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Development of novel HPTLC method for determination of imidazole antifungal drug fenticonazole: Exploring hydrotropy

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ABSTRACT

A novel High-Performance Thin-Layer Chromatography (HPTLC) method was portrayed for the determination of Fenticonazole Nitrate (FTZ) in Bulk and Vaginal Capsules. The estimation of Fenticonazole Nitrate was achieved on aluminium pre-coated sheets of silica gel 60 F_{254} (10 cm × 10 cm) using mobile phase Toluene: Methanol: Triethylamine (4:1:0.5 v/v/v). Densitometry detection of Fenticonazole Nitrate was performed at 254nm. Fenticonazole nitrate demonstrated a strong correlation with a coefficient of correlation of 0.999 over the concentration range of 500 – 3000 ng/band. The R_f value for Fenticonazole Nitrate was found to be 0.65. As per International Conference on Harmonization the established method was successfully validated to various parameters like accuracy, precision, sensitivity, specificity, robustness and shows the satisfactory results for all parameters. The recognized method is simple, accurate, precise, robust, sensitive and economical in nature. This method can be used for quality control analysis of Fenticonazole Nitrate in bulk and vaginal capsules.

1. Introduction

Fenticonazole Nitrate (FTZ) chemically is 1-[2(2,4-dichlorophenyl)-2 {[4 (phenyl sulfanyl) phenyl] methoxy} ethyl] 1H-imidazole (Figure 1). It is an Imidazole antifungal drug used in the treatment of vulvovaginal candidiasis. It is active against a range of organisms including dermatophyte pathogens, Malassezia furfur, and Candida albicans. It acts by three mechanisms such as inhibition of the secretion of protease acid by Candida albicans, damage to the cytoplasmic membrane; and by blocking cytochrome oxidases and peroxidises.[1,2]

FTZ shows less solubility in aqueous solution. So, to increase solubility hydrotropic agents were used. Hydrotropic agent increases the solubility of drugs having poor water solubility by different mechanisms.[3] This hydrotropy concept is mainly applied to the UV-Spectrophotometry for the determination of different pharmaceuticals.[4,5]

A detailed literature revealed few analytical and bio-analytical methods such as UV-Spectrophotometry[6], RP-HPLC[7–9], capillary electrophoresis[10] and LC-MS[2,11,12] reported for the determination of FTZ.

To our knowledge, no efficient and inexpensive HPTLC method for the estimation of FTZ in bulk and vaginal capsule formulation has been discovered till date. The objective of the current work is therefore to develop a simple, sensitive, convenient, precise and cost-effective HPTLC method for evaluating FTZ in bulk and vaginal capsule formulation in compliance with the International Harmonization Conference (ICH) guideline Q2 (R1).

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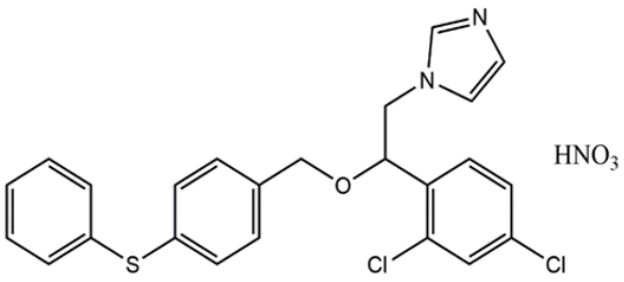

Fig. 1: Chemical structure of FTZ

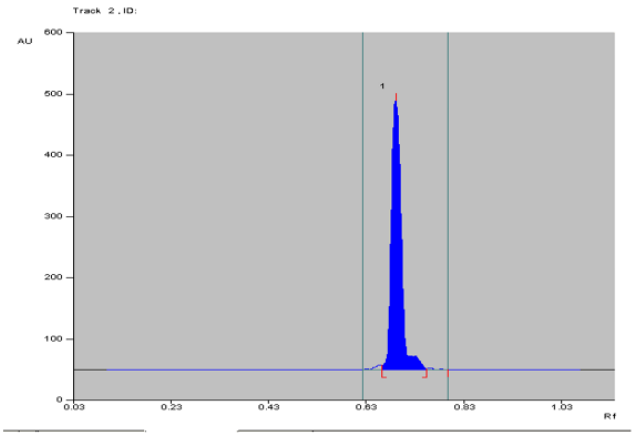

Fig. 2: TLC Densitogram of FTZ (1500 ng/band, R_f 0.65 ± 0.03) drugsolutions in toluene: Methanol: Triethylamine (4: 1: 0.5 v/v).

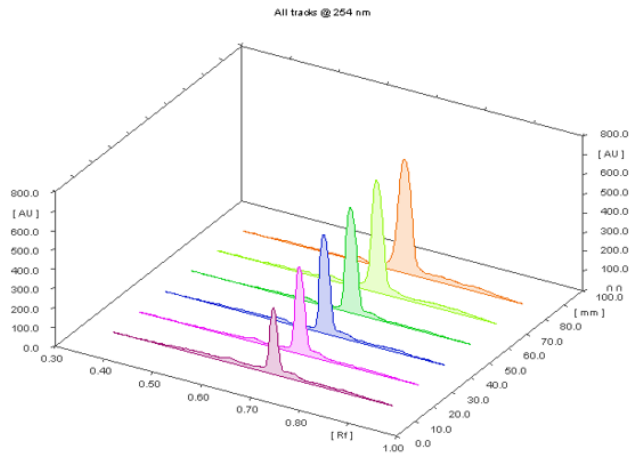

Fig. 3: 3D linearity of FTZ.

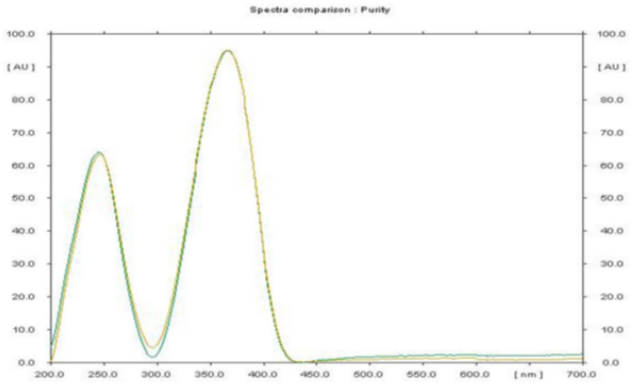

Fig. 4: Peakpurity spectra of FTZ extracted from capsule, scanned at the peak — start, peak- apex, and peak-end positions of the band (Correlation > 0.999).

2. Experimental

2.1. Chemicals and reagents

Pharmaceutical grade FTZ working standard was a generous gift from Glenmark Pharmaceuticals ltd. Mumbai, India. Fixed dose vaginal capsule formulation of FTZ containing 600mg of FTZ procured from local pharmacy store. HPLC grade toluene, methanol and triethylamine are used as mobile phase and procured from Merck. Ltd, Mumbai.

2.2. Instrumentation and chromatographic condition

FTZ study performed by the CAMAG (Muttenz, Switzerland) HPTLC system consisting of a Linomat-5 sample applicator (Muttenz, Switzerland) fitted with a 100-μL Hamilton sample syringe (Bonaduz, Switzerland), a Camag twin trough glass chamber (10 cm × 10 cm) and a Camag TLC Scanner 3, all appliances wired to WinCATS Data Processor Software version 1.3.0. Chromatography was performed over 10 cm × 10 cm NP-HPTLC aluminum-backed plates coated with TLC silica gel 60 F254 layers of 200 μm (Merck, Darmstadt, Germany, supplied by Merck India, Mumbai, India). Using methanol, the plates were prewashed and activated in a hot air oven at 105^0C for 5 minutes. Then samples were applied as 6 mm wide bands with the help of Linomat 5 sample applicator under controlled nitrogen stream, later plate was developed in a pre-saturated Camag twin trough glass chamber which is already saturated with mobile phase vapors for 25min on suitable temperature. The plate was developed to a distance of 80.0 mm using mobile phase Toluene: Methanol: Triethylamine (4: 1:0.5 v/v/v). Camag TLC Scanner-3 linked to WinCATS data processor software version 1.3.0 at 254nm, densitometry scanning was accomplished. The radiation source was a Deuterium lamp that emits constant Ultraviolet radiation between 400-200nm.

2.3. Preliminary solubility studies and selection of solvent

Solubility of FTZ was checked in aqueous solution of different hydrotropic agent including Sodium Benzoate, Sodium Acetate, Sodium Citrate, Urea, Benzalkonium chloride, Pluronic (F68, F127), Potassium Acetate, and Polyethylene Glycol. FTZ was found to be completely soluble in Pluronic F127 solution of various strengths such as 30%, 20%, and 10% (w/v). Minimumconcentration (10% w/v) of Pluronic F127 was chosen for solubilization of drug for entire study.

2.4. Preparation of pluronic F127 (10 % w/v solution

100g of Pluronic F127 was transferred in 1000 ml volumetric flask containing 700 ml water, sonicated for 20 minutes and volume was made up to the mark with water.

2.5. Preparation of Standard Stock Solution

The stock standard solution was formulated by weighing 10 mg of FTZ. The weighed powder was transferred into a 10 ml volumetric flask and diluted up to the limit with Pluronic F127 (10% w/v) to achieve the concentration of 1000 μg/ml.

2.6. Selection and optimization of mobile phase

Firstly, to isolate the spots, single solvents were chosen based on their polarity. Then spots were developed with Toluene: Methanol: Triethylamine (4:1:0.5 v/v/v). In this mobile phase, the drug was separated with good resolution and with sufficient Rf. The Triethylamine was used as modifier into the mobile phase.

2.7. Analysis of bulk material

Accurately weighed 10 mg of FTZ was transferred into 10mL volumetric flask dissolved in methanol, shaken manually and volume was adjusted to mark using same solvent. 1.5 ml further diluted to 10 ml with methanol and appropriate volume of 10 μl of this solution containing 1500 ng/band of FTZ was applied on NP-HPTLC plate. The plate was developed, dried and scanned as described above.

2.8. Analysis of vaginal capsule formulation

Ten vaginal capsule of Fenticonazole nitrate were accurately weighed; average weight was determined and equivalent to 60 mg of Fenticonazole nitrate was transferred into 100 ml of volumetric flask contain 50 ml Pluronic F127 (10%w/v) sonicated for 45 min and volume was adjusted to mark using same solvent. It was filtered through a 0.45μm filter (Millifilter, milford, MA, USA). From the filtrate, an appropriation volume of solution was applied on plate to get concentration 1500 ng/band.

3. Method Validation

The developed HPTLC system was validated according to ICH guideline Q2 (R1) for various parameters such as linearity, accuracy, precision; robustness, detection limit, and quantitation limit. [13]

3.1. Linearity studies of FTZ

Linearity of the established HPTLC method was evaluated by constructing the calibration curves at six concentration levels. Calibration curves were plotted over a concentration range of 500-3000ng/band. Aliquots of standard working solution of FTZ were applied to the plate (0.5, 1, 1.5, 2, 2.5, and 3.0μL/band). The calibration curves were developed by plotting the peak area versus concentration (n = 6) with the help of the winCATS software and Standard deviation (SD), coefficient of determination (r^2), slope and intercept of the calibration curves were estimated to determine linearity of the method.

3.2. Accuracy

The accuracy of the developed HPTLC method was determined by measurement of percent recovery. Recovery trials were conducted at three distinct levels i.e. 80, 100, and 120 %. To the pre-analyzed sample solutions, a known amount of drug standard solution of FTZ was over-spotted at three distinct levels.

3.3. Precision

The precision was evaluated as intra-day, inter-day precision and repeatability. The repeatability of measurement of peak area was determined by performing six replicate measurements of the same using 1500 ng/band concentration for FTZ. Intra-day variation was obtained by determining three different concentrations thrice a day and Inter-day precision was assessed by three different concentrations for three different days, over a week. The intra-day and inter-day variations were assessed at three different concentrations 1000, 1500, 2000 ng/band (FTZ).

3.4. Robustness

Robustness was assessed by introducing small, deliberate changes in the parameters (mobile phase composition, mobile phase volume, development distance, and duration of saturation) of developed HPTLC method.

3.5. Sensitivity (LOD and LOQ)

With reference to the limit of detection (LOD) and limit of quantification (LOQ), the sensitivity of the developed HPTLC method was determined. The noise was measured six times with blank spot scanning of Pluronic F127 (10 % w/v). The drug solution concentration series (10-

500ng/band) was applied to the plate and examined for LOD and LOQ determination. LOD was calculated as 3 times the noise level, and LOQ was calculated as 10 times the noise level. LOD and LOQ were determined experimentally by diluting the known concentrations of FTZ till the average responses were around 3–10 times the standard deviation (SD) of the responses for six replicate determinations.

3.6. Specificity

The specificity of the method was assessed by examining FTZ in presence of additives of FTZ in vaginal capsule formulations. The bands of FTZ in the sample were verified by comparing Rf values and comparative spectra of the sample with those of the standards. The peak purity of FTZ was evaluated by comparing the spectra of standard FTZ and FTZ- isolated from the vaginal capsule at the peak-start (S), peak-apex (A), and at the peak-end (E) points. This peak purity concludes that no impurity was found in the vaginal capsule formulation comparing with the peak of standard drug solution.

Table 1: Linear regression data for calibration curve (n=6)

Parameter	Result
Linear range (ng/band)	500 - 3000
Slope	2.914
Intercept	3700
Correlation coefficient (r^2)	0.999

n= number of determinations

Table 2: Analysis of capsule formulation

	Drug % Amount Found ± SD	% RSD [n=6]
FTZ	99.9 ± 1.32	1.32

n= number of determinations

Table 3: Summary of validation parameter.

Parameter	Result
Linearity Range [ng/band]	500 - 3000
Correlation Coefficient	0.999
Accuracy (% Recovery)	99.68 – 99.57
Precision (% RSD)	1.09 (Intra-day) 1.67 (Inter-day) 1.82 (Repeatability)
Detection limit (ng)	10.14
Quantification Limit (ng)	30.74

4. Result and Discussion

Choice of the most suitable solvent system is the crucial step in HPTLC method development. So, for the proposed HPTLC method various solvent systems were attempted, initially, Toluene: Methanol: Ammonia (4:1:0.5 v/v/v) was used as a mobile phase but tailing of peak obstruct the results. Then spots were developed by replacing the ammonia with triethylamine i.e. Toluene: Methanol: Triethylamine (4:1:0.5 v/v/v). In this mobile phase the drug was separated with good resolution and with suitable R_f 0.65±0.03 (Figure 1). It was seen that chamber saturation time and solvent migration distance are significant in chromatographic separation when tank saturation time is less than 15min and solvent migration distance more than 80mm produced a scattering of analyte band. Therefore, Toluene: Methanol: Triethylamine 4:1:0.5 (v/v/v) proportions with chamber saturation time of 25 min at 25^0C and solvent migration distance of 80mm was used. A well-specified convenient spot of FTZ with appropriate migration 0.65 obtained. Linearity of an analytical method is ability of test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. The established HPTLC method was found to be linear in concentration range of 500-3000ng/band (n=6) confirming obedience of the system to Beer's law.(Figure 3) displays three dimensional overlay of HPTLC densitogram of the calibration bands of FTZ at 254 nm. Linear regression data for the calibration curve was given in (Table 1). The LOD and LOQ for FTZ were found to be 10.14 ng and 30.74ng respectively which shows the adequate sensitivity of the method. Specificity of the method for FTZ was proved from the spectral scan (Figure 4) for FTZ in bulk and in vaginal capsule formulations indicate that there is no merging or co-elution of interfering peaks with FTZ, so there is no interference from any excipients present in vaginal capsule formulation. The accuracy of this HPTLC method was assessed by the implementation of an analytical technique to recovery analysis where the quantity of standard was spiked on pre-analyzed sample solution. The % recovery of FTZ was observed in range of 99.57-99.68. The % recovery values indicate that developed HPTLC method was highly accurate in nature. The precision was done by three parameters intra-day, inter-day, and repeatability precision. The intra-day precision % RSD was 0.25-0.46 and inter-day precision %RSD was 0.57-108. The repeatability % RSD was found to be 0.87. The % RSD less than 2 indicate that method is extremely précised. Analysis of vaginal capsule formulation i.e. capsule assay was done. The % RSD value of vaginal capsule assay was found to be 1.32 denotes that results are within range (Table 2). Robustness was performed by changing four parameters and the % RSD value for all four parameters was found to be in acceptable range. This denotes that method was robust in nature. An overview of the validation parameter is shown in (Table 3).

5. Conclusion

A new HPTLC method was successfully developed for the determination of FTZ in bulk and vaginal capsule formulation. The developed HPTLC method was validated to different parameters as per ICH guidelines and it confirmed that the developed method is simple, accurate, linear, precise, robust, sensitive, and economical in design from the results. This approach can be applied for routine quality control.

6. Source if Funding

None.

7. Conflict of Interest

None.

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