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STABILITY INDICATING CHROMETOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MICONAZOLE AND ORNIDAZOLE IN ITS PHARMACEUTICAL DOSAGE FORM BY HPLC

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ABSTRACT

An accurate, precise and simple HPLC stability indicating assay method for simultaneous estimation of Miconazole and Ornidazole in their pharmaceutical dosage form has been developed and validated. The best separation was achieved on a C_{18} (250 mm × 4.6 mm) i.d., 5µm particles, C_{18}reversed phase column with gradient program, Mobile phase Water: Acetonitrile: Acetic acid (30:70;0.1%v/v), Flow rate 1ml/min, Injection volume 20µl, UV detection was performed at 224 nm. The method was linear over the concentration range of 10-30µg/ml (r=0.999) and 2-6µg/ml(r=0.999) for Miconazole and Ornidazole, respectively. The limit of detection 0.089µg/ml and 0.457µg/ml for Miconazole and Ornidazole, respectively. The limit of quantification 0.270µg/ml and 1.386µg/ml for Miconazole and Ornidazole, respectively. Forced degradation studies was carried out according to ICH guideline in all condition(Acid hydrolysis, Base hydrolysis, Oxidative degradation, Thermal degradation, Photo degradation).

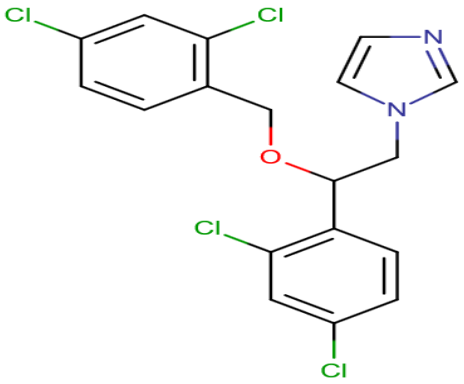
KEYWORDS: RP-HPLC, Validation, Stability, Miconazole, Ornidazole.

INTRODUCTION

MICONAZOLE

Miconazole is an imidazole antifungal agent that is used topically and by intravenous infusion. Molecular formula for miconazole is $C_{18}H_{14}Cl_4N_2O$ and its molecular weight is 416.13. The mechanism of action of miconazole is Miconazole interacts with 14α

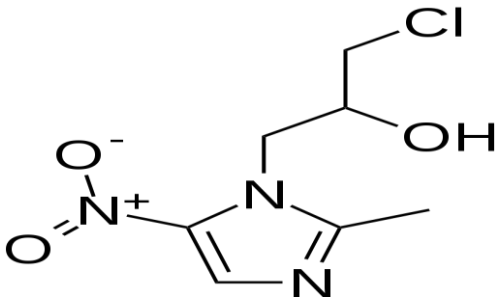
demethylase, a cytochrome P450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. The IUPAC name of miconazole is 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-1-Himidazole.



Miconazole.

Ornidazole

Ornidazole is a drug that cures some protozoan infections. It has been investigated for use in Crohn's disease after bowel resection. The molecular formula for ornidazole is $C_7H_{10}ClN_3O_3$ and its molecular weight is 219.625 g/mol. The IUPAC name of ornidazole is 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol. It produce its action by the inhibition of DNA synthesis resulting in to loss of helical structure.



Ornidazole.

MATERIALS AND METHODS

PRELIMINARY ANALYSIS OF DRUGS

Ornidazole

Description

The sample of Ornidazole was observed for its color and texture.

Melting point

The sample of Ornidazole was taken in capillary and place into the melting point apparatus. Observed the melting point and compared with the reference.

IdentificationTest

Potassium Bromide IR disc was prepared using 1mg of Ornidazole on Hydraulic Pellet Press. This disc was scanned in the region of 4000–400cm^{-1} in FTIR and obtained IR spectrum was compared with the reference spectrum of Ornidazole.

Solubility

The sample of Ornidazole was taken in testtubes and observed for solubility in various solvents like water, methanol, 0.1 N Hcl and 0.1 N NaOH.

Miconazole

Description

The sample of Miconazole was observed for its and texture.

Melting point

The sample of Miconazole was taken in capillary and place into the melting point apparatus. Observed the melting point and compared with the reference.

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Potassium Bromide IR disc was prepared using 1 mg of Miconazole on Hydraulic Pellet Press. This disc was scanned in the region 0f 4000–400cm^{-1} in FTIR and obtained IR spectrum was compared with the reference spectrum of Miconazole.

Solubility

The sample of Miconazole was taken in testtubes and observed for solubility in various solvents like water, methanol, 0.1 N Hcl and 0.1 N NaOH.

Development and Validation for Simultaneous Estimation of Ornidazole and MiconazoleBy RP-HPLC

Apparatus and Instruments

Model: Shimadzu HPLC System

Pump:-LC-20 AT

Column: C_{18} (250 mm × 4.6 mm i.d., 5µm)

Detector: UV Detector

Software: Spinchrom

Analytical balance: Electronic Balance (Shimadzu AUX220)

Reagents and Materials

Ornidazole was procured from Accretion Pharma

Miconazole was procured from Accretion Pharma

Water-HPLC gradefrom Finar

Methanol-HPLC grade from Merk

Acetonitrile –HPLC grade from Merck

Glacial acetic acid-from Finar

Preparation of standard solutions

(A) Miconazolestandard stock solution: (40µg/mL)

A 40 mg of Miconazole was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with methanol. Take 10ml from this solution and transfer to 100ml volumetric flask and volume was made up with the methanol.

(B) Ornidazole standard stock solution: (200µg/mL)

A 20 mg of Ornidazole was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with methanol.

(C) Preparation of standard solution of binary mixtures ofMiconazole(4µg/mL) and Ornidazole (20µg/mL)

Take 1 mL from the Miconazole stock solution and 1mL from Ornidazole stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Selection of wavelength

Standard solution of Miconazole(4µg/mL) and Standard solution of Ornidazole(20µg/mL) in Methanol were scanned between 200-400 nm using UV-visible spectrophotometer.

Both solutions were scanned between 200 - 400 nm.

Wavelength was selected from the overlay spectra of above solutions.

Chromatographic separation

Standard solutions of 2-6µg/ml of Miconazole and 10-30µg/ml of Ornidazole were injected in column with 20µl micro-syringe. The chromatogram was run for appropriate minutes with mobile phase Water: Acetonitrile: Acetic acid (30:70:0.1).

The detection was carried out at wavelength 224nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc were recorded using software.

System suitability test

It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Chromatographic conditions

Column: C_{18} (250 mm × 4.6 mm i.d., 5µm).
Mobile Phase: Water: Acetonitrile: Acetic acid (30:70:0.1).
Flow Rate: 1.0 ml/min.
DetectionWavelength: 224nm.
Runtime: 10.0min
Injectionvolume: 20.0µl

Stability-Indicating Method

1. Acid degradation

Acid decomposition studies were performed by taking the 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N HCl solutions was added and mixed well and put for 4hrs at Room temperature. Then the volume was adjusted with diluent to get Ornidazole (20µg/mL) and Miconazole (4µg/mL).

2. Base degradation

Basic decomposition studies were performed by taking the 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed

well and put for 4 hrs at Room temperature. Then the volume was adjusted with diluent to get Ornidazole (20µg/mL) and Miconazole (4µg/mL).

3. Oxidative degradation

Oxidative decomposition studies were performed by refluxing the 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 3% H_2O_2 solutions was added and mixed well and put for 4hrs at Room temperature. Then the volume was adjusted with diluent to get Ornidazole (20µg/mL) and Miconazole (4µg/mL).

4. Photo Degradation

Photo Degradation studies were performed by weigh powder and exposed to UV chamber for 48 hrs. the powder was removed from the UV chamber for proper dilution and chromatograms were taken.

5. Thermal degradation

Thermal Degradation studies were performed by weigh powder and exposed to dry heat in an oven at 70°C for 4 hrs. The powder was removed from the oven for proper dilution and chromatograms were taken.

Validation of Developed RP-HPLC method

1. Linearity

The linearity for Miconazole and Ornidazole were assessed by analysis of combined standard solution in range of 2-6 µg/ml and 10-30 µg/ml respectively, 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Miconazole(40µg/ml) and Ornidazole(200 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 2,3,4,5 and 6 µg/ml and 10,15,20,25 and 30 µg/ml for Miconazole and Ornidazole respectively In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

2. Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

Repeatability

Standard solution containing Miconazole (4 µg/ml) and Ornidazole (20 µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

1. Intra-day precision

Standard solution containing (2,4,6 µg/ml) of Miconazole and (10,20,30 µg/ml) of Ornidazole were analyzed three times on the same day and % R.S.D was calculated.

2. Inter-day precision

Standard solution containing (2,4,6 µg/ml) of Miconazole and (10,20,30 µg/ml) of Ornidazole were analyzed three times on the different day and % R.S.D was calculated.

3. LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

$$LOD = 3.3 \times (SD/Slope)$$

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,

$$LOQ = 10 \times (SD/Slope)$$

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

4. Accuracy

For Miconazole

2 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 224 nm. The amount of Miconazole was calculated at each level and % recoveries were computed.

For Ornidazole

10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 224 nm. The amount of Ornidazole was calculated at each level and % recoveries were computed.

5. Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH was changed ((±0.2).
3. Ratio of Mobile phase was changed (±2) Water: Acetonitrile (32:68) and Water: Acetonitrile (28:72).

6. Analysis of formulation

Take Crushed Tablet powder equivalent to 4 mg of Miconazole and 20 mg of Ornidazole was transferred to a 100 ml volumetric flask and made up volume up to the mark with mobile phase. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with mobile phase. The solution was injected 20 µl. The areas of resulting peak were measured at 224 nm.

RESULT

Stability Indicating RP-HPLC Method Development And Validation For Simultaneous Estimation of Miconazole and Ornidazole In Their Combined Dosage Form.

IDENTIFICATION OF DRUGS

Melting point

Melting point of Miconazole: 157-163°C.

Melting point of Ornidazole: 76-78°C.

This Value is same as that of the literature citation.

Infrared spectroscopy

A pellet of the drug and KBr (Spectroscopic grade) was prepared using hydraulic pellet press at a pressure of 7-10 tones. FT-IR was scanned from 400-4000 cm^{-1}. Following peaks were observed.

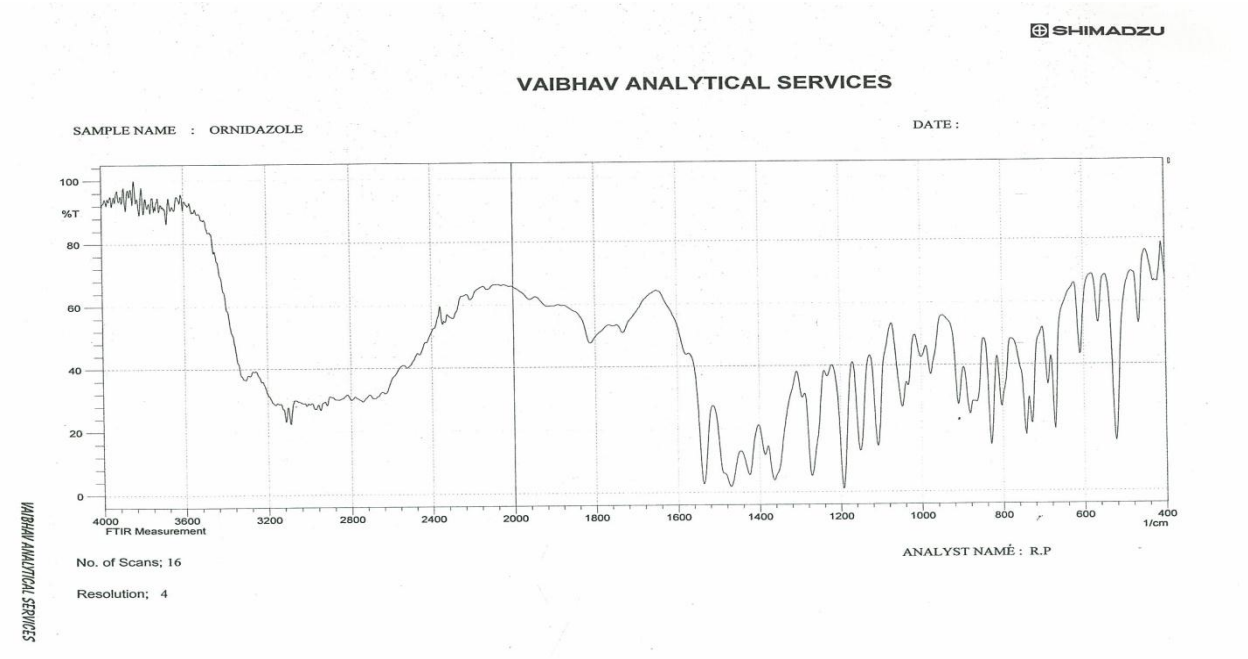

Figure 1: IR Spectra of Sample Ornidazole.

Functional group	Frequency
NO_2 (Aromatic)	1500
C-N	1200
C=C	1250
C-Cl	750

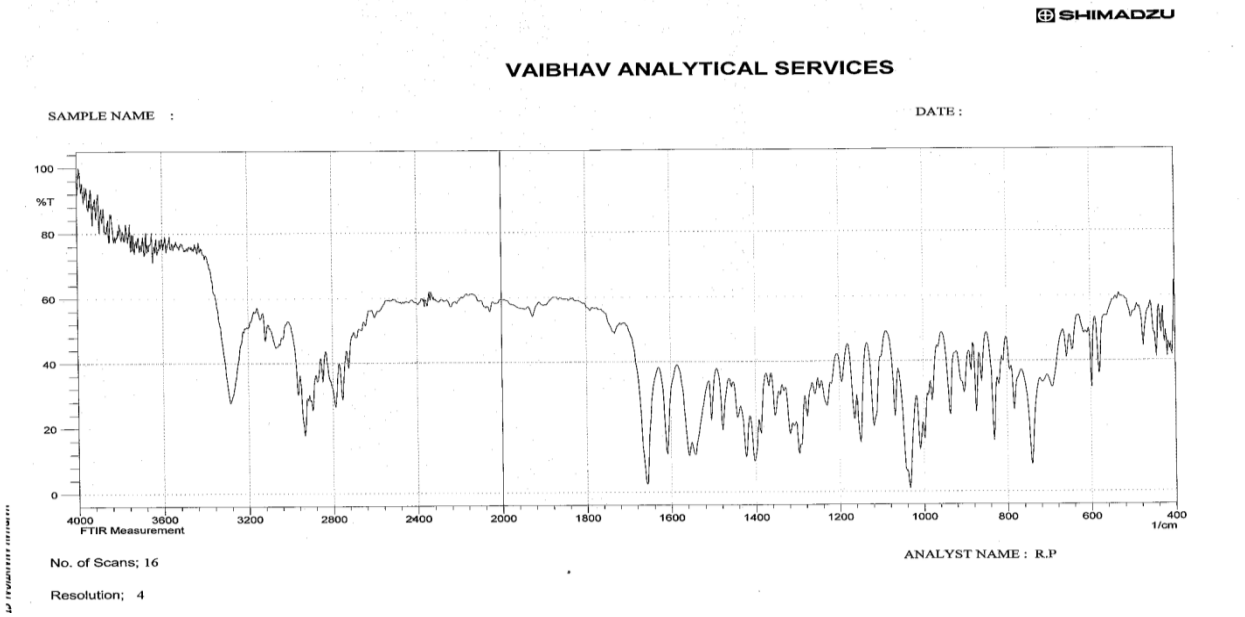

Figure 2: IR Spectra of sample Miconazole.

Functional group	Frequency
C-O	1050
C-N	1300
C=C	1690
C-Cl	730

SOLUBILITY STUDY

Table 1: Solubility of Miconazole and Ornidazole.

SOLVENT	SOLUBILITY	
	Miconazole	**Ornidazole**
Water	Insoluble	Slightly soluble
0.1 N NaOH	Insoluble	Slightly soluble
0.1 N HCl	Insoluble	Insoluble
Methanol	Soluble	Freely soluble

Method Development and Validation For Simultaneous Estimation Of Miconazole and Ornidazole By RP-HPLC

Selection of Elution Mode

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. C_{18} column is least polar compare to C_4 and C_8 columns. Here, A 250 x 4.6 mm column of 5.0 µm packing was selected for separation of Miconazole and Ornidazole. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability.

Selection of wavelength

Both Miconazole and Ornidazole show reasonably good response at 224 nm.

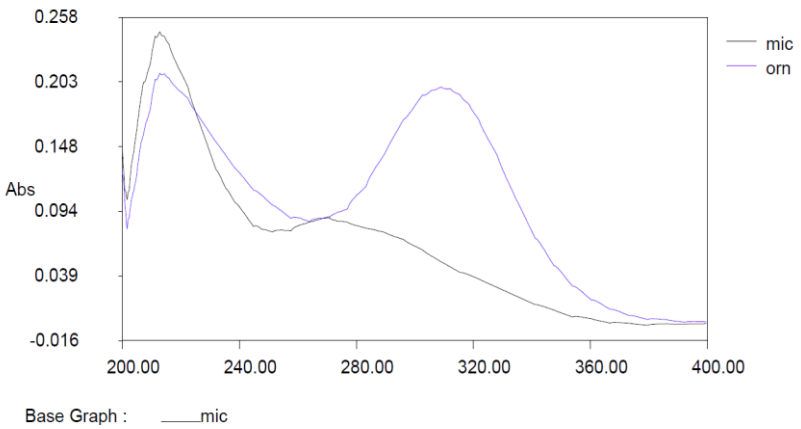

Figure 3: Overlay UV Spectrum of Miconazole and Ornidazoleshowing selection of wavelength detection.

Selection of Mobile Phase

Table 2: Effect of different mobile phase compositions on the separation of Miconazole and Ornidazole.

Mobile Phase	Flow Rate (ml/min)	Ratio	Retention Time (min) Ornidazole	Retention Time(min) Miconazole	Remark
Water: Methanol	1.0	50:50	-	-	No peak observed
Water: Methanol	1.0	30:70	-	-	No peak observed
Water: Methanol	1.0	10:90	-	-	Still not any peak find
Water: Acetonitrile	1.0	50:50	-	-	Still not any peak observed by replacing the methanol with Acetonitrile
Water: Acetonitrile	1.0	30:70	13.190	-	One peak Observed
Water: Acetonitrile	1.0	30:70	13.053	-	Peak of Ornidazole Confirmed
Water-Acetonitrile	1.0	30:70	-	-	Observed peak was not of Miconazole
Water Acetonitrile	1.0	10:90	8.547	-	Retention time reduced
Water: Acetonitrile: Acetic acid	1.0	30:70:0.05	5.893	7.197	Second peak Observed by using Acetic acid
Water: Acetonitrile: Acetic acid	1.0	30:70:0.05	-	7.223	Peak of Miconazole Confirmed
Water: Acetonitrile: Acetic acid	1.0	40:60:0.05	7.497	8.983	Resolution is not so good
Water: Acetonitrile: Acetic acid	1.0	40:60:0.1	7.803	11.100	Resolution increased but run time is high
Water: Acetonitrile: Acetic acid	1.0	30:70:0.1	4.640	7.633	Run time decreased
Water: Acetonitrile: Acetic acid	1.0	25:75:0.1	3.860	5.127	Resolution decreased

After considering the varying combinations of various mobile phases, Water: Acetonitrile: Acetic acid (30:70:0.1) was finalized as it was showing good peak shapes and a significant amount of resolution.

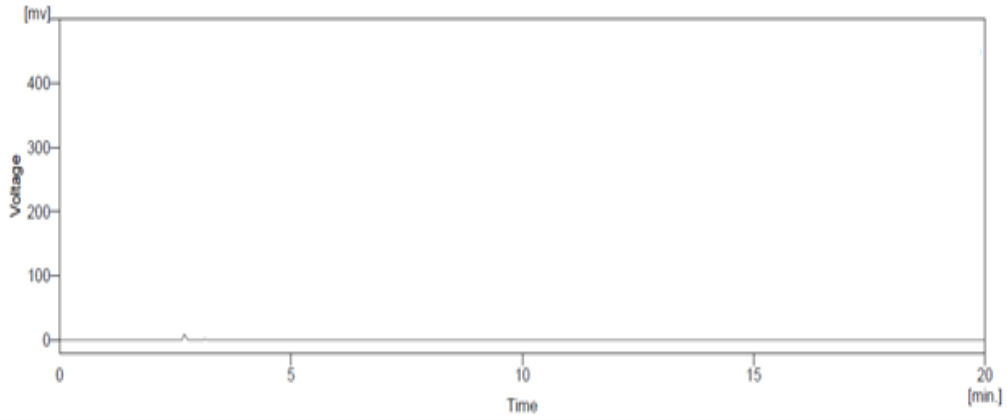

Figure 4: Chromatogram of Miconazole and Ornidazolein water : Methanol (50:50v/v) (Flow rate-1.0 ml/min).

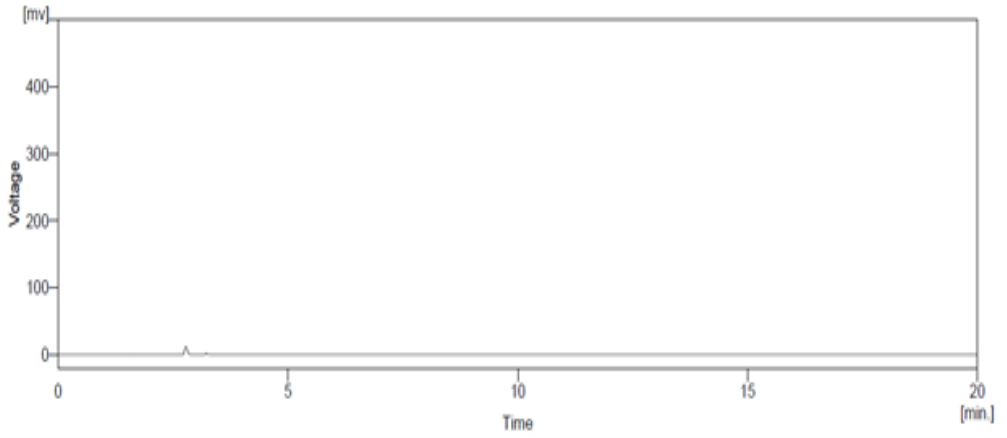

Figure 5: Chromatogram of Miconazole and Ornidazole in water : Methanol (30:70v/v) (Flow rate-1.0 ml/min).

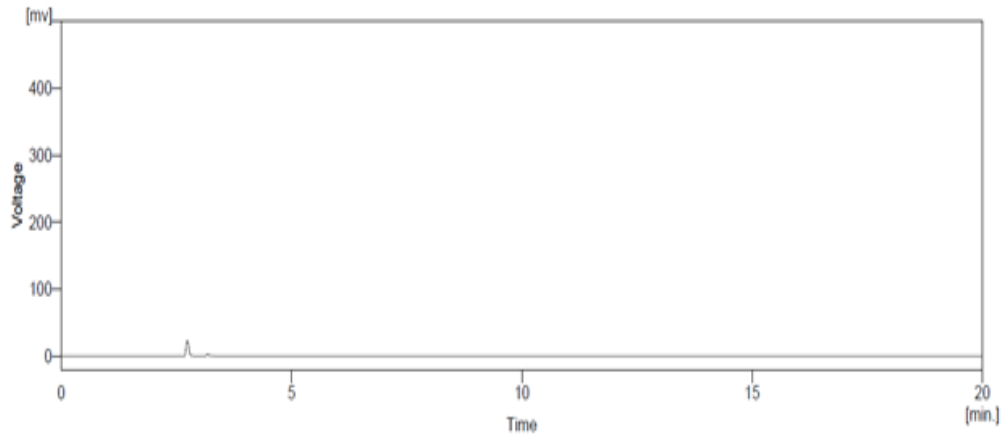

Figure 6: Chromatogram of Miconazole and Ornidazolein water : Methanol (10:90v/v) (Flow rate-1.0 ml/min).

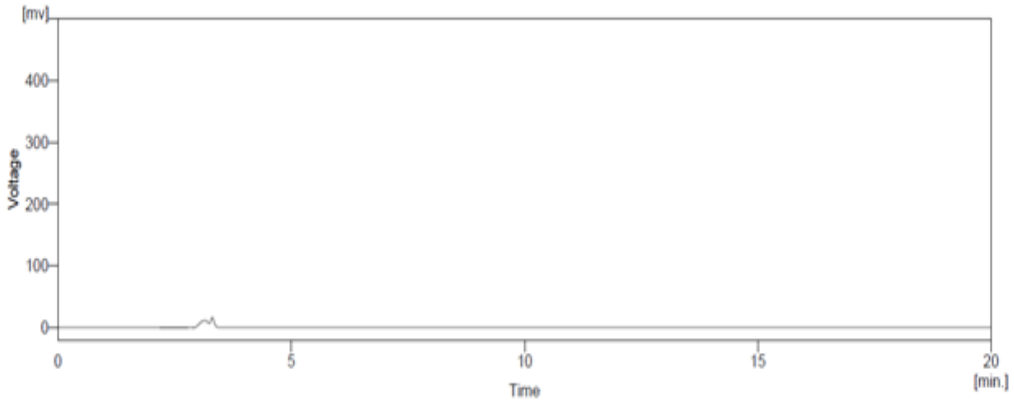

Figure 7: Chromatogram of Miconazole and Ornidazole in water: Acetonitrile (50:50v/v) (Flow rate-1.0 ml/min).

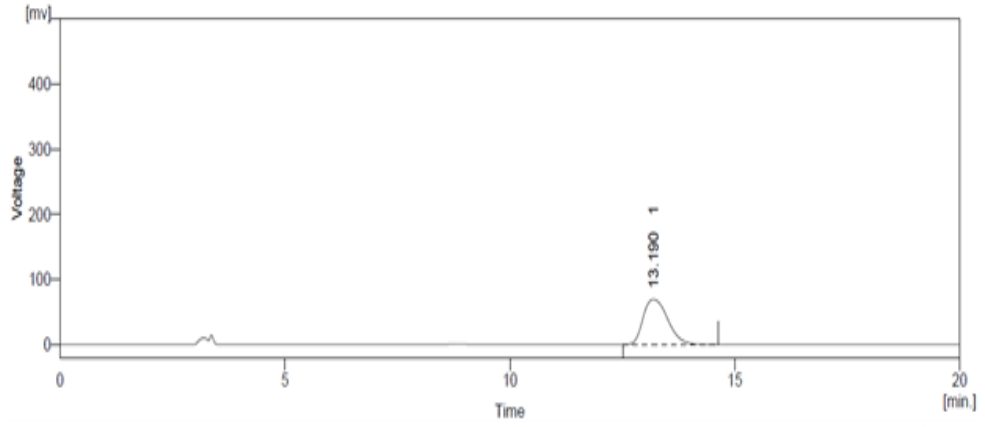

Figure 8: Chromatogram of Miconazole and Ornidazole in water : Acetonitrile (30:70v/v) (Flow rate-1.0 ml/min).

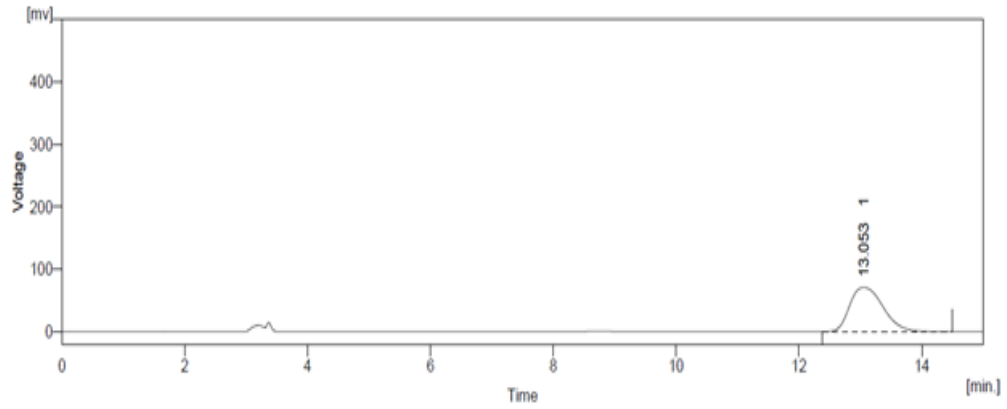

Figure 9: Chromatogram of Ornidazole in water : Acetonitrile (30:70v/v) (Flow rate-1.0 ml/min).

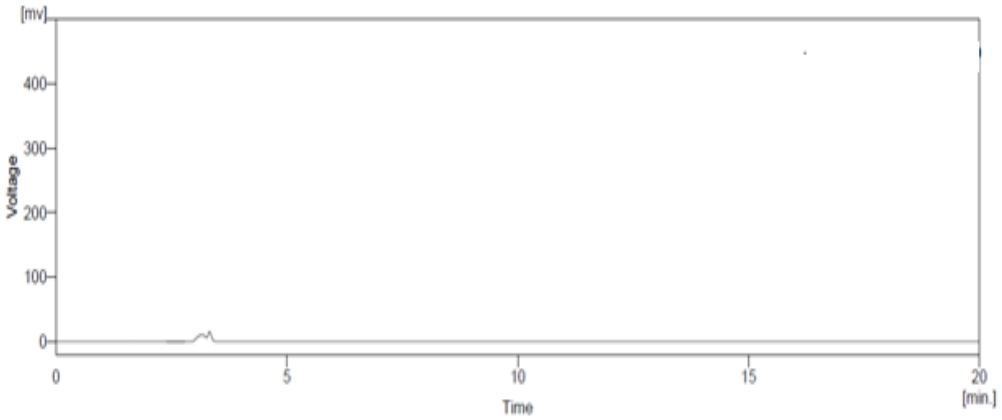

Figure 10: Chromatogram of Ornidazole and Miconazole in water: Acetonitrile (30:70v/v) (Flow rate-1.0 ml/min).

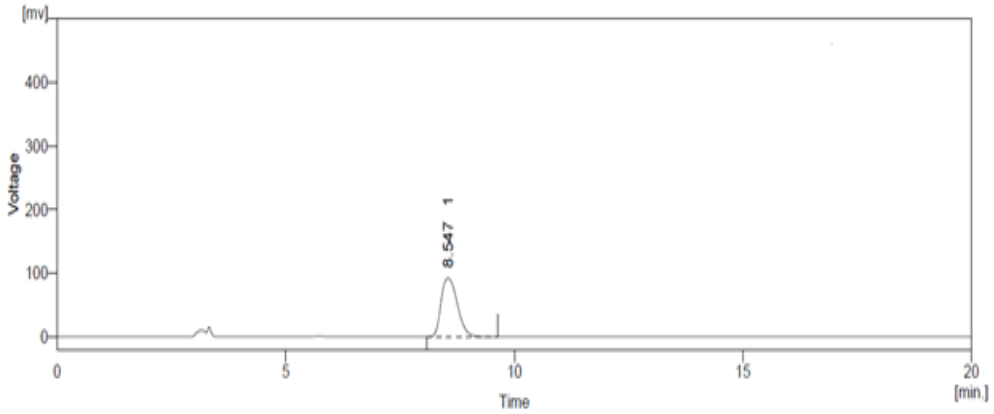

Figure 11: Chromatogram of Ornidazole and Miconazolein water: Acetonitrile (10:90v/v) (Flow rate-1.0 ml/min).

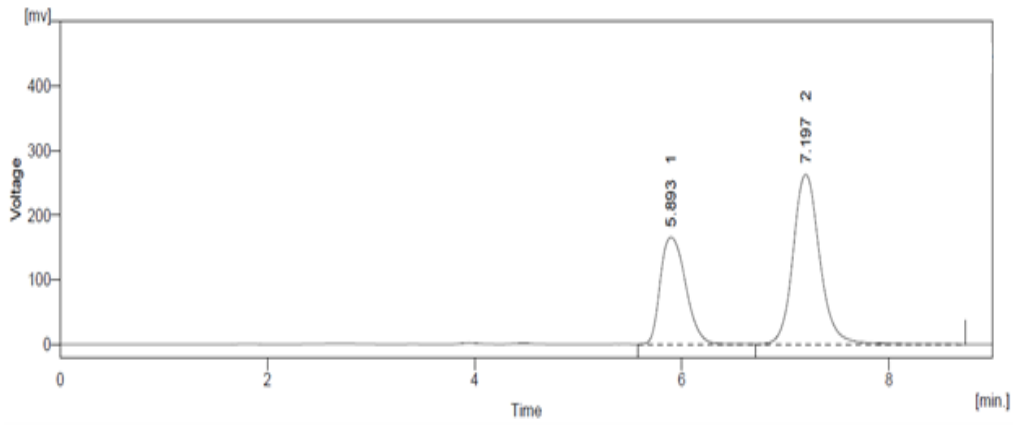

Figure 12: Chromatogram of Ornidazole and Miconazole in water: Acetonitrile: Acetic acid (30:70:0.05 v/v) (Flow rate-1.0 ml/min).

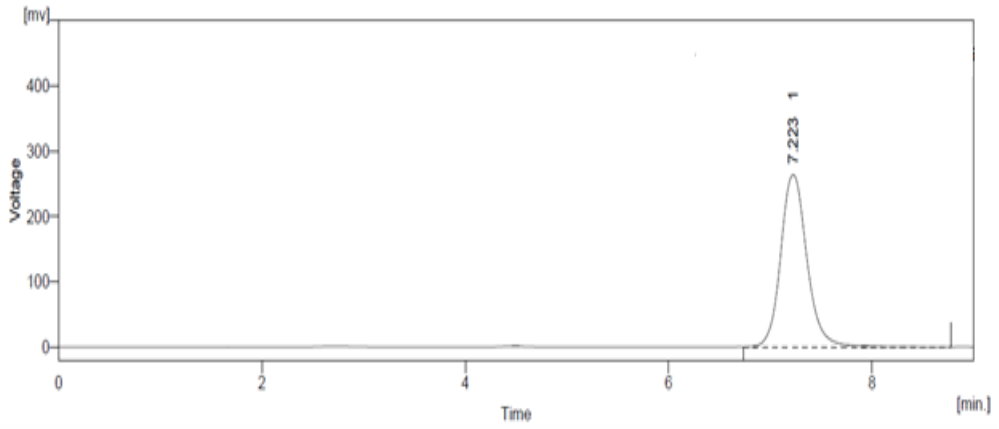

Figure 13: Chromatogram of Miconazole in water: Acetonitrile: Acetic acid (30:70:0.05 v/v) (Flow rate-1.0 ml/min).

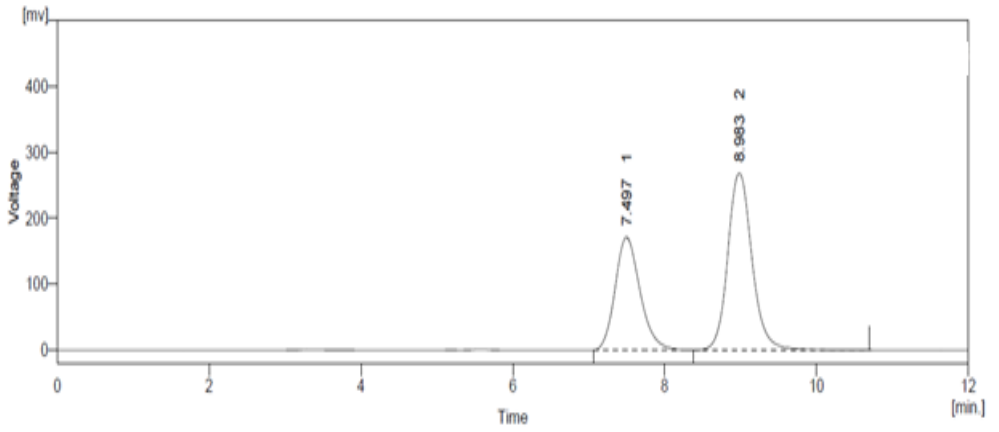

Figure 14: Chromatogram of Ornidazole and Miconazole in water: Acetonitrile: Acetic acid (40:60:0.05 v/v) (Flow rate-1.0 ml/min).

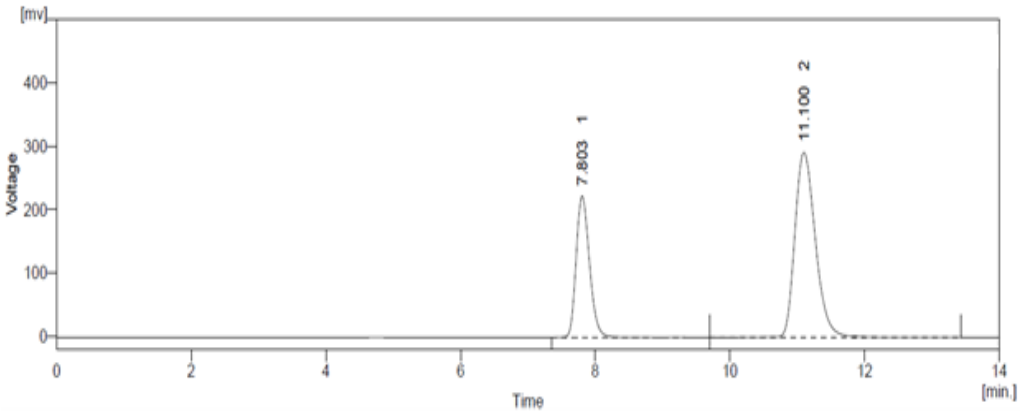

Figure 15: Chromatogram of Ornidazole and Miconazole in water : Acetonitrile: Acetic acid (40:60:0.1 v/v) (Flow rate-1.0 ml/min).

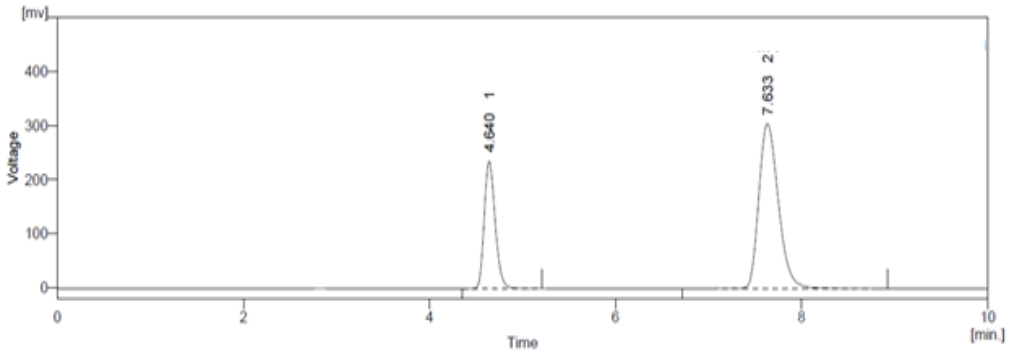

Figure 16: Chromatogram of Ornidazole and Miconazole in water: Acetonitrile: Acetic acid (30:70:0.1 v/v) (Flow rate-1.0 ml/min). (Final).

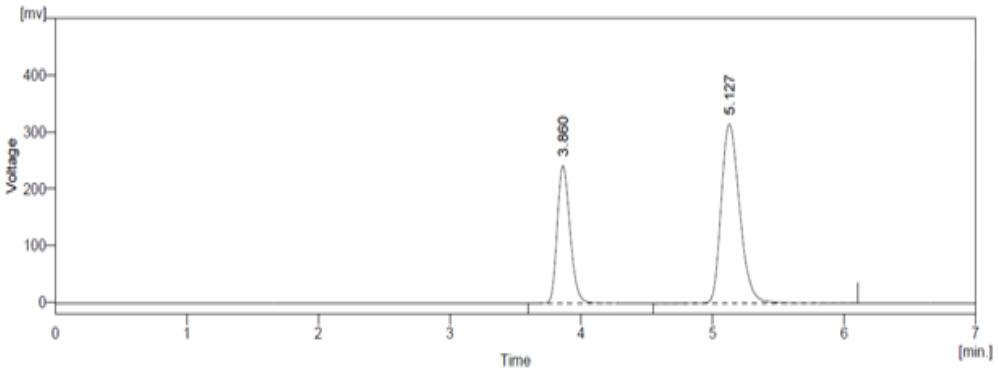

Figure 17: Chromatogram of Ornidazole and Miconazole in water: Acetonitrile: Acetic acid (25:75:0.1 v/v) (Flow rate-1.0 ml/min).

Chromatography

The mobile phase water: Acetonitrile: Acetic acid (30:70:0.1 v/v)was selected because it was found to ideally resolve the peaks with retention time (RT) 4.640 min and 7.633 min for Ornidazole and Miconazole respectively and the same is shown in fig. 6.18.

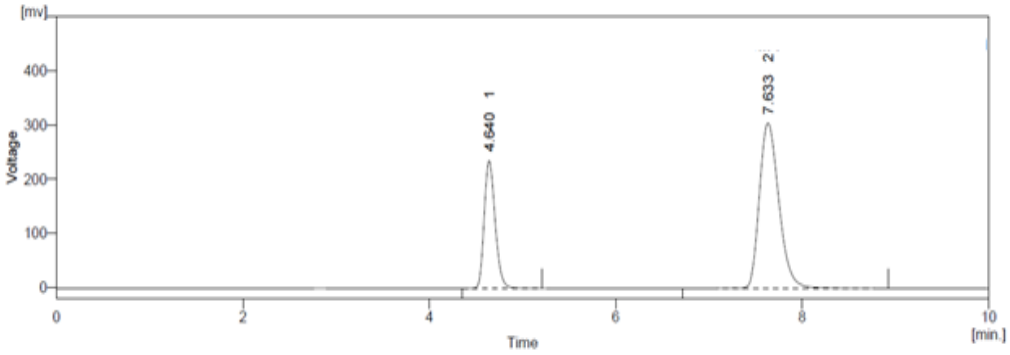

Figure 18: Chromatogram of Ornidazole and Miconazole in water: Acetonitrile: Acetic acid (30:70:0.1 v/v) (Flow rate-1.0 ml/min).

Observed values for system suitability test

1. **Resolution (Rs):** Resolution was observed 9.877, depicted in Table 6.3.
2. **Column efficiency (N):** Number of plates observed for Ornidazole and Miconazolewere 7058 and 6283, respectively, depicted in Table 6.3.
3. **Symmetry factor (S):** Tailing factor observed for Ornidazole and Miconazolewere 1.367 and 1.404, respectively, depicted in Table 6.3.

Table 3: Results for system suitability test.

Parameters	Data observed	
	Ornidazole	Miconazole
Theoretical plates per column	7058	6283
Symmetry factor/Tailing factor	1.367	1.404
Resolution	9.877	

Stability Indicating Method for Simultaneous Estimation of Ornidazole and Miconazole done By RP-HPLC

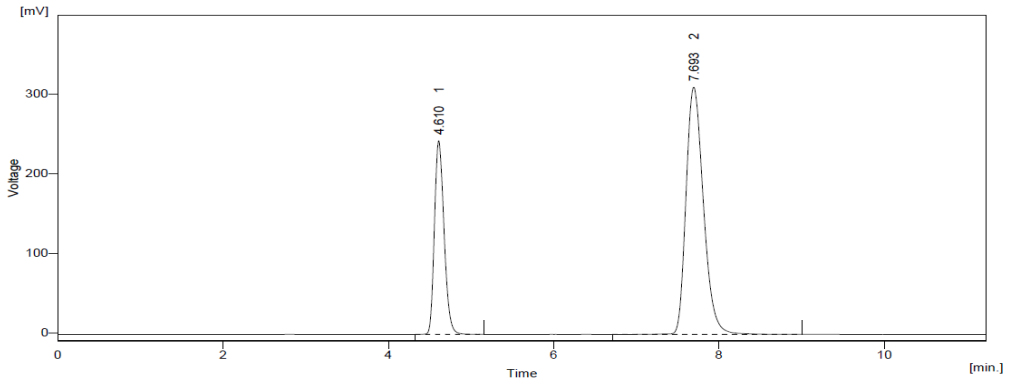

Figure 19: Chromatogram of stability study of Miconazole and Ornidazole Standard.

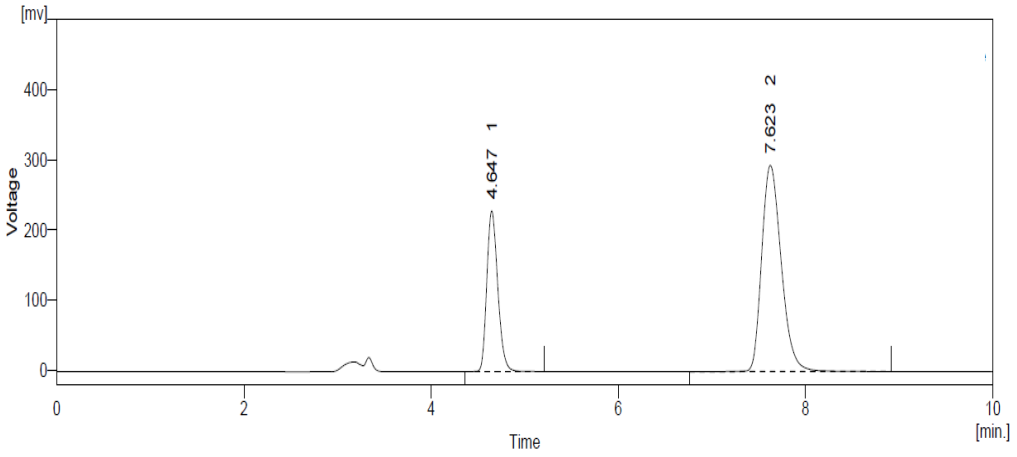

Figure 20: Chromatogram of stability study of Miconazole and Ornidazole sample.

1. Acid degradation

Acid decomposition studies were performed by taking the 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N HCl solutions was added and mixed well and put for 4hrs at Room temperature. Then the volume was adjusted with diluent to get Ornidazole (20µg/mL) and Miconazole (4µg/mL).

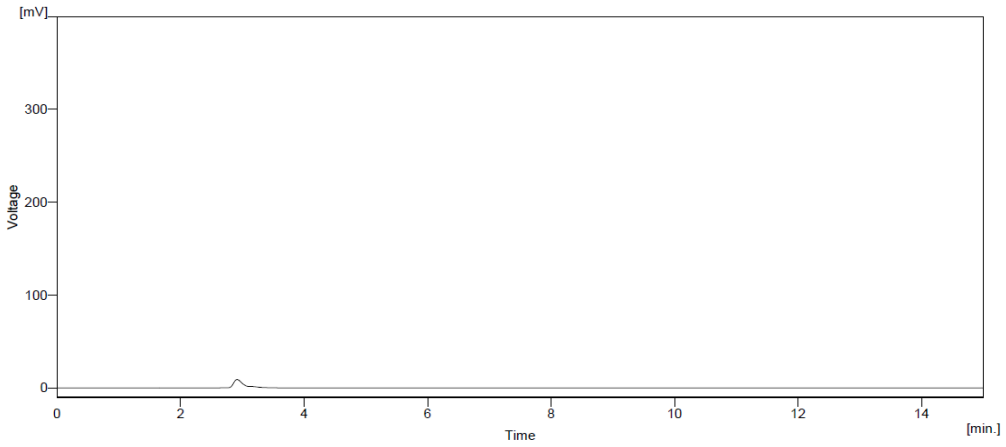

Figure 21: Chromatogram of Acid Degradation Blank.

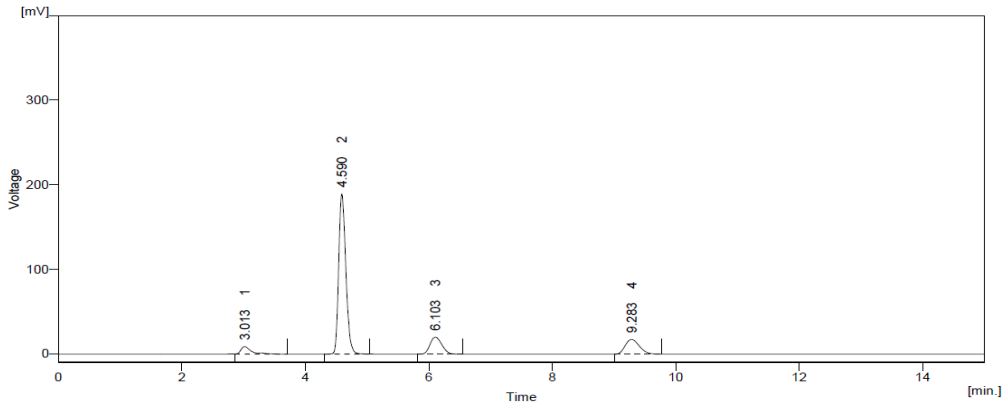

Figure 22: Chromatogram of Ornidazole Acid Degradation Standard.

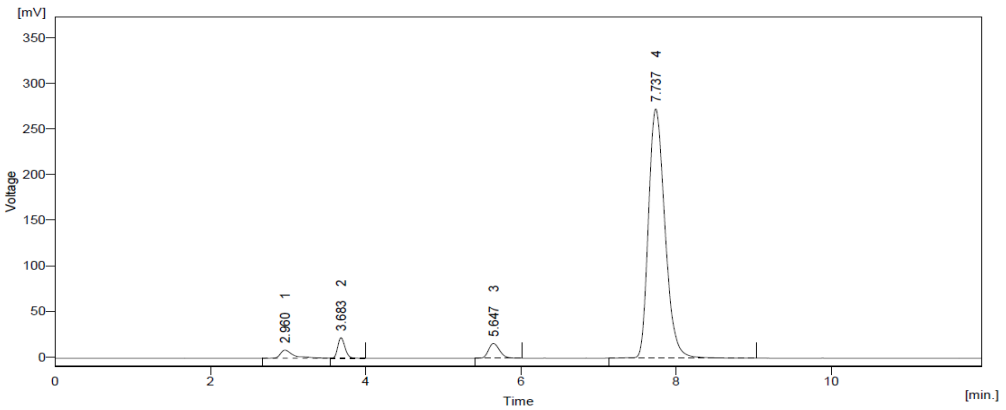

Figure 23: Chromatogram of Miconazole Acid Degradation Standard.

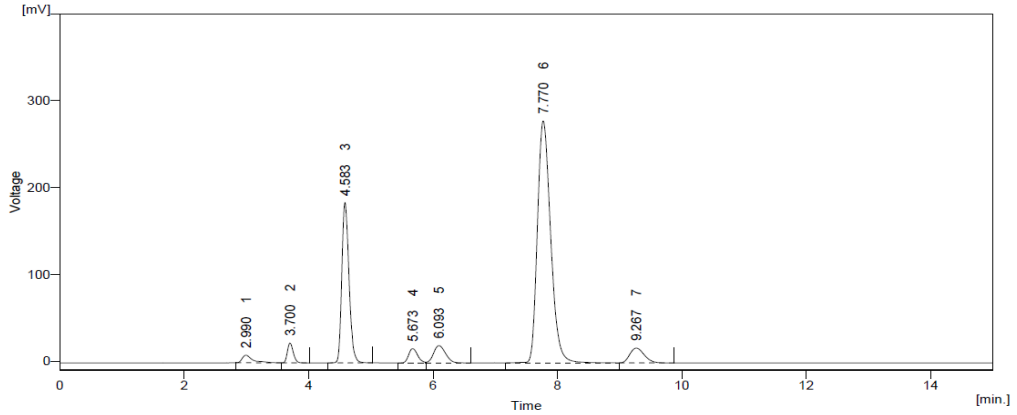

Figure 24: Chromatogram of Miconazole and Ornidazole Acid Degradation Sample.

2. Base degradation

Basic decomposition studies were performed by taking the 1ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 0.1 N NaOH solutions was added and mixed well and put for 4 hrs at Room temperature. Then the volume was adjusted with diluent to get Ornidazole (20µg/mL) and Miconazole (4µg/mL).

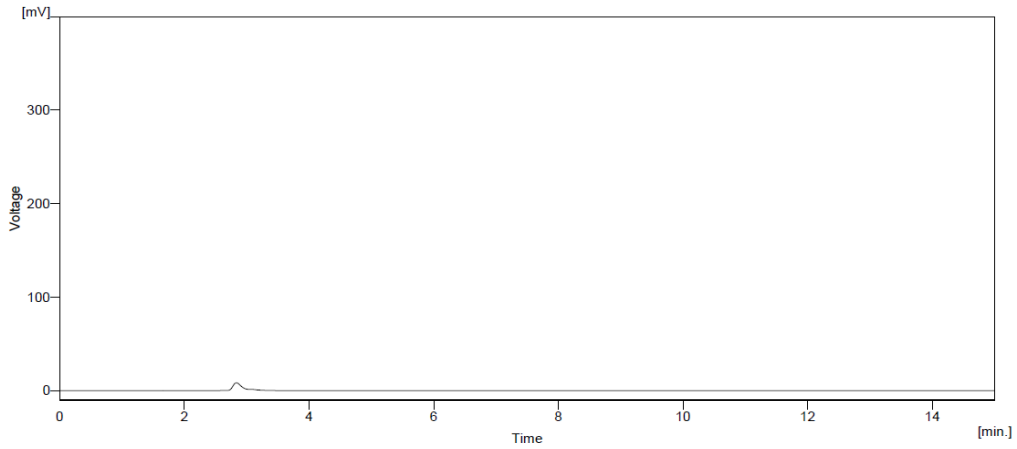

Figure 25: Chromatogram of Base Degradation Blank.

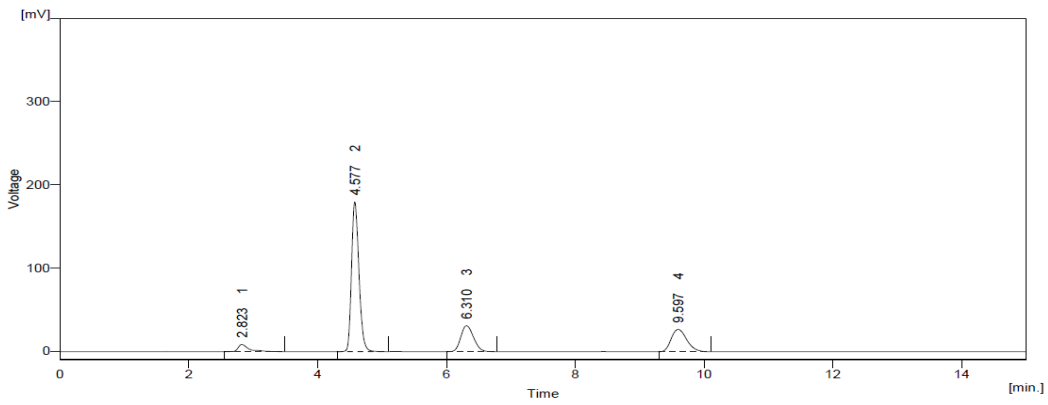

Figure 26: Chromatogram of Ornidazole Base Degradation Standard.

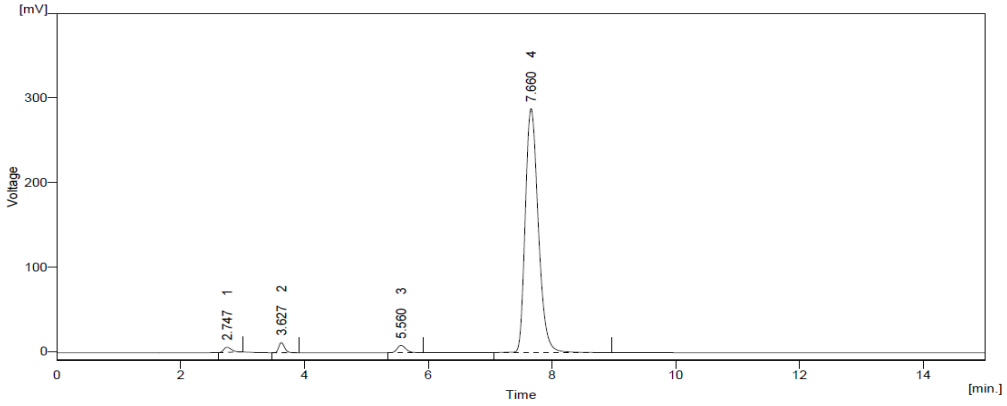

Figure 27: Chromatogram of Miconazole Base Degradation Standard.

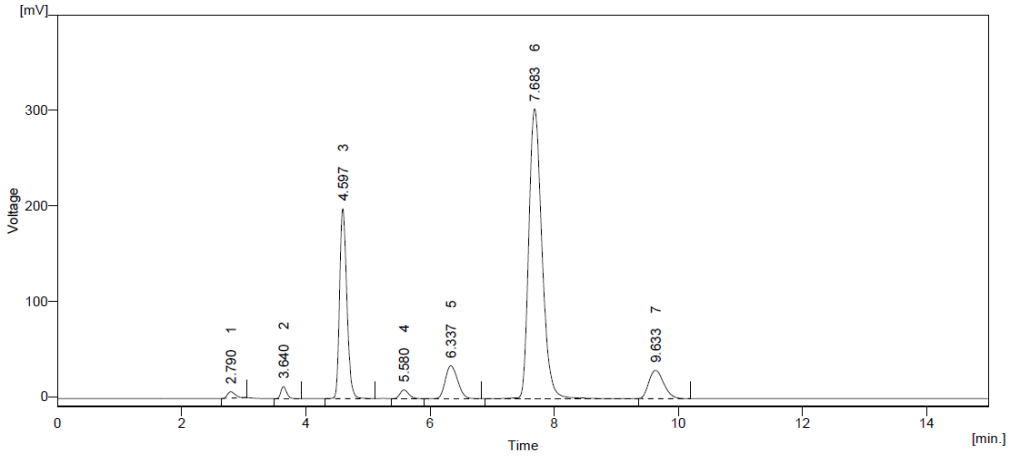

Figure 28: Chromatogram of Miconazole and Ornidazole Base Degradation Sample.

3. Oxidative degradation

Oxidative decomposition studies were performed by taking the 1 ml of stock solution was transferred in to 10 ml of volumetric flask. 2 ml of 3% H_2O_2 solutions was added and mixed well and put for 4hrs at Room temperature. Then the volume was adjusted with diluent to get Ornidazole (20µg/mL) and Miconazole (4µg/mL).

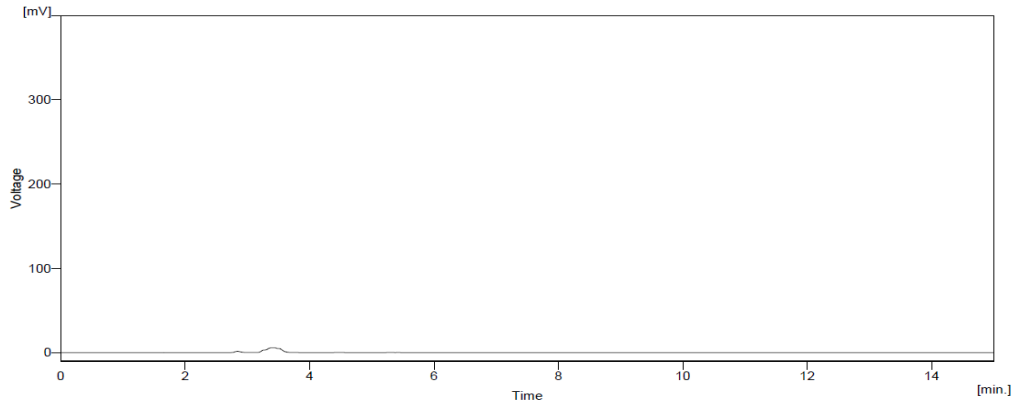

Figure 29: Chromatogram of Oxidation Degradation Blank.

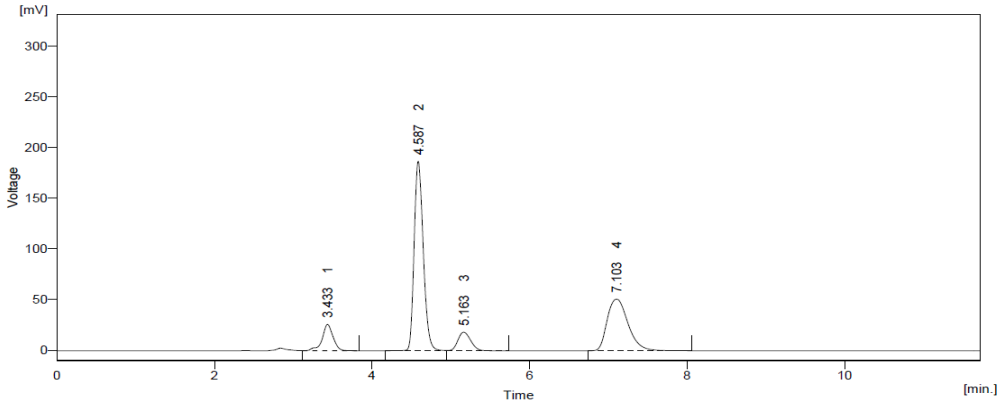

Figure 30: Chromatogram of Ornidazole Oxidation Degradation Standard.

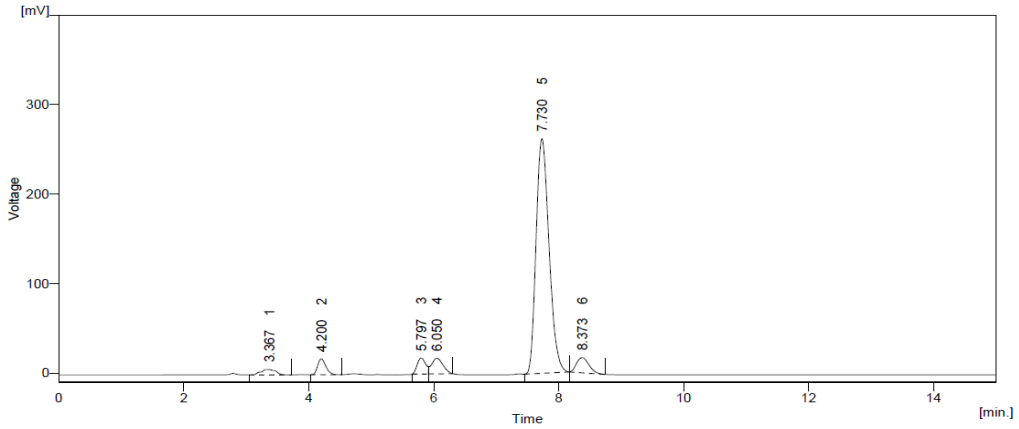

Figure 31: Chromatogram of Miconazole Oxidation Degradation Standard.

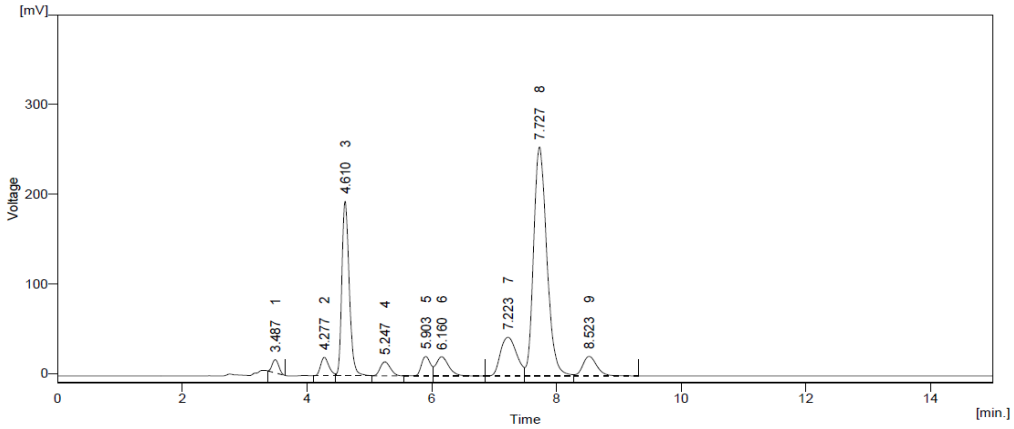

Figure 32: Chromatogram of Miconazole and Ornidazole Oxidation Degradation sample.

4. Thermal degradation

Miconazole Thermal Degradation: Miconzole was taken 100mg in a petri dish and kept it in the oven at 70 ^{0}C for 4hrs, After time period the miconazole was kept out and from this 40mg of miconazole was transferred in 100ml volumetric flask and volume was made up with mobile

phase, From this solution taken 0.1ml and transferred to 10ml volumetric flask to make miconazole 4µg/ml.

Ornidazole Thermal Degradation: Ornidazole was taken 500mg in a petri dish and kept it in the oven at 70 ^{0}C for 4hrs, After time period the ornidazole was kept out and from this 20mg of ornidazole was transferred in 100ml volumetric flask and volume was made up with mobile phase, From this solution taken 1ml and transferred to 10ml volumetric flask to make ornidazole 20µg/ml.

Tablet Thermal Degradation: Tablet powder taken in a petri dish and put it in the oven at 70 ^{0}C for 4hrs, After time period the tablet powderequivalent to 20mg of ornidazole and 4mg of miconazole was transferred in 100ml volumetric flask and volume was made up with mobile phase, from this solution taken 1ml and transferred to 10ml volumetric flask to make ornidazole 20µg/ml and miconazole 4µg/ml.

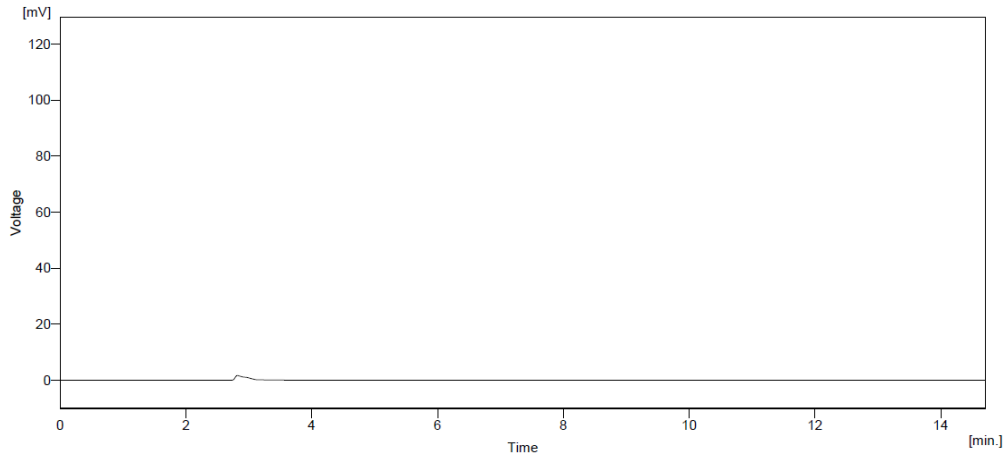

Figure 33: Chromatogram of Themal Degradation Blank.

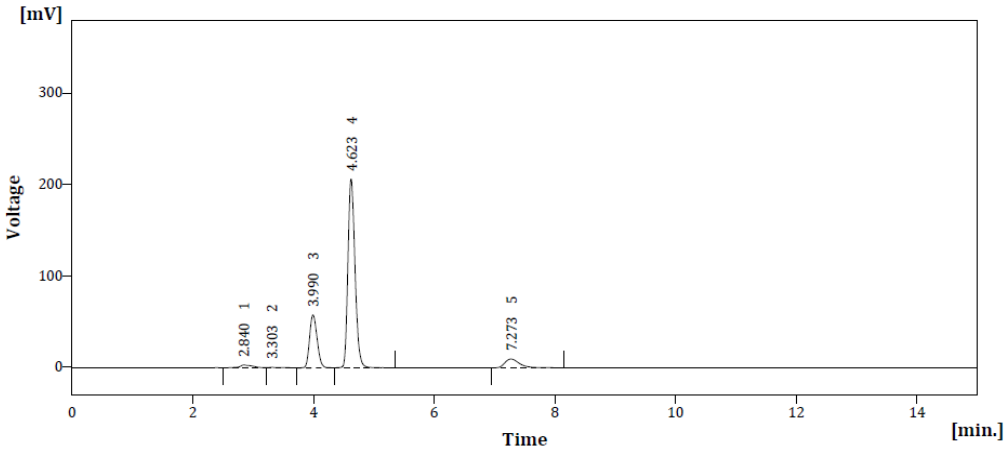

Figure 34: Chromatogram of Ornidazole Thermal Degradation Standard.

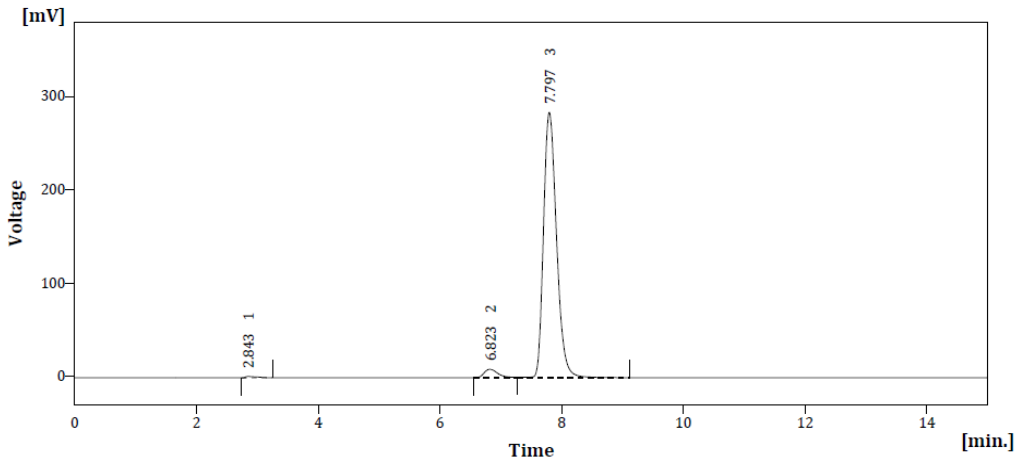

Figure 35: Chromatogram of Miconazole Thermal Degradation Standard.

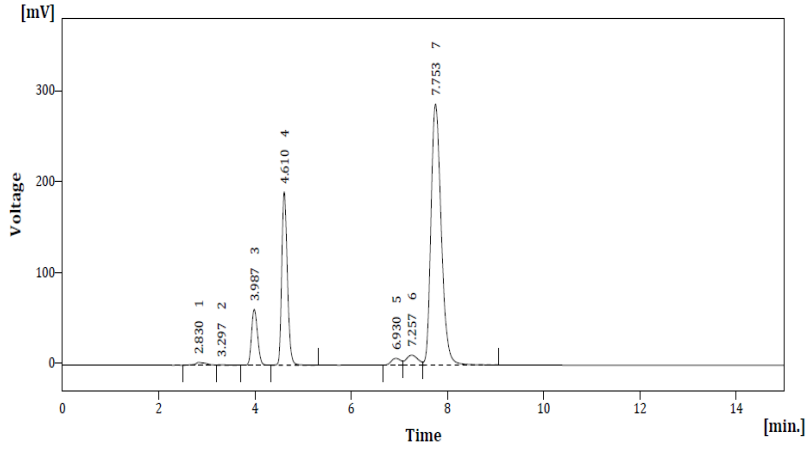

Figure 36: Chromatogram of Miconazole and Ornidazole Thermal Degradation sample.

5. Photo degradation

Miconazole Photo Degradation: Photo Degradationstudies were performed by taken 100mg of miconazole in petri dish and kept it in the UV chamber for 48 hours. After the time period the 40mgmiconazole was transferred in 100ml volumetric flask and volume was made up with mobilephase, from this solution taken 0.1ml and transferred to 10ml volumetric flask to make miconazole 4µg/ml.

OrnidazolePhotoDegradation: Taken 500mg of ornidazole in a petri dish and kept it in the UV chamber for 48 hours, after time period the 20mgornidazole was transferred in 100ml volumetric flask and volume was made up with mobile phase, from this solution taken 1ml and transferred to 10ml volumetric flask to make ornidazole 20µg/ml.

Tablet PhotoDegradation: Tablet Powder taken in a petri dish and kept it in the UV chamber for 48 hours, after time period the tablet powder equivalent to 20mg of ornidazole and 4mg of miconazole was transferred in 100ml volumetric flask and volume was made up with mobile phase, from this solution taken 1ml and transferred to 10ml volumetric flask to make ornidazole 20µg/ml and miconazole 4µg/ml.

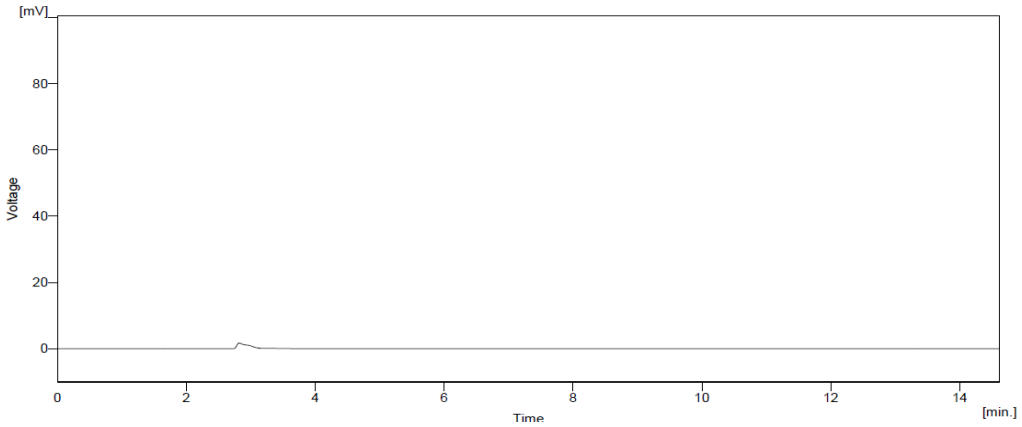

Figure 37: Chromatogram of Photo Degradation Blank.

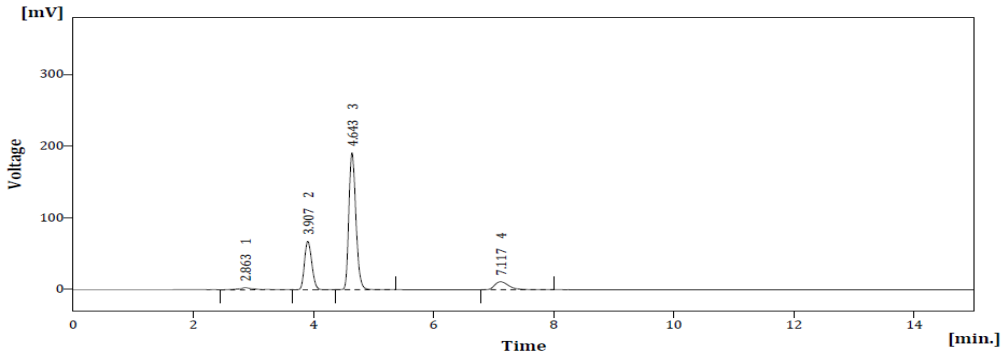

Figure 38: Chromatogram of Ornidazole Photo Degradation Standard.

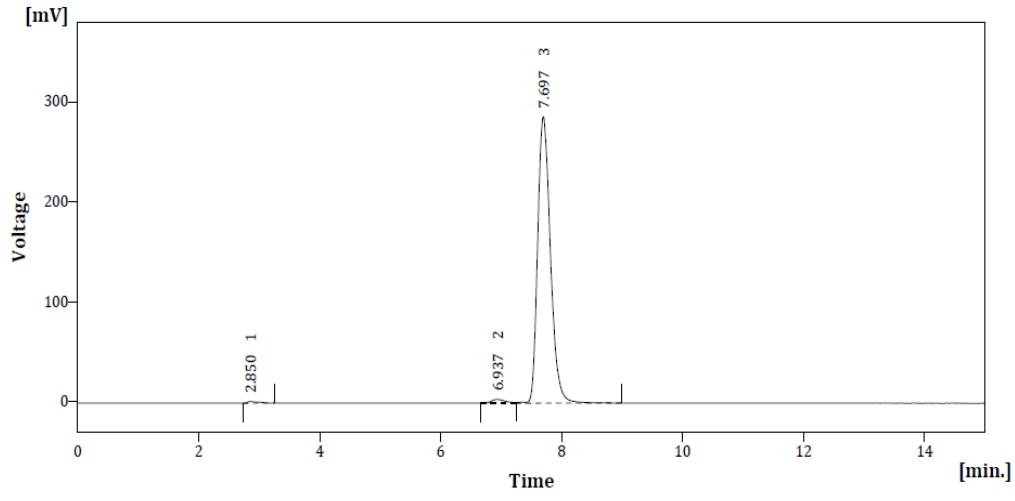

Figure 39: Chromatogram of Miconazole Photo Degradation Standard.

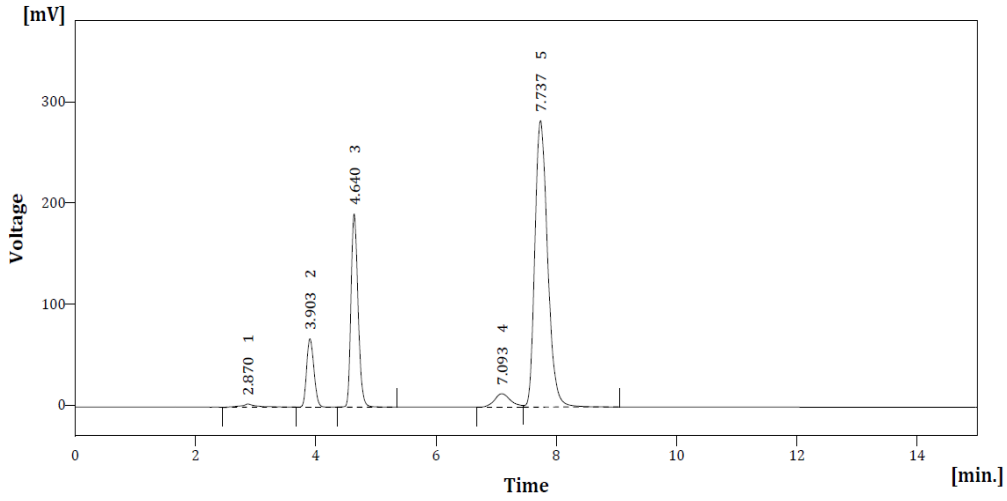

Figure 40: Chromatogram of Miconazole and Ornidazole Photo Degradation sample.

Calculation for Stability

Table 4: Miconazole and Ornidazolestd for stability.

Drugs	Area
Miconazole	4556.680
Ornidazole	1972.384

Table 5: Miconazole % Degradation.

Miconazole				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	3985.55	12.53	3824.52	16.07
Base	4009.62	12.01	4055.90	10.99
Thermal	3924.74	13.86	3948.53	13.35
Oxidation	3772.82	17.20	3772.95	17.20
Photo	3802.51	16.55	3773.79	17.18

Table 6: Ornidazole % Degradation.

Ornidazole				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**	**Area**	**%Degradation**
Acid	1526.70	22.60	1485.16	24.70
Base	1447.20	26.63	1508.77	23.51
Thermal	1684.41	14.60	1548.44	21.49
Oxidation	1510.93	23.40	1595.94	19.09
Photo	1567.57	20.53	1561.88	20.79

Method validation

1. Specificity

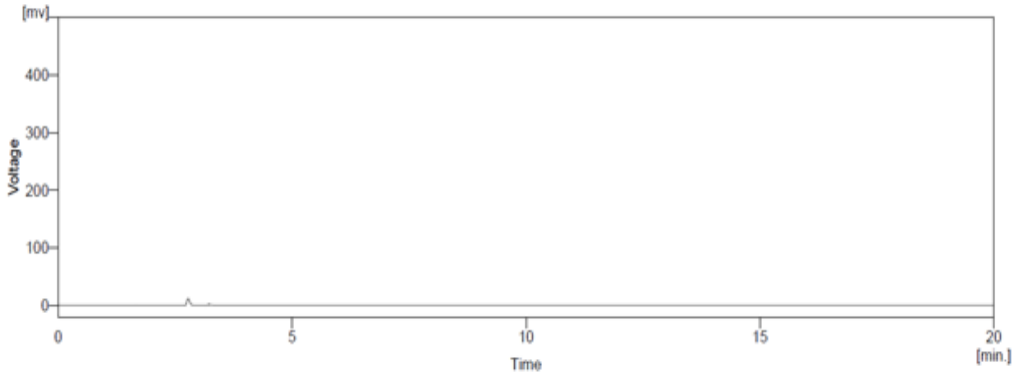

Figure 41: Chromatogram of Ornidazole and Miconazole blank.

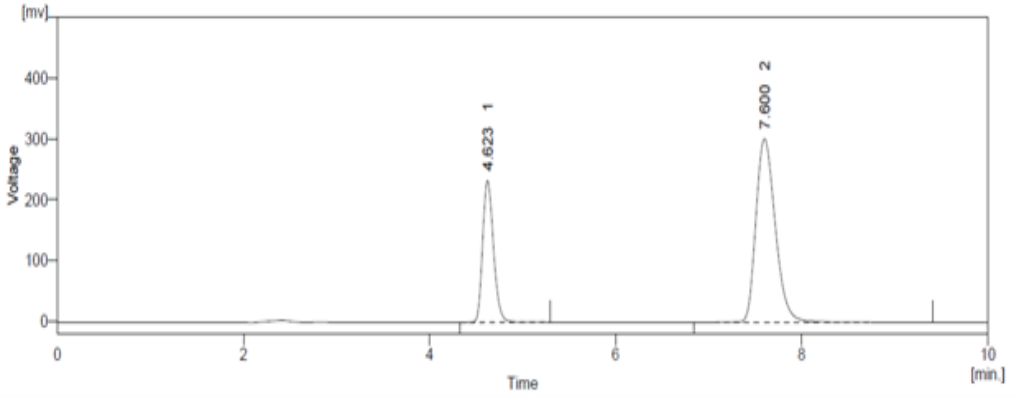

Figure 42: Chromatogram of Ornidazole and Miconazole std.

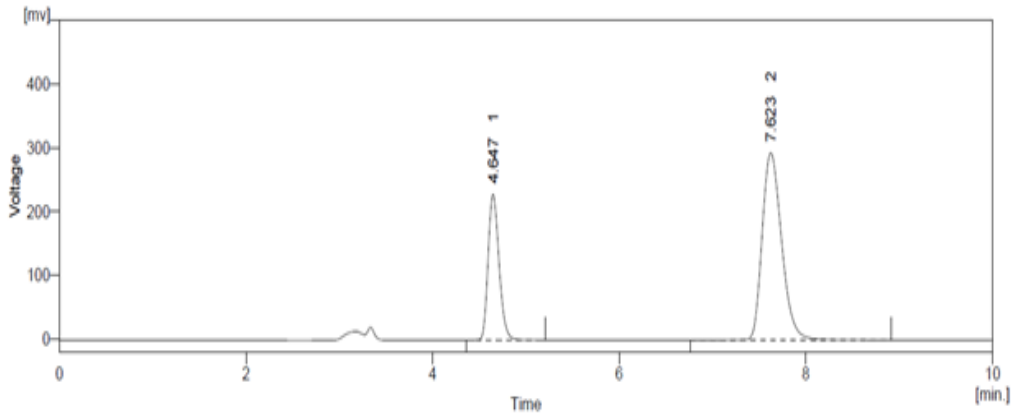

Figure 43: Chromatogram of Ornidazole and Miconazole sample.

The Chromatograms of ornidazole and miconazole standards and ornidazole and miconazole sample show no interference with the chromatogram of ornidazole and miconazole blank, so the developed method is specific.

2. Linearity and Range

The linearity for Miconazole and Ornidazole were assessed by analysis of combined standard solution in range of 2-6 µg/ml and 10-30 µg/ml respectively. Correlation co-efficient for calibration curve Miconazole and Ornidazolewas found to be 0.999 and 0.999 Respectively. The regression line equation For Miconazole: y = 94.49x + 7.213

1095x - 0.293 and For Ornidazole: y = 1095x - 0.293

Table 7: Linearity data for Miconazole

Sr.No	Concentration(µg/ml)	Area
1	2	2213.258
2	3	3247.411
3	4	4389.165
4	5	5500.228
5	6	6566.309

Table 8: Linearity data for Ornidazole.

Sr.No	Concentration(µg/ml)	Area
1	10	958.938
2	15	1408.078
3	20	1902.745
4	25	2380.279
5	30	2835.108

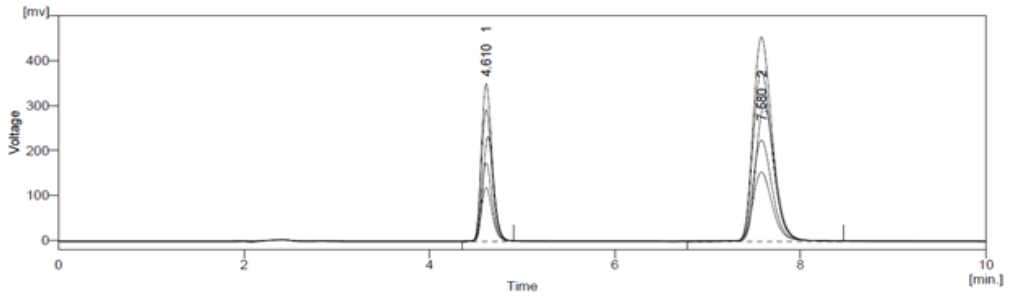

Figure 44: Overlay chromatogram of different concentrations of binary mixtures of Ornidazole and Miconazole.

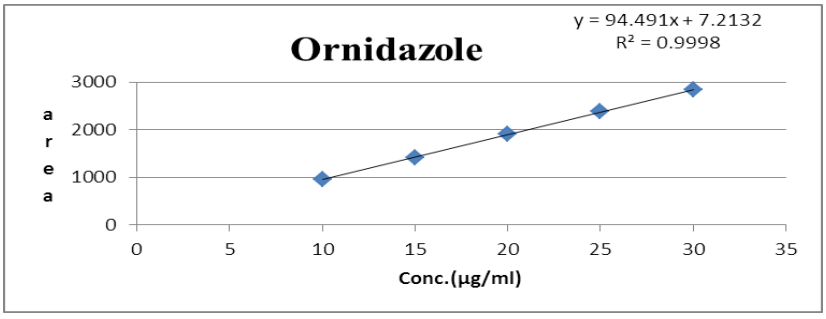

Figure 45: Calibration Curve of Ornidazole(10-30µg/ml).

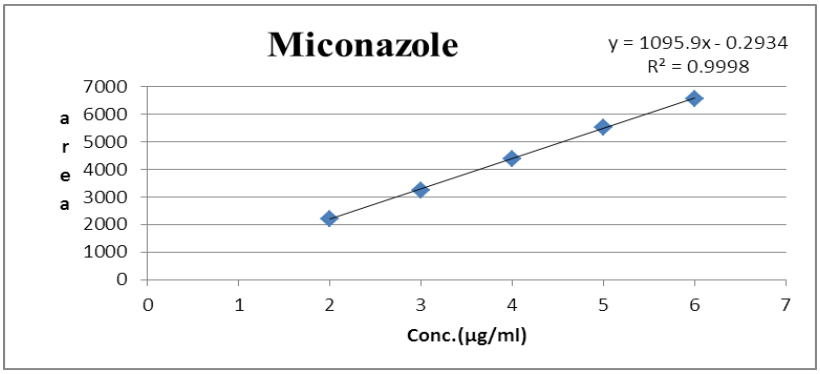

Figure 46: Calibration Curve of Miconazole (2-6µg/ml).

3. Precision

1. Repeatability

The data for repeatability of peak area measurement for ornidazole and miconazole, based on six measurements of same solution of ornidazole and miconazole are depicted in table 6.9 and 6.10 The % RSD for ornidazole and miconazole was found to be 1.047 and 1.025 respectively.

Table 9: Repeatability data for Miconazole.

Miconazole				
Sr No.	**Conc.(µg/ml).**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	4	4455.173	4524.246±46.378	1.025
		4513.279		
		4572.287		
		4581.162		
		4510.149		
		4513.426		

Table 10: Repeatability data for Ornidazole.

Ornidazole				
Sr No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	20	1931.397	1967.800 ±20.601	1.047
		1956.649		
		1982.221		
		1986.177		
		1978.174		
		1972.183		

2. Intraday precision

The data for intraday precision for Ornidazole and Miconazole is shown in table 6.11. The % R.S.D. for Intraday precision was found to be 0.355-0.720. for Miconazole and 0.210-0.503 for Ornidazole.

Table 11: Intraday precision data for estimation of Ornidazole and Miconazole.

	Miconazole			Ornidazole		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	2	2222.284 ± 7.899	0.355	10	964.060± 4.850	0.503
2	4	4373.692± 31.499	0.720	20	1901.293± 3.988	0.210
3	6	6626.107± 32.590	0.492	30	2868.606± 11.677	0.407

3. Interday precision

The data for intraday precision for Ornidazole and Miconazole is shown in table 6.12. The % R.S.D. for interday precision was found to be 0.893-1.753 for Miconazole and 0.706-1.055 for Ornidazole.

Table 12: Interday precision data for estimation of Ornidazole and Miconazole.

	Miconazole			Ornidazole		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	2	2197.614±38.513	1.753	10	956.349±10.089	1.055
2	4	4394.436±54.215	1.234	20	1910.88±13.485	0.706
3	6	6621.246±59.156	0.893	30	2869.411±25.619	0.893

4. LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where,

SD = Standard deviation of intercepts

Limit of Detection

Table 13: Limit of Detection data for Miconazole and Ornidazole.

Miconazole	Ornidazole
LOD = 3.3 x (SD / Slope) = 3.3 x (29.588/1095) = 0.089 µg/ml	LOD = 3.3 x (SD / Slope) = 3.3 x (13.095/94.49) = 0.457 µg/ml

Limit of Quantification

Table 14: Limit of Quantification data for Miconazole and Ornidazole.

Miconazole	Ornidazole
LOQ = 10 x (SD / Slope) = 10 x (29.588/1095) = 0.270 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (13.095/94.49) = 1.386 µg/ml

5. Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 6.13 and 6.14. Percentage recovery for Miconazole was 100.900-101.458%, while for Ornidazole, it was found to be in range of 100.365-100.837%.

Table 15: Recovery data for Miconazole.

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	2	1.6	1.599	99.949	100.837± 1.020
2		2	1.6	1.610	100.610	
3		2	1.6	1.631	101.952	
4	100 %	2	2	2.027	101.357	101.365 ± 0.499
5		2	2	2.037	101.868	
6		2	2	2.017	100.871	
7	120 %	2	2.4	2.423	100.971	100.566 ± 0.669
8		2	2.4	2.395	99.795	
9		2	2.4	2.422	100.933	

Table 16: Recovery data for Ornidazole.

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	10	8	8.078	100.981	100.900 ± 0.858
2		10	8	8.137	101.716	
3		10	8	8.000	100.005	
4	100 %	10	10	10.115	101.154	101.458 ± 0.264
5		10	10	10.159	101.588	
6		10	10	10.163	101.631	
7	120 %	10	12	12.095	100.795	101.119 ± 0.405
8		10	12	12.119	100.988	
9		10	12	12.189	101.573	

6. Robustness

The effect of changes was found to be within the acceptance criteria as shown in table 6.17 and table 6.18**.**The % RSD should be less than 2%.

Table 17: Robustness data for Ornidazole Standard.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	2101.021	1730.423	2117.848	1742.516	2128.385	1746.755
2	2095.276	1742.601	2141.178	1765.224	2147.520	1741.008
3	2117.829	1759.198	2136.900	1750.734	2158.256	1749.384
% R.S.D	0.557	0.828	0.583	0.656	0.706	0.245

Table 18: Robustness data for Miconazole Standard.

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	4845.413	3989.822	4884.326	4017.779	4908.634	4021.760
2	4808.714	4017.775	4938.065	4070.195	4952.731	4001.271
3	4884.146	4066.037	4882.869	4041.699	4977.577	4033.556
% R.S.D	0.778	0.958	0.642	0.649	0.706	0.406

7. Analysis of marketed formulation by developed method.

Applicability of the proposed method was tested by analyzing the commercially available Tablet formulation Candifem. The results are shown in table 6.19.

Table 19: Analysis of marketed formulation.

Tablet	Lable value		Assay (% of label claim*) Mean ± S. D.	
	Miconazole	Ornidazole	% Miconazole	% Ornidazole
Candifem	100mg	500mg	98.975±0.808 2.218124 2.218124 ± 1.5289	99.901 ± 0.763

The assay results were comparable to labeled value of each drug in Tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

DISCUSSION

Miconazole is an imidazole antifungal agent that is used topically and by intravenous infusion.

Ornidazole is a drug that cures some protozoan infections. It has been investigated for use in Crohn's disease after bowel resection RP-HPLC method was developed for simultaneous estimation Ornidazole and Miconazole. In RP-HPLC method, good resolution and separation

of two drugs was achieved. Water: Acetonitrile: Acetic acid (30:70:0.1) was used as mobile phase. Retention time of Ornidazole and Miconazole were found to be 4.640 and 7.633min respectively with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Ornidazole and Miconazole in tablets. Forced degradation study of Ornidazole and Miconazole was performed by RP-HPLC method which includes Acid, Base, Oxidative, Photo and Thermal degradation. Results of degradation were found within limit.

CONCLUSION

The above developed stability indicating RP-HPLC method was validated in terms of accuracy, precision, linearity, robustness and reproducibility. This method was found to be simple, rapid, accurate, robust. Precise and reproducible. This method can be applied for routine quantitative analysis of miconazole and ornidazole.

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