/V/V) where used. Finally, good resolution as well as sharp and symmetrical peak with improved $R_F$ values were obtained from the mobile phase containing toluene: chloroform: ethanol in the ratio of 5:4:1 (V/V/V).

**Method validation**

Validation of the optimized method HPTLC method was carried out according to the ICH guidelines [7].

**Linearity**

The linearity of the response to Posaconazole was assessed in the concentration ranges100–500 ng/band. Linearity of the method was studied by injecting five concentrations of the drug to the HPTLC plates. The plate was then developed using the mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

**Precision**

Precision of the method was demonstrated by repeatability and intermediate precision studies. Repeatability (% RSD) was determined by analysis of Posaconazole at the concentration of 250 ng/band.

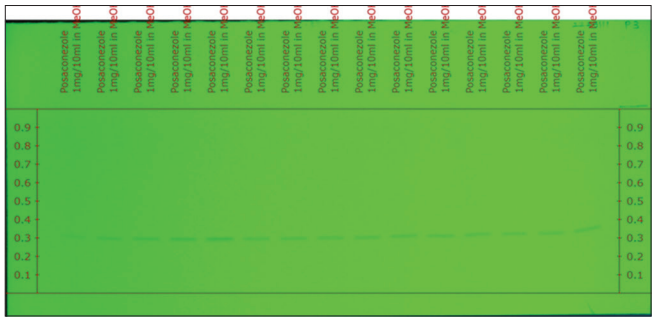

**Fig. 2: Images of the HPTLC plates taken at 265 nm**

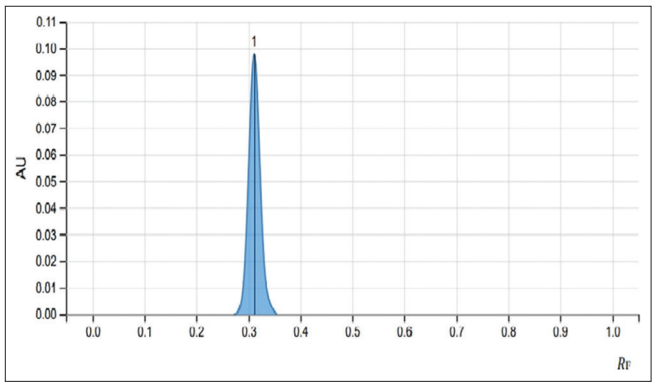


**Fig. 3: HPTLC densitogram under optimized conditions showing $R_F$ values of 0.331 for Posaconazole (250 ng/band)**

**Accuracy**

The accuracy of the method was determined by the analysis of standard addition at three different levels, that is, multiple-level recovery studies. The pre-analyzed samples were spiked with 80%, 100%, and 120% of the standard drug and the mixture was reanalyzed by the proposed method. This was done to verify the recovery of the drug at different levels in the formulation.

**Specificity**

The specificity of the method was ascertained by analyzing standard drug and test samples. The band for Posaconazole in the sample was confirmed by comparing the $R_F$ value and spectrum of the spot with that of a standard. The peak purity of Posaconazole was determined by comparing the spectrum at three different regions of the spot, that is, peak start (S), peak apex (M), and peak end (E).

**Sensitivity**

The sensitivity of the developed method is expressed as limit of detection (LOD), the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value under the experimental conditions, as well as limit of quantification (LOQ), which is the lowest amount of analyte that can be detected and quantified with suitable precision, accuracy, and reproducibility. The LOD and LOQ are calculated based on the standard deviation of the regression lines and slope of the calibration curves using the below equations:

$$LOD = 3.3 \times \sigma/S,$$

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

Where σ is the standard deviation of the regression line and *S* is the slope of the calibration curve.

**Robustness**

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as changing the mobile phase composition and chamber saturation period. The amount of mobile phase was varied over the range of ±5 ml, the chamber saturation period was varied over the range of ±5 min and the distance travelled was varied over the range of ±5 mm.

**Analysis of marketed formulation**

To determine the content of Posaconazole in a conventional tablet (brand name: Picasa GR tablet (Intas pharmaceuticals ltd) label claim: 100 mg Posaconazole per tablet, 10 tablets were weighed, their mean weight was taken and finely powdered. The weight of the powder equivalent to 10 mg of Posaconazole was transferred into a 100 mL volumetric flask 50 mL of methanol was added. The prepared solution was sonicated for 15 min, cooled and the volume was completed to 100 ml. The resulting solution of 100 µg/mLfor Posaconazole was centrifuged at 3000 rpm for 5 min and the supernatant was collected. The analysis was repeated 6 times and the possibility of excipients interference with the analysis was examined.

## RESULTS AND DISCUSSION

Results of the validation studies on the estimation of the method developed for Posaconazole in the present study involving toluene:

**Table 1: Linearity data for Posaconazole**

| Concentration (ng/band) | Peak area |
|---|---|
| 100 | 0.00132 |
| 200 | 0.00216 |
| 300 | 0.00296 |
| 400 | 0.00373 |
| 500 | 0.00445 |

chloroform: ethanol (5:4:1 V/V/V) as the mobile phase for HPTLC are given as follows.

**Method validation**

*Linearity*

Linear relationships were observed by plotting drug concentrations against peak areas for the compound. Posaconazole showed linear response in the concentration range of 100–500 ng/band. The corresponding linear regression equation was y = 8E-06x + 0.0006 for Posaconazole with correlation coefficient of the calibration plot was 0.9991 as shown in Fig. 4 and 5.

*Precision*

Results of the system precision and method precision experiments are shown in the Tables 2 and 3 The developed method was found to be precise as the %RSD values for precision studies were <2%, respectively, as recommended by ICH guidelines.

*Specificity*

The chromatogram of the pharmaceutical formulation obtained using the developed method showed only one peaks at $R_F$ of for Posaconazole and was found to be at the same $R_F$ for the standard drugs. The peak purity of Posaconazole was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the band. The purity exceeded 0.999 for all peaks, indicating the specificity of the method in the presence of various excipient.

*Accuracy*

When used to evaluate the recovery after spiking with three concentrations of standard, 80%, 100%, and 120%. The proposed method showed good percentage recovery rates between 98 and 102%. The results of the recovery studies and its statistical validation are given in Table 4.

*Sensitivity*

Using the trend line equations derived from the experiments, the sensitivity of the method in terms of LOD and LOQ was calculated based on the standard deviation of the regression lines and slope of calibration curves. The LOD and LOQ were found to be 10.45 and 31.67 ng/band, respectively, for Posaconazole, indicating the sensitivity of the proposed method.

**Table 2: System precision data for Posaconazole**

| Serial number | Concentration (ng/band) | Peak area |
|---|---|---|
| 1 | 250 | 0.00254 |
| 2 | 250 | 0.00255 |
| 3 | 250 | 0.00253 |
| 4 | 250 | 0.00250 |
| 5 | 250 | 0.00256 |
| 6 | 250 | 0.00257 |
| Mean | 0.002541667 | |
| SD | 0.0000248328 | |
| Percentage RSD | 0.977027175 | |

SD: Standard deviation, RSD: Relative SD

**Table 3: Method precision data for Posaconazole**

| Serial number | Concentration (ng/band) | Peak area |
|---|---|---|
| 1 | 250 | 0.00253 |
| 2 | 250 | 0.0026 |
| 3 | 250 | 0.00261 |
| 4 | 250 | 0.00254 |
| 5 | 250 | 0.00258 |
| 6 | 250 | 0.00261 |
| Mean | 0.002578333 | |
| SD | 0.0000354495 | |
| Percentage RSD | 1.374899596 | |

SD: Standard deviation, RSD: Relative SD

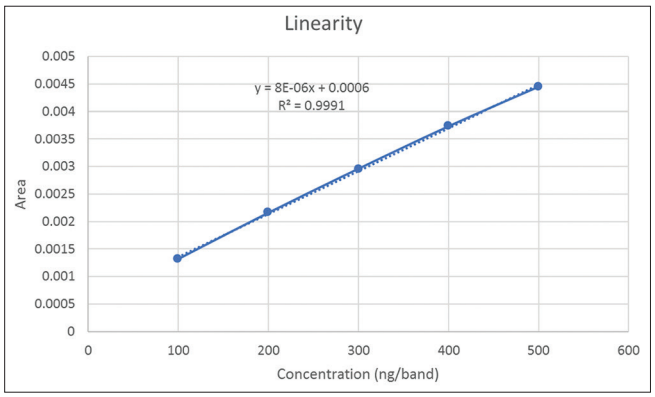


**Fig. 4: Calibration curve of Posaconazole**

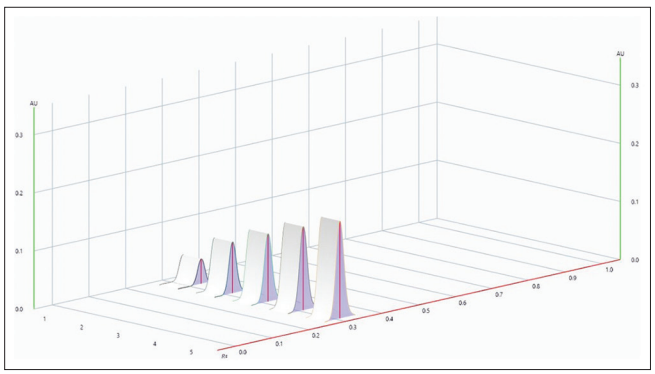

**Fig. 5: Three-dimensional densitogram for the linearity of Posaconazole at 265 nm**

**Table 4: Result from recovery study of Posaconazole**

| Percentage level | Amount spiked (ng/band) | Area | Mean of area | Amount recovered (ng/band) | Recovery (%) | SD of area | Percentage RSD of area |
|---|---|---|---|---|---|---|---|
| 80 | 160 | 0.004520<br>0.004582<br>0.004581 | 0.004561 | 161.93 | 101.21 | 0.00003551 | 0.7785 |
| 100 | 200 | 0.004913<br>0.004962<br>0.004933 | 0.004936 | 201.12 | 100.56 | 0.00002463 | 0.4991 |
| 120 | 240 | 0.005303<br>0.005211<br>0.005285 | 0.0052663 | 244.87 | 102.03 | 0.00004875 | 0.9258 |

SD: Standard deviation, RSD: Relative SD

**Table 5: Robustness results of the proposed high-performance thin layer chromatography method**

| Ratio | $R_F$ | Area±SD (ng/band) | Percentage RSD |
|---|---|---|---|
| **Change in the mobile phase ratio (5:4:1±0.2 in toluene content)** | | | |
| 4.8:4:1 | 0.317 | 0.002526±0.00002309 | 0.9140 |
| 5:4:1 | 0.331 | 0.002533±0.00002082 | 0.8217 |
| 5.2:4:1 | 0.310 | 0.002553±0.00003512 | 1.3754 |
| **Change in saturation time (20 min±5 min)** | | | |
| 15 min | 0.311 | 0.002566±0.00003786 | 1.4750 |
| 20 min | 0.330 | 0.002576±0.000035118 | 1.3629 |
| 15 min | 0.313 | 0.002577±0.00002804 | 1.0878 |

The $R_F$ and standard deviation of peak areas were calculated for each parameter and the percentage RSD was found to be<2%. The low values of the percentage RSD and no significant changes in the $R_F$, as shown in the [Table 4], indicate the robustness of the method. SD: Standard deviation, RSD: Relative SD

**Table 6: Analysis of marketed formulation**

| Formulation | Drug | Percentage assay |
|---|---|---|
| Picasa GR tablet 100 mg | Posaconazole | 101.3 |

**Robustness**

*Analysis of marketed formulation*

The analysis of the tablet formulation containing 100 mg of Posaconazole showed good agreement with the label claims (Table 6), thereby suggesting that there was no interference from any of the excipients which are generally present in the tablets. This indicated that the method can be used for routine quality control while testing the tablet formulation.

## CONCLUSION

The present method was found to be simple, specific, precise, accurate, reliable, and selective, saving both cost and time. This study reports a simple fully validated HPTLC protocol for the quantification of Posaconazole in pharmaceutical formulation. It demonstrates that the method can accurately quantify the drug content of tablet formulation without excipient interference or the necessity of a drug extraction step before analysis. Thus, the developed method can be implemented in quality control laboratories for the routine analysis of drug in their pharmaceutical formulation.

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## AUTHORS CONTRIBUTION

All authors are have equally contributed in research work.

## CONFLICTS OF INTERST

The authors declare that they have no conflicts of interest.

## AUTHORS FUNDING

Nil.

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