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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TINIDAZOLE AND FLUCONAZOLE AND ITS APPLICABILITY IN MARKETED DOSAGE FORM

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ABSTRACT

Objective: The current method is focused on the development and validation of a simple, rapid, precise and robust stability indicating High Performance Thin Layer Chromatography for the simultaneous estimation of anti-infective drugs Tinidazole and Fluconazole in bulk and its pharmaceutical dosage form. The method was tailored to analyse the drugs in their commercial dosage form (tablets) with no interference from ingredients.

Methods: Chromatographic separation was performed over precoated TLC plates (60 F_{254}, 20 cm × 10 cm, 250 µm thickness, Merck) via a linear ascending technique using toluene: acetonitrile as the mobile phase in the ratio 6:4 v/v. Detection and quantification was achieved at the isobestic point of the two drugs, which was observed at 263 nm through Spectro-densitometric analysis. Analytical performance of the proposed HPTLC method was validated according to the ICH guidelines with respect to the linearity, accuracy, precision, detection and quantitation limits, robustness and specificity.

Results: Tinidazole and Fluconazole were well separated and identified with an Rf value of about 0.46±0.03 and 0.75±0.05, respectively. The calibration curves were linear over a concentration range of 800-1200ng/spot for Tinidazole and 60-90ng/spot for Fluconazole with correlation coefficients (r^2) more than 0.998. The above-developed method was validated as per ICH guidelines Q2(R1) and was found to be precise, sensitive, accurate and robust.

Conclusion: The validated stability indicating HPTLC method was found to be simple, precise, accurate and sensitive for the concurrent quantification of Tinidazole and Fluconazole in pharmaceutical dosage form and can be released into quality control for regular analysis.

Keywords: Fluconazole, Tinidazole, High-performance thin layer chromatography, Pharmaceutical dosage form, Stability, Validation

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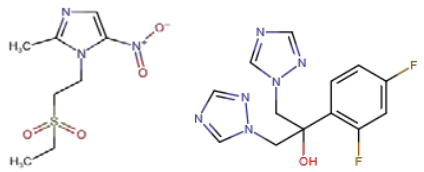
INTRODUCTION

A fungus affects the tissue and causes infection, which is known as a fungal infection. Infections with fungi can start on the skin and extend to the bones, tissues, organs, or the entire body. A bacterial infection is a disease in which harmful bacteria multiply and cause illness in the body. It can infect and multiply in any area of the body pretty rapidly. The selected drug combination for the study belongs to the class of anti-infective drug, which is primarily employed in the treatment of several bacterial and fungal infections, such as amoebiasis, giardiasis and trichomoniasis.

The membranes of fungal cells are critical for their survival because they prevent undesired substances from entering the cells and stop the leakage of cell contents. Fluconazole is an antifungal that kills fungus by damaging their cell membranes. Tinidazole is a broad-spectrum antibiotic that kills gram-negative and gram-positive bacteria that grow aerobically (with oxygen) and anaerobically (without oxygen). It harms bacteria and protozoa's DNA (genetic material) and prevents the creation of new DNA. As a result, microorganisms are killed and the infection is cleared.

Tinidazole is chemically1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole [1and 2], while Fluconazole is 2-(2,4-difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl)propan-2-ol [3 and 4]. The chemical structure of Tinidazole and Fluconazole are represented in fig. 1.

HPTLC is a well-known and adaptable separation technology based on the idea of adsorption. It is a form of planar chromatography [5]. It has proved a very useful technique because of its low operating cost, high sample throughput and the need for minimum sample clean-up [6]. The mobile phase solvent moves according to capillary action. Different components of the solution separate according to their affinities toward the adsorbent [7].



(a) (b)

Fig. 1: Chemical structure of (a) Tinidazole (b) Fluconazole

Literature survey reveals that few analytical methods have already been reported for the quantification of Tinidazole and Fluconazole in pharmaceutical formulations and biological fluids by employing UV and HPLC, individually [8-12] or in combination [13-17] and a HPTLC [18] for the simultaneous measurement of these compound's in pharmaceutical formulations has been published.

The major goal of this study was to create a simple, quick, accurate, specific and cost-effective HPTLC method for estimating Tinidazole and Fluconazole in bilk and markets formulations. The HPTLC method was created and verified in accordance with the ICH guidelines for analytical method validation [19].

MATERIALS AND METHODS

Materials

Standard materials of Tinidazole and Fluconazole were obtained as gift samples from Fourrtz (India) Laboratories, Pvt., Ltd., Chennai. FLUCOTI tablets containing 1000 mg of Tinidazole and 75 mg of Fluconazole was selected for sample analysis, which is procured from the local market. Silica gel 60 F_{254} TLC plates (20x20 cm, layer

thickness 0.2 mm, Merck, Germany) were employed as stationary phase in the analysis. Reagents utilized during the study process were procured from SD fine chemicals Ltd., and Merck laboratories. Throughout the analysis process double distilled water was used and glassware's of class "A" grade were employed.

The current research aims in the development and validation of a simple, accurate, precise and sensitive stability indicating HPTLC method for the quantification of Tinidazole and Fluconazole.

Instruments and software's employed

Instruments used comprises of Camag HPTLC Sample Applicator–Linomat V, Twin trough Chamber, Camag HPTLC Scanner, Camag HPTLC Document photo, Hamilton syringe (100 µl), Shimadzu libror AEG-220 weighing balance and ultra-sonic bath. Software's such as win CATS (for handling HPTLC) and Microsoft excel (for statistical analysis) were employed in the study.

Solubility studies

Solubility characters of Tinidazole and Fluconazole were studies and both the selected drugs were found to be freely soluble in methanol and partially in distilled water. Hence, methanol was employed as the solvent for solubilization and for further dilutions.

Standard stock solutions

Tinidazole and Fluconazole standard stock solutions were prepared separately by accurately weighing 20 mg and 10 mg of Tinidazole and Fluconazole, respectively, into two separate 10 ml standard flasks. Methanol was added in half the volume and the solutions were sonicated for 10 min before the final volume was made up with methanol. Tinidazole and Fluconazole concentration of 2 mg/ml (2000 µg/ml) and 1 mg/ml (1000 µg/ml) were obtained, respectively.

Determination of λmax

Tinidazole and Fluconazole stock solutions were diluted individually with methanol to yield a concentration of 10 µg/ml. In the UV region of 400–200 nm, the solutions were scanned.

Preparation of working solution

5 ml of Tinidazole and 0.75 ml of Fluconazole standard stock solution was pipette out into a 10 ml volumetric flask and the volume was made up to 10 ml with Methanol and mixed well to get a final concentration containing 1000 and 75µg/ml of Tinidazole and Fluconazole respectively. 2 µl of the above solution was spotted on the precoated TLC plate and subjected to development. The plate after development, was dried and scanned at 263 nm. The peak areas of the standard drugs were recorded.

Optimized chromatographic conditions

Optimization of HPTLC conditions were based upon various preliminarytrails carried as tabulated in table 1 and 2. Toluene and Acetonitrile in the ratio of 6:4 v/v was selected as the best suited solvent for the ideal separation of Tinidazole and Fluconazole, with a total development time of about 15 min at ambient temperature. The resolved spots were detected at 263 nm under densitometer and the Rf values of Tinidazole and Fluconazole were identified as 0.46 and 0.75, respectively.

A mixed reference solution containing Tinidazole (1000 µg/ml) and Fluconazole (75 µg/ml) was prepared for further investigation.

2 µl of the aforementioned solution was spotted and developed on an HPTLC plate precoated with Silica gel 60 GF254 on Aluminum sheets. After development, the plates were dried and scanned at 263 nm.

Assay procedure for marketed formulation

Weighed and powdered twenty Flucoti tablets containing Tinidazole (1000 mg) and Fluconazole (75 mg). An amount of tablets powder equivalent to 0.05g Fluconazole was weighed and placed in a 50 ml volumetric flask, 20 ml methanol was added and sonicated for 10 min, and the volume was made to 50 ml with methanol and thoroughly mixed. The solution above has been filtered. The filtrate was diluted appropriately before being used for further investigation.

2 µl of the aforementioned solution was spotted on a precoated TLC plate and developed. After development, the plate was dried and scanned at 263 nm. The sample's peak area was measured.

Amount present in synthetic mixture = Peak area of Sample/Peak area of Std x Cs x D_F

where,Cs-Concentration of Standard

D_F-Dilution Factor

Statistical comparison of the results obtained by applying the proposed method and the reported method for the analysis of Tinidazole and Fluconazole in pharmaceutical formulation were performed.

Method validation

Linearity

Appropriate aliquots of Tinidazole and Fluconazole standard stock solutions were diluted up to the mark with Methanol in five different 10 ml volumetric flasks to obtain final concentrations ranging from 800-1200 µg/ml for Tinidazole and 60-90 µg/ml for Fluconazole.

Linearity solution 1 contains 800 µg/ml of Tinidazole and 60 µg/ml of Fluconazole. Linearity solution 2 contains a concentration of 900 µg/ml and 67 µg/ml of Tinidazole and Fluconazole, respectively. Linearity solution 3 is diluted to contain 1000 µg/ml and 75 µg/ml of Tinidazole and Fluconazole. A concentration of 1100 µg/ml of Tinidazole and 82 µg/ml of Fluconazole in Linearity solution 4 and finally the linearity solution 5 is diluted suitably to contain 1200 µg/ml and 90 µg/ml of Tinidazole and Fluconazole respectively. On the precoated TLC plate, 2 µl of the aforesaid solutions were spotted. The plate went through an ascending development process. After drying, the plate was scanned at 263 nm. The peak regions were measured and calibration curves were built. In the concentration ranges of 1600-2400 ng/spot for Tinidazole and 120-180 ng/spot for Fluconazole, linearity obeyed Beer's Law.

Sensitivity of the proposed method was assessed by computing detection limit (LOD) and quantification limit (LOQ) from the obtained linearity data.

Precision

The intra-day and inter-day precision investigations (intermediate precision) were conducted by calculating the equivalent responses six times on the same day and three times on three distinct days at 100% concentration (1000 µg/ml Tinidazole and 75 µg/ml Fluconazole). 2 µl of the aforesaid solution were spotted six times on the precoated TLC plate and developed. After development, the plates were dried and scanned at 263 nm and the peak areas were recorded.

Accuracy

Recovery studies using the conventional addition method was performed to test the approach's accuracy at 80 %, 100 %, and 120 % of target concentration levels. The amount of drug recovered was calculated using the following formula.

$$\% \text{ Recovery} = \frac{\text{Amount Recovered - Amount added}}{\text{Amount present}} * 100$$

Robustness

The robustness of the proposed approach was determined by examining its ability to remain unaffected by minor but deliberate variations in procedure conditions. As a result, parameters such as detection wavelength (±2 nm), mobile phase composition (Toluene: acetonitrile–6.0±0.2: 4.0±0.2 v/v), development distance (7, 8 and 9 cm) and chamber saturation time (15, 30 and 45 min) were varied and their effect on quantification was investigated.

Stability studies

A working stock solution containing 1000 and 75 µg/ml of Tinidazole and Fluconazole, respectively. This solution was utilized to demonstrate the stability indicating property and specificity of the proposed approach through forced deterioration. After applying

six repetitions to each degradation study, the average peak area of Tinidazole and Fluconazole was obtained.

Unstressed condition

On the precoated TLC plate, 2 µl of the working stock solution was applied and developed. After development, the plates were dried and scanned at 263 nm. The highest points were marked on a map.

Degradation caused by acid and base

Refluxing the working stock solution containing in 0.1M hydrochloric acid at 80 °C for 12 h was employed for acid decomposition. The alkaline decomposition was performed in 0.1M sodium hydroxide, which was then refluxed at 40 °C for 12 h.

Degradation caused by peroxide

Initial tests were carried out in 1 % hydrogen peroxide at room temperature for 12 h to investigate hydrogen peroxide-induced deterioration. After that, the drugs were exposed to 3 % and 30% hydrogen peroxide at room temperature for 12 h.

Dry heat and wet heat degradation product

To evaluate dry heat degradation, the working standard stock solution containing a mixture of drugs was held in reflux at 80 °C for 12 h, and for wet heat degradation, the working standard stock solution containing a mixture of drugs was kept in reflux at 80 °C for 12 h.

Photochemical degradation product

The photochemical stability of the drugs were studied by exposing the working stock solution, as well as solid drug to direct sun light, UV light (254 nm) and dark light for 12 h on a wooden plank and kept on a terrace.

2 µl of all the above solutions were spotted on the precoated TLC plate and subjected to development. The plates after development were dried and scanned at 263 nm and the peak areas were recorded.

RESULTS AND DISCUSSION

Thin layer chromatography advanced rapidly and gained widespread recognition as a significant analytical instrument for both qualitative and quantitative ways of analysis, and it became a well-established method for drug detection in mixtures [20]. The application of HPTLC is well-liked and accepted all across the world. Many strategies are being developed in order to standardize assay methods. When compared to other chromatographic tools, HPTLC remains one step ahead. It has a wide range of applications in pharmaceutical research, including stability, impurities, synthetic medicines, pharmacokinetics, enantiomeric purity, and drug monitoring in biological fluids [21].

According to ICH guideline "Stability testing of new drug substances and products," stress testing is required to elucidate the inherent stability characteristics of the active substance, so the drugs were subjected to oxidation and alkaline degradation.

Overlay UV spectra of tinidazole and fluconazole

The λ_{max} of Tinidazole and Fluconazole was found to be 267 nm and 320 nm, respectively. The isobestic point was discovered to be 263 nm, and this wavelength was chosen for the simultaneous estimation of Tinidazole and Fluconazole. The Overlay UV spectrum of Tinidazole and Fluconazole is depicted in fig. 2.

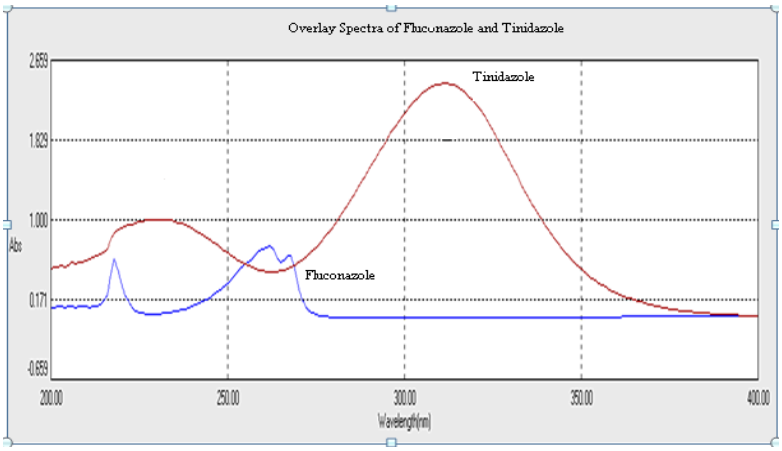


Fig. 2: Overlay UV spectra of tinidazole and fluconazole

Optimization of chromatographic conditions

The solubility studies revealed Tinidazole and Fluconazole were soluble in methanol, and hence the method development started with 100% methanol as the mobile phase. Further solvents of varying polarity [22], such as chloroform, toluene, ethyl acetate, and acetonitrile, were tested to assess the chromatographic behavior of the selected analytes and the findings are presented in table 1.

With the previous knowledge and the trial runs conducted, diverse mobile phase systems such as methanol: acetonitrile, ethyl acetate: acetonitrile, chloroform: acetonitrile, and toluene: acetonitrile in different ratios were explored, and the results are depicted in table 2. A good, reasonable separation with compact spots and ideal Rf values of 0.46 and 0.75 for Tinidazole and Fluconazole, respectively, was obtained with toluene: acetonitrile as the mobile phase in a ratio of 6:4 v/v.

Table 1: Initial trials with neat solvents

S. No.	Mobile phase	Tinidazole	Fluconazole
1.	Methanol	Movement of spot to the solvent front	Movement of spot to the solvent front
2.	Chloroform	Partial movement of the spot just above the baseline	Spreading of the spot slightly above baseline
3.	Toluene	Movement of the spot below the solvent front	Movement of the spot to the solvent front
4.	Ethyl acetate	Changes in the position of the spot immediately above the baseline	With tailing and a trace quantity of sample in the baseline, the spot moves partially
5.	Acetonitrile	Spot movement was not observed	Spot movement was not observed

Table 2: Initial trials with combination of solvents

S. No.	Mobile phase	Tinidazole	Fluconazole
1.	Methanol: Acetonitrile (5:5 v/v)	Drugs showed optimal movement	The drugs spots appeared to be dispersed
2.	Ethylacetate: Acetonitrile (5:5 v/v)	Optimal movement of the drug spot to the middle of the plate	Tailing of the spot at the middle of the plate
3.	Chloroform: Acetonitrile (5:5 v/v)	Optimal movement of the drug spot to the middle of the plate	Movement of spot to the solvent front
4.	Toluene: Acetonitrile (5:5 v/v)	Optimal movement of both the drug spots were observed, even the resolution can be improved	
5.	Toluene: Acetonitrile (6:4 v/v)	Optimal movement of both the drug spots were observed with good resolution	

When compared to the sole known HPTLC method [18], were, complicated and hazardous solvents are used, and the approach does not emphasize detailed information about drug stability except for alkali degradation.

The overlain spectra of the standard spots placed on silica gel were obtained on the HPTLC apparatus to choose the analytical wavelength for the quantification of the drugs. Based on the overlain spectra, both Tinidazole and Fluconazole showed high absorbance at about 263 nm; this was chosen as the analytical wavelength for further analysis.

The absence of a peak in the blank mobile phase densitogram confirmed the purity of the standard peaks obtained with the proposed mobile phase.

A mixed standard solution containing Tinidazole (1000µg/ml) and Fluconazole (75µg/ml) was prepared and used for further analysis. The recorded standard densitogram is represented in fig. 3.

Assay of tinidazole and fluconazole in marketed formulation (Tablets)

The new approach was used to analyze the commercial product FLUCOTI tablets (containing Tinidazole (1000 mg) and Fluconazole (75 mg). The sample was processed as described in the procedure for analysis of Tinidazole and Fluconazole.

The densitogram of the tablet sample revealed only two peaks, with Rf values of 0.44 and 0.76 for Tinidazole and Fluconazole, respectively, indicating that the excipients in the tablet formulation did not interact. By comparing the peak areas of the sample to the standard, the Tinidazole and Fluconazole content was determined. The % RSD was found to be less than 2%. Table 3 shows the amount of drug present, and fig. 4 depicts the densitogram for the assay.

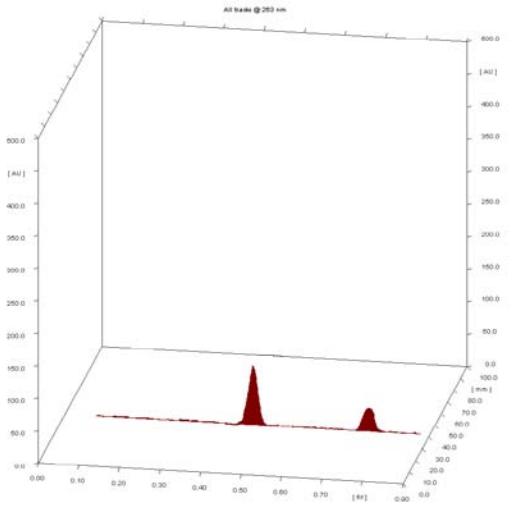


Fig. 3: Densitogram of standard solution of tinidazole and fluconazole

Table 3: Assay of tinidazole and fluconazole in marketed formulation

Drug	Label claim (mg)	Amount estimated (mg)	% Assay (n=3)	mean±SD*	%RSD
Tinidazole	1000	998.98	98.98	98.98±0.15	0.15
Fluconazole	75	78.14	98.14	99.34±1.31	1.31

*mean±SD(n=3) = average of three determinations

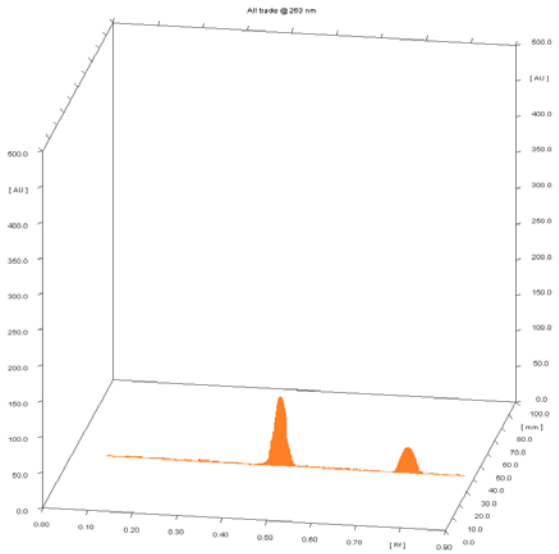


Fig. 4: Assay densitogram of tinidazole and fluconazole in marketed formulation

The results of the suggested HPTLC densitometric method for determining Tinidazole and Fluconazole in its pharmaceutical formulation were statistically compared to those of the described HPTLC method [18]. The estimated t-and F-values were found to be lower than the theoretical ones, indicating accuracy and precision at a 95% confidence level and the results are tabulated in table 4.

Table 4: Statistical comparison of the results obtained by applying the proposed method and the reported method for the analysis of tinidazole and fluconazole in pharmaceutical formulation

Parameter	Tinidazole		Fluconazole	
	Reported method[b]	Proposed method[a]	Reported method[b]	Proposed method[a]
mean±SD*	99.17±0.84	99.49±0.33	99.04±0.70	99.11±0.80
n	3	3	3	3
T-Test (2.776)	0.610		0.107	
F-Test (19)	6.197		1.30	

[a]The values between parenthesis are corresponding to the theoretical values of t and F (P = 0.05), [b]Determination-Fluconazole and Tinidazole–Reported method [18], *mean±SD (n=3) = average of three determinations

Validation

Linearity and range

Peak areas were discovered to have a stronger linear connection with concentration than peak heights. The r^2 for Tinidazole was 0.999, whereas the r^2 for Fluconazole was 0.9993. Calibration graphs were created for Tinidazole in the concentration range of 1600-2400 ng/spot and Fluconazole in the concentration range of 120-180 ng/spot. The correlation coefficients, y-intercepts, and slopes of the two drugs, regression lines were calculated.

The linearity data are tabulated in table 5 and the overlay densitogram and linearity plots were represented in fig. 5 and 6 (a and b), respectively. LOD and LOQ were calculated from the linearity data and the results were tabulated in table 6. Linearity range reported in the proposed method is wider than the reported method [18].

Table 5: Linearity of tinidazole and fluconazole

S. No.	Drugs	Concentration (ng/spot)	Peak area
1.	Tinidazole	1600	3611.4
		1800	4115.5
		2000	4572.3
		2200	4999.5
		2400	5442.8
2.	Fluconazole	120	792.1
		134	875.1
		150	979.6
		164	1078.2
		180	1181.9

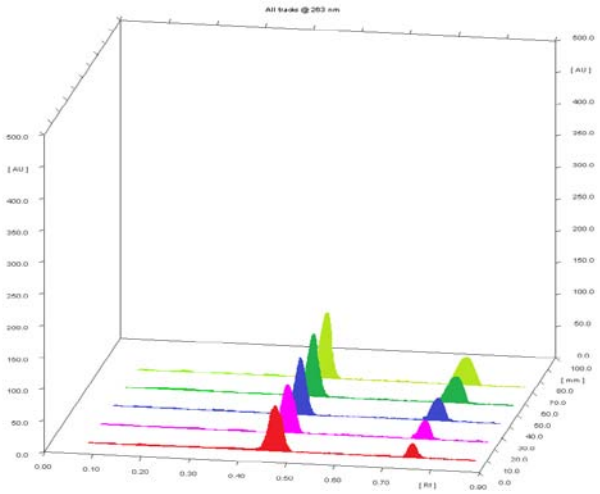
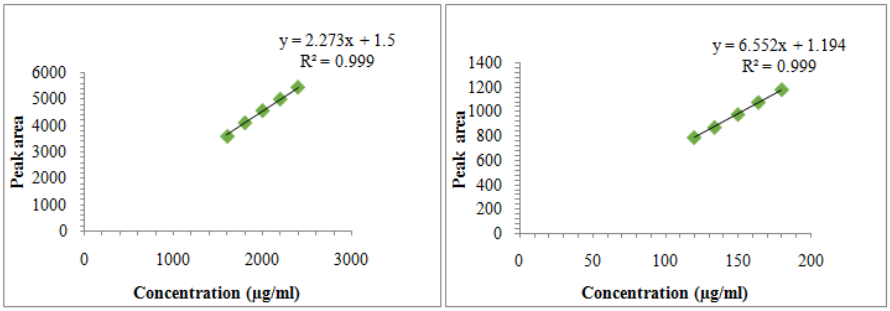


Fig. 5: Overlay densitogram of tinidazole and fluconazole showing linearity

Table 6: System suitability parameters

Drug	Linearity range (ng/spot)	Regression equation	R^2	Slope	Intercept	LOD (ng)	LOQ (ng)
Tinidazole	1600-2400	y=2.2734x+1.5	0.999	2.273	1.5	0.0452	0.0044
Fluconazole	120-180	y=6.552x+1.1943	0.999	6.552	1.194	0.1370	0.0136

The proposed method is found to be more sensitive with less reported LOD and LOQ values than the method reported [18].



(a) **(b)**

Fig. 6: Linearity plot of (a) Tinidazole (b) Fluconazole

Precision

The precision of the devised approach was expressed in terms of the peak area's RSD. The results demonstrated that the intra and inter-day variation of the results at concentrations of 1000 µg/ml for Tinidazole and 75 µg/ml for Fluconazole were within acceptable limits. The coefficients of variation for the method's inter-day and intra-day precision were determined to be less than 2% for both medications and more precise than the reported method [18]. Table 7 summarizes the findings of precision studies.

Table 7: Intra and Inter day precision study of tinidazole and fluconazole

Repeatability	Drug	Concentration (µg/ml)	Amount found in µg/ml (n=6)	(mean±SD)*	%RSD
Intra-day	Tinidazole	1000	1000.21 1001.31 999.45 1002.87 1000.94 1002.54	1001.22±1.31	0.13
	Fluconazole	75	72.41 74.11 72.49 72.19 74.57 72.89	73.11±0.99	1.35
Inter-day	Tinidazole	1000	998.34 1001.87 1002.97 1002.54 1002.11 1002.87	1001.78±1.73	0.17
	Fluconazole	75	72.54 73.11 72.58 72.48 74.57 72.89	73.02±0.79	1.08

*mean±SD (n=6) = average of six determinations

Table 8: Accuracy study of tinidazole and fluconazole

Drug	Levels of recovery (%)	Amount initially present (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	mean±SD*
Tinidazole	80	500	300	798.21	98.21	99.54±1.94
	100	500	500	998.65	98.65	
	120	500	700	1201.77	101.77	
Fluconazole	80	50	10	58.64	98.64	99.53±0.95
	100	50	15	79.41	99.41	
	120	50	40	90.54	100.54	

*mean±SD (n=3) = average of three determinations

Accuracy

The method's accuracy was evaluated at 80 %, 100 %, and 120 % of target concentration accuracy was tested. The percentage recovery was found to be within limits (98-102 % w/w) and the results were tabulated and displayed in table 8.

Robustness

A ruggedness test is a component of method validation and can be included in the precision evaluation. Repeatability and reproducibility are connected to ruggedness [23]. The proposed method's robustness was assessed by looking into its capacity to

remain unaffected by modest but deliberate procedure variations. In any of these trials, the differences in circumstances had no effect on the separation or quantification of the drugs tested.

Furthermore, the relative standard deviation of peak regions did not exceed 2%, indicating that the approach was robust enough. The results of robustness study data's are tabulated in table 9.

Table 9: Robustness study of tinidazole and fluconazole

Drugs	Parameters	Variations											
		Detection wavelength, 263±2 nm			Mobile phase composition (Toluene: acetonitrile– 6.0±0.2: 4.0±0.2 v/v)			Development distance (cm)			Time of saturation (min)		
		261	263	265	5.8:4.2	6:4	6.2:3.8	7	8	9	15	30	45
Tinidazole	Rf	0.45	0.46	0.46	0.45	0.46	0.46	0.45	0.46	0.47	0.46	0.46	0.46
	Peak area±SD*	4681.4 ±3.48	4585.9 ±13.04	4573.8 ±8.99	4579.0 ±12.07	4569.8 ±5.67	4974.8 ±40.72	4625.0± 66.29	4616.6± 61.33	4620.7± 58.51	4672.1 ±35.22	4600.8 ±84.94	4547.4 ±61.95
	%RSD	0.07	0.28	0.19	0.26	0.12	0.81	1.43	1.32	1.26	0.75	1.84	1.36
Fluconazole	Rf	0.75	0.75	0.76	0.75	0.75	0.76	0.74	0.76	0.78	0.75	0.75	0.76
	Peak area±SD*	980.4 ±5.35	984.9 ±0.55	996.9 ±1.40	981.9 ±5.10	978.5 ±5.74	985.9 ±1.19	979.2 ±5.39	970.2 ±6.38	967.2 ±19.19	986.0 ±1.34	966.2 ±1.02	976.4 ±2.11
	%RSD	0.54	0.05	0.14	0.52	0.58	0.12	0.55	0.65	1.98	0.13	0.10	0.21

*mean±SD (n=3) = average of three determinations

Stability indicating study

Chemical stability of pharmaceutical compounds is a major concern since it impacts the drug's safety and efficacy. Knowledge of molecular stability aids in the selection of appropriate formulation and packaging, as well as giving adequate storage conditions and shelf life, which is required for regulatory paperwork [24].

Acid-induced degradation product

When compared to alkali, the rate of breakdown in acid was slower. After heating the drug solution with 0.1M hydrochloric acid at 40 °C for 12 h, no degradation was noticed, thus, the temperature was increased to 80 °C. After heating the drug solution with 0.1M hydrochloric acid at 80 °C for 12 h, 80 % deterioration was observed in Tinidazole and 40 % in Fluconazole.

Base-induced degradation product

The drugs have shown to be extremely sensitive to alkaline degradation. The reaction in 0.1M sodium hydroxide at 80 °C was so fast that it degraded approximately 80% of the drugs in just 24 h. The drugs were completely degraded in 18 h after being refluxed with 0.1M sodium hydroxide at 40 °C for 12 h.

Hydrogen peroxide-induced degradation product

The drugs did not show any degradation and were found to be stable for 6 h with 1% and 3% hydrogen peroxide. Hence, the drugs were exposed to 30% H_2O_2 for 12 h at room temperature. On exposure, 25% degradation was observed in Tinidazole and 75% in Fluconazole.

Dry and wet heat degradation product

The standard drugs in solid form were placed in an oven at 105 °C for 12 h to study dry heat degradation, and for wet heat degradation, the drugs were kept in reflux at 80 °C for 12 h.

Photochemical degradation product

The drugs were found to be highly labile to photochemical degradation. The drugs under investigation were exposed by preparing a reference solution containing 1000 µg/ml of Tinidazole and 75 µg/ml of Fluconazole, as well as solid drugs separately to direct sunlight, UV light (254 nm), and dark light for 12 h. The results of stability data of Tinidazole and Fluconazole were tabulated in table 10. The stability studies data presented in the current method depicts the behavior of the selected drugs in different stress conditions in comparison with the reported method [18].

Table 10: Forced degradation study data

Condition	Time (h)	*Drug recovered (%) (*mean±SD)		*Drug decomposed (%) (*mean±SD)		*Rf (*mean±SD)	
		Tinidazole	Fluconazole	Tinidazole	Fluconazole	Tinidazole	Fluconazole
Un Stressed	12	99.98±1.94	98.14±0.85	-	-	0.46±1.47	0.75±0.54
Hydrolysis							
Acid 0.1 M HCl	12	85.14±1.11	45.89±1.47	14.86±0.21	54.11±0.21	0.46±0.11	0.75±0.14
Base 0.1 M NaOH	12	32.55±	34.53±0.57	67.45±0.68	65.47±1.11	0.45±1.32	0.76±0.19
Oxidation							
H_2O_2 (30% v/v solution)	12	74.59±0.74	28.66±0.11	25.41±0.61	71.34±0.51	0.44±0.44	0.74±1.57
Thermal							
Dry heat (oven 105°C)	12	30.49±1.21	52.53±0.65	69.51±0.24	47.47±1.21	0.44±0.21	0.76±1.54
Wet heat (Reflux 80°C)	12	30.51±0.23	23.19±0.11	69.49±1.24	76.81±2.01	0.46±0.21	0.75±1.52
Photo degradation							
• Sun light							
1. Solution	12	75.05±1.12	69.89±0.52	24.95±0.11	30.11±1.65	0.45±0.26	0.70±0.54
2. Dry powder	12	21.19±0.89	23.32±0.25	78.81±0.24	76.68±2.11	0.46±1.02	0.75±1.24
• UV light							
1. Solution	12	61.52±0.21	58.08±0.87	38.48±1.24	41.92±1.24	0.47±1.25	0.74±0.57
2. Dry powder	12	21.81±0.54	23.66±0.11	78.19±2.04	76.34±0.28	0.46±0.95	0.73±0.23
• Dark							
1. Solution	12	69.84±2.14	70.13±0.36	30.16±0.35	29.87±0.69	0.47±0.74	0.77±0.58
2. Dry powder	12	71.77±0.21	74.49±0.56	28.234±1.24	25.51±1.65	0.47±1.88	0.77±0.47

*mean±SD(n=3) = average of three determinations

CONCLUSION

As no simple and rapid methods are reported for the simultaneous estimation of Tinidazole and Fluconazole, a stability-indicating HPTLC method was developed and validated for the determination of Tinidazole and Fluconazole in co-formulations on pre-coated silica gel HPTLC plates using simple mobile phase. The optimized method was observed to be simple, quick, selective, sensitive, and suitable for determining Tinidazole and Fluconazole simultaneously. The HPTLC method has several advantages over liquid chromatographic methods, including the ability to analyze a sample and a standard on the same plate, a short system equilibrium time, multiple/repeated scanning of chromatograms, a higher mobile phase pH, a large sample capacity, a short run time, a low solution consumption, and no prior solvent treatment such as filtration and degassing. The drugs could be evaluated in the presence of their degradation products, according to the stability indicating properties established in accordance with ICH guidelines, and thus can be used in the industry for the simultaneous estimation of Tinidazole and Fluconazole and their degradation products in stability samples.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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