Analytical Method Development and Validation of Ornidazole and Miconazole Nitrate in Bulk and Tablet Dosage Form

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ABSTRACT:
An HPTLC method has been optimized and validated for the simultaneous estimation of ornidazole and miconazole nitrate in bulk and tablet dosage form. The antibiotic ornidazole breaks down the DNA of bacteria and other infectious microbes, the antifungal drug miconazole nitrate prevents the growth of fungus by preventing them from developing their protective outer layer. Together, they efficiently treat the condition and promote in getting rid of fungal diseases. The mobile phase of nhexane: methanol (8: 2 v/v) was chosen because it provided good resolution and acceptable peak characteristics. The samples were applied using a sample applicator and a 100 µL sample syringe on a precoated silica gel plate 60 F_{254} (10 × 10) with a 250 µm thickness, forming bands of 6 mm width with 8 mm between each band. Using a 20 mm/sec scanning speed and a slit size of 5 mm by 0.45 mm, development took about 15 minutes. At 310 nm, densitometry scanning was carried out on every development that used the WINCATS software. The radiation emitted came from a deuterium lamp. R_f value was found to be 0.27 ± 0.15 for miconazole and 0.74 ± 0.12 for ornidazole. The method fulfilled, every validation specifications described in the ICH Q2 R1 guidelines, and it was determined to be linear for the drugs ornidazole and miconazole nitrate, with correlation coefficients of 0.9987 and 0.9985, respectively. For Miconazole Nitrate, the linearity range was 100–600 ng/band, while for Ornidazole, it was 500–3000 ng/band. The method's percent RSD was determined to be no higher than 2%, making it precise, accurate, and robust. In order to regularly analyse drug samples, the specified method can be implemented.

Keywords: Ornidazole, Miconazole Nitrate, Antifungal, HPTLC, Methanol, n-hexane, etc.

1. INTRODUCTION:

MICONAZOLE NITRATE

Miconazole nitrate, an imidazole antifungal compound which belongs to the BCS class II, It is applied topically and can also be given intravenously. The chemical nomenclature for miconazole nitrate is $C_{18}H_{14}C_{l4}N_2O$, and its molecular weight is 416.13 g/mol. As per IUPAC, miconazole nitrate can also be referred to as 1-[2-(2,4dichlorophenyl)-2-[(2,4-dichlorophenyl) methoxy ethyl] imidazole nitric acid. Miconazole nitrate exhibits a melting point between 85° to 95 °C. It is a solid white powder by nature. The pKa value of it is 2.4. The wavelength at which miconazole exhibits its maximum absorbance is 242.6 nm. It dissolves in acetonitrile and methanol but only very slightly soluble in water. Miconazole nitrate's mechanism of action involves its interaction with 14 demethylase, a cytochrome P450 enzyme that is required, so as to change lanosterol into ergosterol. Because ergosterol is a crucial component of membranes and a key component of membranes, as Miconazole nitrate inhibit ergosterol synthesis of increases cellular permeability, which results in the leakage of the contents inside of the fungus cell thus inhibit fungal growth. [1,2]

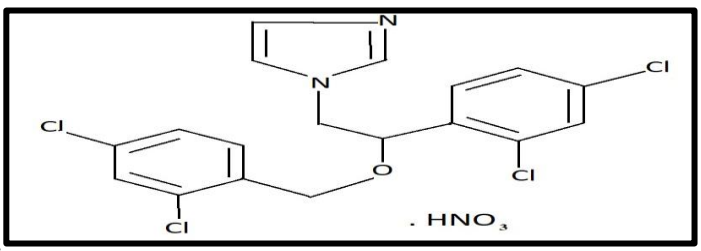

Fig. No.1 Miconazole Nitrate

ORNIDAZOLE

Ornidazole belongs to BCS Class II. Protozoan infections can be treated with ornidazole. It is a 5-nitro imidazole-group agent with antifungal activity. It has been investigated for use in treating Crohn's disease post intestinal resection. Ornidazole has the molecular weight of 219.625 g/mol and the chemical formula $C_7H_{10}ClN_3O_3$. In IUPAC nomenclature, ornidazole is known as 1-chloro-3-(2-methyl-5-nitro-1H-imidazol1-yl)propan-2-ol. 90% of ornidazole is unable to dissolve in water, but able to dissolve in ethanol, methanol, dimethyl formamide, and dimethyl sulfoxide. Its melting point is between 85 to 95°C. It is a solid, off-white to white powder in its physical form. The partition coefficient (pKa) value of ornidazole is 6.77. The wavelength at which highest drug absorbance is observed, is at 318 nm for ornidazole. By targeting microbial DNA, which causes the loss of helical structure and destruction of DNA from microbes, it prevents the synthesis of DNA of microorganism and inhibits its growth. [3,4]

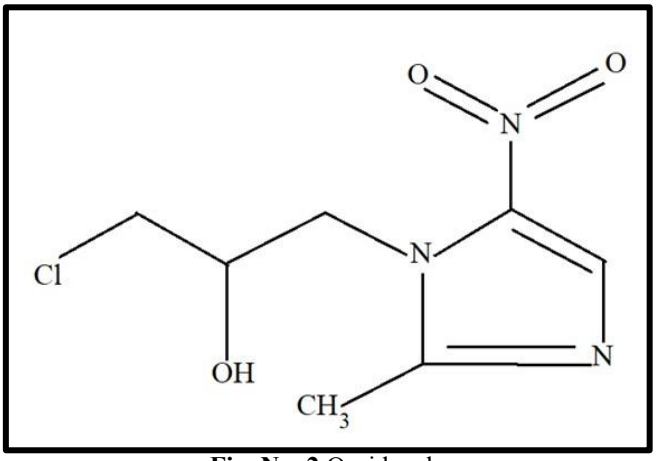

Fig. No. 2 Ornidazole

MICONAZOLE + ORNIDAZOLE belongs to the group of antifungals BCS Class II drugs. It is used to treat vaginal infections in female populations On the other hand, this drug treats vaginal candidiasis and bacterial vaginosis in female patients, while for male patients who have trichomoniasis and balanitis are treated with it. Balanitis, a disorder that affects men, can cause the foreskin and genital part to become inflamed and uncomfortable. Trichomonasis is a sexually transmitted illness (STD) brought on by the parasite “Trichomonas vaginalis”. The fungus responsible for vaginal candidiasis is “Candida albicans”, a vaginal yeast infection. Bacterial vaginosis alters the normal equilibrium of vaginal bacteria.

In the same way as the antibiotic ornidazole breaks down the DNA of bacteria and other infectious microbes, the antifungal drug miconazole nitrate prevents the growth of fungus by preventing them from forming their protective outer coat.

Together, they efficiently treat your illness and aid in the healing of fungal diseases.

There are various marked formulations of Ornidazole I.P. and Miconazole I.P. combination to treat such fungal infections.

2. MATERIALS AND METHODS

Instrumentation

A. Instrument Details 1. Camag HPTLC System

- Linomat - 5 sample applicator
- Camag TLC Scanner 3
- Win CATS software V- 1.4.2
- 100 µL sample syringe (Hamilton, Bonaduz, Switzerland)

Using a CAMAG Linomat 5 sample applicator, precoated silica gel aluminium plate 60 F_{254} (10 × 10) with a thickness of 250 mm (E. MERCK, Darmstadt, Germany). The slit size was 5 mm × 0.45 mm, and the scanning speed was 20 mm/sec. In a twin trough glass container(CAMAG, Muttenz, Switzerland) sized about 10 cm by 10 cm, linear ascending development was carried out. Final chromatographic mobile phase for final optimization was Methanol : n-hexane (8: 2 v/v). and 310 nm was detection wavelength.

2. UV-Visible Double beam spectrophotometer (Jasco Model V-550) with single Monochromator.

Materials

Drug Sample.

Miconazole Nitrate and Ornidazole gift samples were offered by "Indoco Remedies Ltd."

A nearby drugstore provided the “Candifem” tablet. Each uncoated tablet contains (Miconazole Nitrate 100 mg and Ornidazole 500 mg). Manufactured by: Meyer Organics

Pvt. Ltd”. Analytical grade chemicals and reagents were utilized. [5,6]

3. Method Development

3.1. Selection of mobile phase and chromatographic conditions:

Experiments of chromatographic separation were carried out by utilizing the working standard solution of Miconazole (200 ng/band) and Ornidazole (1000 ng/band). Initially, various trials were carried out using various solvents in different proportions on HPTLC plates, to obtain the desired system suitability parameters. After few trials, n-Hexane: Methanol (8: 2 v/v) was chosen as the mobile phase which gave good resolution and acceptable peak parameters. Other chromatographic parameters such as chamber saturation time, detection wavelength, run length, distance between tracks, were optimized for achieving repeatable Rf values and a symmetrical drug peak shape. [7,8]

Table No. 1. Chromatographic Conditions:

Paramater	Description
Bands Width	6 mm
Space between bands	8 mm
Sample syringe	100 µL (Hamilton, Bonaduz, Switzerland)
Stationary Phase	Precoated silica gel aluminum plate 60 F_{254} (10 × 10) with thickness of 250 µm.
Sample application	CAMAG Linomat 5 sample applicator
Scanning speed	20 mm/sec
Chamber saturation time	15 min
Length of chromatogram run	8 cm
Development time	15 min
Development	10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland)
Densitometry scanning	CAMAG thin layer chromatography scanner at 310 nm
Software used	WINCATS software version 1.4.2.
Source of radiation	Continuous UV radiation from a Deuterium lamp ranging in wavelength from 200 to 400 nm

Preparation of Standard stock solution:

Separately, standard stock solutions of miconazole and ornidazole were made by combining 10 mg of each std drug with 10 ml of methanol to achieve a concentration of 1000 µg/ml. A working standard solution comprising 100 µg/ml (100 ng/ml) of each miconazole and ornidazole, separately in methanol, was created from the corresponding standard stock solution.[9,10]

Selection of Detection Wavelength:

In order to obtain a spectrum, even more dilutions of the standard stock solution were created utilizing methanol and scanned between 200 and 400 nm. Both drugs were found to have significant absorbance at 310 nm. [11,12] **(Fig. No. 3)**

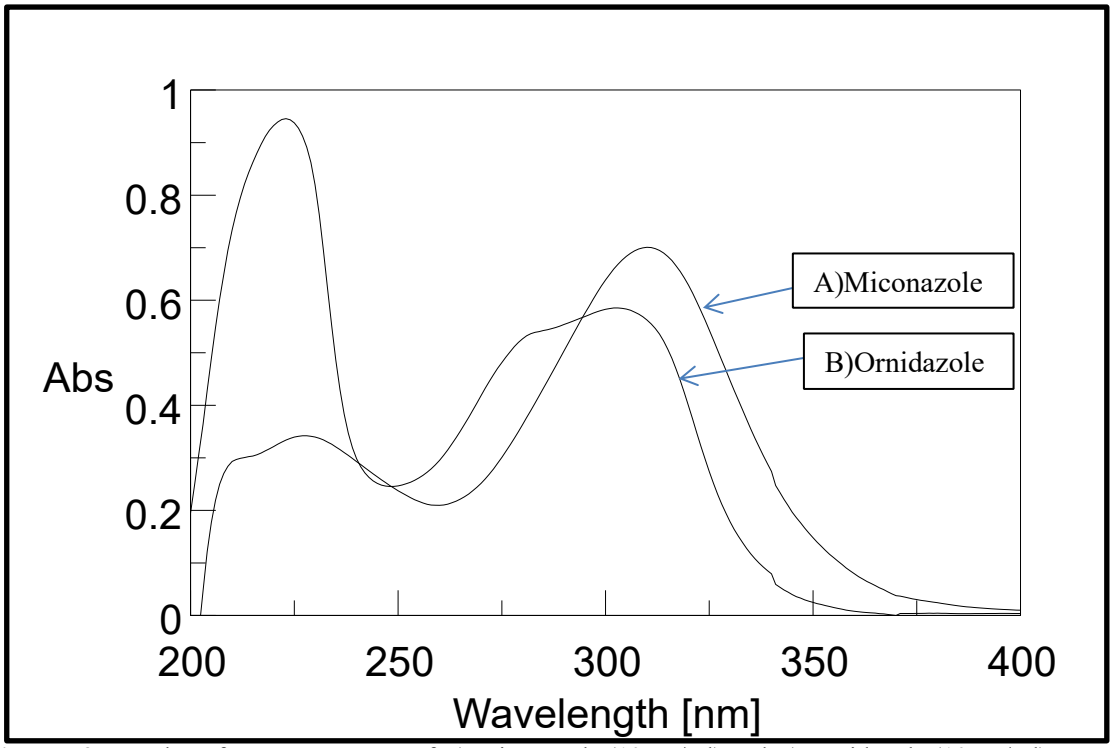

Fig. No. 3: Overlay of UV-VIS Spectra of A) Miconazole (10 µg/ml) and B) Ornidazole (10 µg/ml)

Preparation of sample solution (Tablet Formulation Analysis):

Twenty (20) medicines, each containing 100 mg of miconazole and 500 mg of ornidazole (Brand Name: Candifem), were weighed and powdered. 50 mg of ornidazole, which is the equal of 10 mg of miconazole, were moved to a volumetric

flask of 10 ml diluted with methanol, and then the volume was adjusted to 10 ml (1000 g/ml of miconazole and 5000 g/ml of ornidazole). To obtain the final concentration of 100 µg/ml of miconazole and 500 µg/ml of ornidazole, the solution was filtered and further dilutions were done using mobile phase. On a TLC plate, 2 µl volume was applied, and it was developed under the optimal conditions. [13,14]

Validation

To ensure effective chromatographic separation, accuracy, precision, specificity, and recovery, it is considered necessary to validate an analytical method. According to ICH recommendations, the developed HPTLC method was subsequently validated for a number of parameters.

System suitability parameter

System suitability is for ensuring that the chromatographic system's repeatability and resolution are appropriate for the evalution of the drug. On a TLC plate, 200 ng/band of miconazole and 1000 ng/band of ornidazole were administered three times as a part of system suitability test, under optimal chromatographic conditions, and the retardation factor of multiple applications was observed by TLC Scanner.

Specificity

To determine the specificity, peak purity profiling experiments were used for the approach. The peak purity values greater than 0.998 indicates that no other peak of a degradation product or impurity interfered with the experiment.

Linearity

By plotting calibration curves at six varied drug concentrations, method linearity was investigated. Working solution samples of MIC and ORD 100 µg/ml and 500 µg/ml were applied to the pre-coated plate at concentrations ranging from 100-600 ng/band for Miconazole and 500-3000 ng/band for Ornidazole. The mobile phase, n-hexane: methanol (8:2), was used to allow the plate to grow. The resulting peak areas were then plotted against drugs concentrations to create calibration curves. Consequently collected data were subjected to least square regression analysis in order to verify the linearity of the devised methodology and compare anticipated and experimental values against their respective 95% confidence intervals.

Range

The range is what exists between the analyte's upper and lower concentrations when a method of analysis has appropriate level of linearity, accuracy, and precision.

Precision

The precision is repeatability in the test results. Intra-day and inter-day variance studies were used as indicates of the method's precision. In the intra-day trials, the % RSD was calculated after 3 replicates of 3 distinct concentrations were analyzed in a single day. For the interday variation investigations, the % Relative Standard Deviation was calculated after examination of three distinct concentrations over the course of three consecutive days.

Accuracy

Accuracy is the closeness to the test results. Recovery experiments were conducted by adding standard drugs to sample at three distinct levels in order to evaluate the method's accuracy i.e. 50, 100 and 150 %. Basic concentrations of sample chosen were 2 µl of 100 µg/ml of Miconazole and 2 µl of 500 µg/ml of Ornidazole. These solutions were applied on TLC plates in triplicate to obtain the densitogram. The drug concentrations of Miconazole and Ornidazole were calculated by using linearity equations of Miconazole and Ornidazole.

Sensitivity

Limits of detection and quantification were used to predict the sensitivity of the HPTLC technique. Therefore, using the following equation, the values of LOD and LOQ were calculated from the values of standard deviation (SD) of the response measured to the slope of the linearity curve.

$$LOD = \frac{3.3\,\sigma}{S}$$

$$LOQ = \frac{10\,\sigma}{S}$$

Where,σ = Standard Deviation of response for the lowest concentration in the range. S = Slope of the calibration curve. [15,16]

Robustness

It illustrates how effective the technique is under usual circumstance and gives a reflection of the method's resistance to small but intentional alterations in method parameters. If measurements are affected by alteration in such settings, analytical circumstances should be carefully controlled, or a caution should be written into the protocol.

The robustness of the approach was confirmed by conducting the study under various wavelength, chamber saturation, and time from application to development conditions and examining the impacts on area. [17,18]

Results

System Suitability test

For system suitability injection of Methanol blank, Miconazole, Ornidazole and Mixture blank were given. On repeated application retention factor of Miconazole and Ornidazole were found to be:

Miconazole = 0.27 ± 0.15 Ornidazole = 0.74 ± 0.12 as given in Table No. 2

Densitogram of Standard mixture of Miconazole (200 ng/band) and Ornidazole (1000 ng/band) and densitogram of sample mixture of Miconazole (200 ng/band) and

Ornidazole (1000 ng/band) were found to be exact identical. Shown in Fig. No. 4. [19,20]

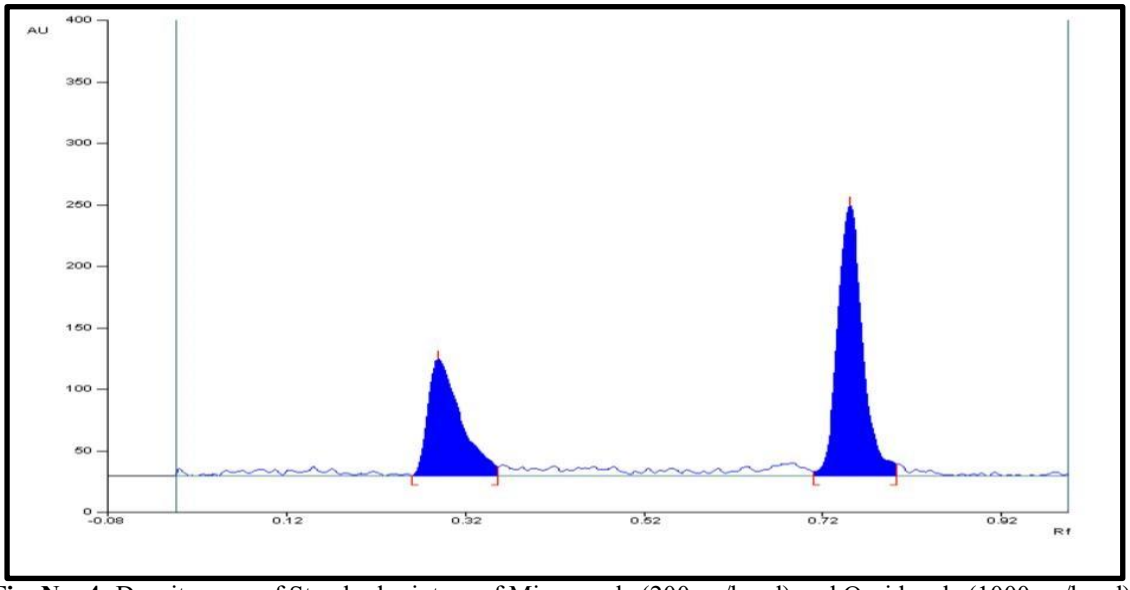

Fig. No. 4: Densitogram of Standard mixture of Miconazole (200 ng/band) and Ornidazole (1000 ng/band).

Table No. 2: System suitability

Name	Conc. (ng/band)	R_f Mean ± % RSD	Std. Peak Area (Mean)	Asymmetry
MIC	200	0.27 ± 0.15	3249	1.00 1.21 1.10
ORN	1000	0.74 ± 0.12	4780	0.9 0.6 0.5

Linearity & Range:

Data of MIC and ORN linearity were collected (Table No. 3). The calibration curve is shown in Figure No. 5a and 5b. The developed approach was linear in the range of 100

- 600 ng/band for MIC and 500 – 3000 ng/band for ORN with correlation coefficients of

0.9987 and 0.9985, respectively. The result shows that linearity satisfies its specific set of acceptance criteria.

Table No. 3 : Linear regression data of MIC and ORN

Parameters	MIC	ORN
Linearity range (ng/band)	100 - 600 ng/band	500 – 3000 ng/band

Straight line equation	y= 5.611x + 85.81	y = 2.753x + 1215
Correlation co-efficient(r^2)	0.9987	0.9985
Slope	5.611	2.753
Intercept	85.81	1215
LOD (ng/band)	8.308 ng/band	40.5 ng/band.
LOQ (ng/band)	26.25 ng/ band.	125.832 ng/band.

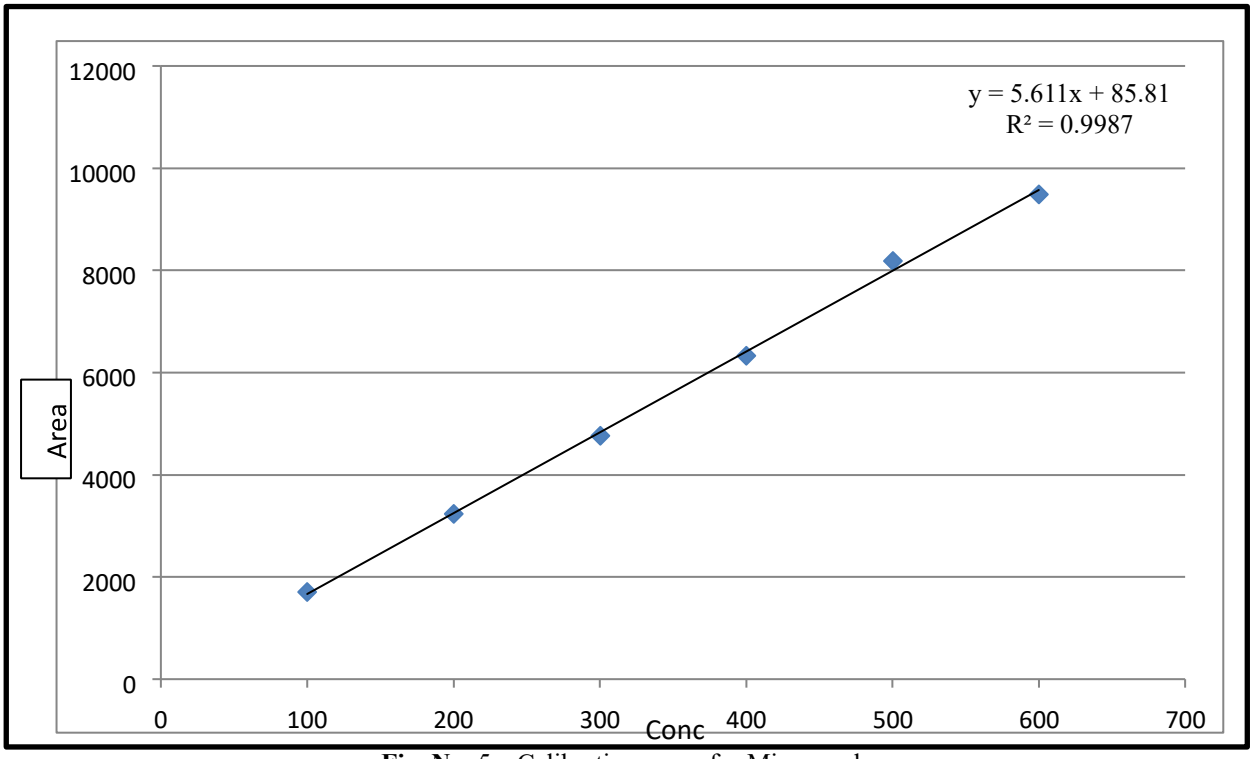

Fig. No. 5a: Calibration curve for Miconazole

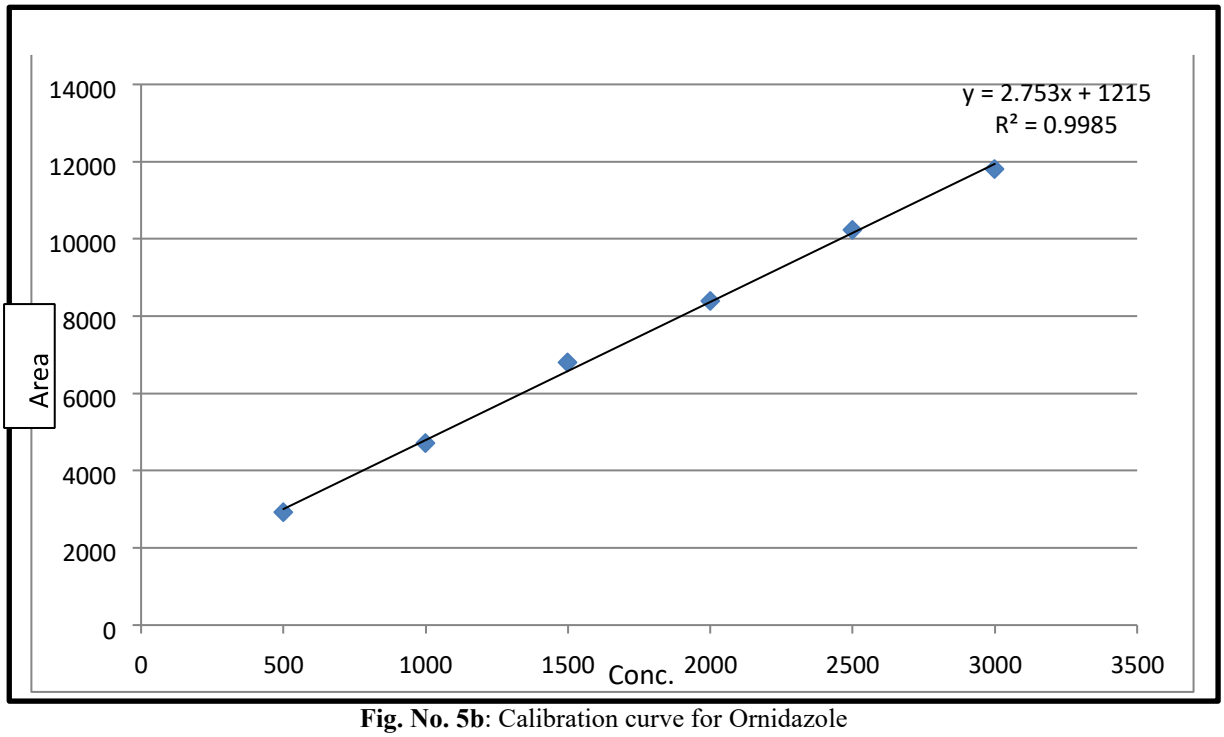

Fig. No. 5b: Calibration curve for Ornidazole

Specificity

Peak purity profiling experiments were done to test the method's specificity. The experiment is shown by peak purity values was found greater than 0.998. Precision

For MIC and ORN, which satisfied the requirements for the High Performance Thin Layer Chromatography method, the % RSD for repeated Standard solutions for the repeatability experiments was less than 2.0%. Results for intra-day & interday precision study for Miconazole are shown in (Table No. 4a) while results of interday & intra-day precision for Ornidazole is given in (Table No. 4b). This demonstrates accuracy of technique.

Table No. 4a. Precision study for Miconazole

	Intraday				Interday		
MIC	Conc.(ng/band)	Area	SD	% RSD	Area	SD	% RSD
	200	3234.5	0.409	±0.411	32427	1.692	±1.139
	400	6436.5	0.235	±0.211	6459	0.474	±0.574
	600	9687.0	0.976	±0.922	9608	0.606	±0.870

Table No. 4b. Precision study for Ornidazole

Intraday					Interday		
ORN	Conc.(ng/band)	Area	SD	% RSD	Area	SD	% RSD
	1000	4687	0.643	±0.642	4840	0.515	±0.641616
	2000	9596	0.393	±0.392	9644	0.234	±0.392
	3000	14326	0.378	±0.379	14282	0.450	±0.379

Accuracy

The recovery percentage values were within the permissible range of 90-110% with minor % RSD for MIC and ORN, showing the method's accuracy and suitability for evaluating commercial formulations (Table No. 5).

Table No. 5. Accuracy study for Miconazole & Ornidazole

MIC					**ORN**			
Level	Sample	Std	SD	±% RSD	Sample	Std	SD	±% RSD
50%	200	100	0.792	± 0.981	1000	500	0.544	± 0.641
100%	200	200	0.359	± 0.735	1000	1000	0.979	± 0.426
150%	200	300	0773	± 0.820	1000	1500	0.260	± 0.598

Robustness

The average values of % RSD of response for MIC and ORD determination under changed circumstances appearing less than 2% showed the procedure's robustness.
(Table 6).

Table No. 6: Robustness study for Miconazole & Ornidazole.

DRUG	**% RSD Found for Robustness Study (Peak Area)**								
	Wavelength			**Chamber Saturation Time (Min)**			**Time form application to development (min)**		
	309	**310**	**311**	**14**	**15**	**16**	**0**	**30**	**60**
Miconazole	0.303	0.0471	1.4430	0.317	0.478	0.853	1.423	0.446	0.522
Ornidazole	0.516	0.631	0.463	0.750	0.647	1.205	0.877	1.031	0.637

LOD & LOQ

The Limit of Detection & Limit of quantitation for MIC and ORN, determined from the linearity curve. LOD of MIC was obtained to be 8.308 ng/band & LOD of Ornidazole 40.5 ng/band. While LOQ of Miconazole was found 26.25 ng/band & LOQ of ORN was 125.832 ng/band. These values show the method's respectable sensitivity.

Assay of marketed formulation

The suitability of the provided technique was evaluated by assessing the commercially available formulation Candifem Tablet (Table No. 7). The % assay for MIC and ORN was calculated using six duplicates of the test sample. MIC and ORN tested on average with results of 99.19% and 99.84%, respectively. These results show that the approach is appropriate for testing commercially available formulations because they fall within the ideal range of 98-102%. [21,22]

Table No. 7. Assay of Formulation

Labeled Claim		**%Assay**	
MIC	**ORD**	**MIC**	**ORD**
100 mg	**500 mg**		
Mean % Assay		99.195	99.841

SD	0.466	0.750
% RSD	0.568	0.751

Summary
On the working standard solution of miconazole (200 ng/band) and ornidazole (1000 ng/band), chromatographic separation procedures were carried out. To determine the ideal system suitability qualities, tests were first conducted on HPTLC plates employing a variety of solvents at varying concentrations. After numerous trials, the mobile phase of n-Hexane: Methanol (8: 2 v/v) was selected because it provided desirable resolution and appropriate peak parameters. Fig. No. 4 The samples were spotted using a sample syringe and a CAMAG Linomat 5 sample applicator on a precoated silica gel aluminium plate 60 F_{254}. A 20 mm/sec scanning velocity was used. The linear ascending development had been done using the mobile phase. For mobile phase, 15 minutes was the optimum chamber saturation period. The process of developing of the chromatogram took around 15 minutes, and the length of the run was 8 cm. All new findings were scanned for densitometry using a CAMAG thin layer chromatography scanner set to 310 nm and the WINCATS software version 1.4.2. The deuterium lamp used as the radiation a resource continuously emitted UV light with a wavelength ranging between 200 and 400 nm. As given in (Table No. 1).

Table No. 8. Summary of Validation Parameters

Sr. No.	Validation Parameter	Results	
		Miconazole	**Ornidazole**
1.	Linearity	y = 15.811x +85.81 R^2 = 0.9987	y = 3.575 x + 1215 R^2 = 0.9985
2.	Range	100-600 ng/band	500 - 3000 ng/band
3.	Assay (Mean ± % RSD)	99.595± 0.468	99.841± 0.751
4.	Precision	% RSD	% RSD
	A) Intraday precision	0.416 – 0.922 %	0.379 – 0.642 %
	B) Interday precision	0.574 – 1.139%	0.234 – 0.508 %
5.	**Accuracy**	**% Recovery**	**% Recovery**
	50%	100.464 ± 0.981	99.572 ± 0.641
	100%	100.023 ± 0.735	101.109 ± 0.416
	150%	100.326 ± 0.820	99.978 ± 0.598
6.	LOD	8.308 ng/ band	41.525 ng/band
7.	LOQ	25.176 ng/band	125.832 ng/band
8.	Specificity	Specific	Specific
9.	Robustness	Robust	Robust

All the validation parameters were found under acceptance criteria.

Conclusion:

- The current analytical method has been verified using the ICH standards and complies with the necessary standards for acceptance.
- For determining, MIC and ORN the method is specific, linear, rapid, accurate, and economical.
- A more recent technique has been developed for HPTLC.
- It is economical method because the amount and expenditure of the solvent utilized are less than what is currently reported in other publications.
- The method was thoroughly validated, and the results for each of the evaluated method validation parameters were satisfactory.
- Without the presence of excipients, miconazole nitrate and ornidazole in their combination dose form can be routinely analyzed using HPTLC techniques.
- It was determined that the procedure offered sufficient evidence for the drug's label claim.

Ethics Committee Approval: The ethics committee approval not required for the proposed research. We were not used any kind of human being and animal matrices.

Informed Consent: Not applicable

Authorship Contributions Concept: V.V.K., Design: S.S.B., Data Collection P.A.D.
Data Processing, N.G.S.
Analysis or Interpretation: S.S.B, N.G.S., Literature Search: S.S.B, V.V.K., Writing: S.S.B, P.A.D.

Conflict of Interest: No conflict of interest was declared by the authors.
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