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Development and Validation of Analytical Method for Estimation of Antifungal Drug in Solid Dosage form by HPLC

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Abstract: *A simple, accurate, precise, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of antifungal drug in solid dosage form. The chromatographic separation was achieved using a C18 column with an appropriate mobile phase composition under optimized chromatographic conditions. The mobile phase was pumped at a constant flow rate and the analyte was detected using a UV detector at a suitable wavelength. The retention time of the drug was found to be satisfactory with good peak symmetry and resolution.*

The developed method was validated according to International Council for Harmonisation guidelines for various analytical parameters including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method exhibited excellent linearity over the selected concentration range with a correlation coefficient close to 1. Accuracy studies showed satisfactory percentage recovery, while precision studies demonstrated low percentage relative standard deviation (%RSD), indicating reproducibility of the method. The robustness study confirmed that small deliberate changes in chromatographic conditions did not significantly affect the analytical performance. The proposed RP-HPLC method was found to be simple, rapid, economical, and reliable for routine quantitative analysis of the antifungal drug in pharmaceutical solid dosage forms. Hence, the method can be successfully applied for quality control and stability testing in pharmaceutical industries and research laboratories..

Keywords: *RP-HPLC*

I. INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. Analytical Chemistry plays an important role in the resolution of a chemical compound into its proximate or ultimate parts, determination of its elements or of the foreign substances it may contain. Its application extends to all parts of an industrial society.1-6

HISTORY OF ANALYTICAL CHEMISTRY

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the world around us. The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860. Most of the major developments in analytical chemistry took place after 1900. During late 20th century analytical chemistry found wide application in forensic, environmental, industrial and medical field.

Importance of Analytical Chemistry:

1) It finds numerous applications in various disciplines of chemistry.

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2) It finds wide applications in other fields of related sciences.
Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.
1. Qualitative analysis deals with the identification of elements, ions or compounds present in the sample.

A. Quantitative analysis

Quantitative analytical measurement plays a vital role in many research areas in chemistry, biochemistry, biology, geology and other sciences. It deals with the determination of how much amount of one or more constituents are present in the sample.1-6

METHOD DEVELOPMENT

Method development is a challenging and time-consuming process requiring much experience, creativity, logical thinking, and experimentation. With all the software and automated systems available today, method development is still very much a trial-and-error approach, expedited by a logical sequence of generic scouting runs and fine-tuning steps to achieve the requisite resolution and method performance.7

EXPERIMENTAL WORK

Material and instruments:

Materials:

The drugs used for the present investigation were obtained from Arrow Chem Mumbai.

Details of Pure drug:

Table No. 8: Details of API

Drug	Supplied By	Quantity	Purity (Assay)
Itraconazole	Arrow Chem Mumbai.	10 G	99.8 % W/W

Table No. 9: Details of marketed Preparation

Brand Name	Mfd By	Content	Quantity
Itaspor Capsule	Intas Pharmaceutica	Itraconazole 100mg	10 tablets

The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

Reagents and chemicals:

All reagents and chemicals used were of AR grade and HPLC grade.
1. Methanol (HPLC grade).
2. Acetonitrile (HPLC grade)
3. Disodium hydrogen phosphate (AR grade).
4. Distilled Water (HPLC grade).
5. Triethylamine (HPLC grade).
6. Ortho Phosphoric Acid (HPLC grade).

Instruments:

Table No. 10: Instruments Used

Sr. No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Waters 600	996 PDA Detector

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3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	1.5 L 50

Study of Functional Group by Using Infra Red Spectroscopy: Determination of wavelength maxima

An accurately weighed quantity of itraconazole (10 mg) was transferred into a 100 mL volumetric flask and dissolved in methanol. The volume was made up to the mark with methanol to obtain a stock solution of 100 µg/mL.

Further dilution was carried out to obtain a working solution of 10 µg/mL.

The solution was scanned in the UV range of 200–400 nm using methanol as blank. The maximum absorbance (λmax) of itraconazole was found to be approximately 264 nm, which was selected for further analysis.

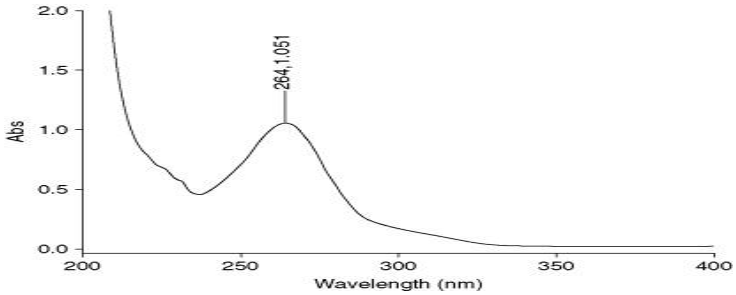

Fig. No. 8: Wavelength Maxima for Itraconazole.

Development of HPLC method for estimation of Itraconazole

Method Development Strategy:

Selection of Common Solvent (Diluents)

Methanol was selected as the common solvent for preparation of standard and sample solutions based on its good solubility for itraconazole. Further dilutions were carried out using the mobile phase.

Preparation of standard stock solution:

Accurately weighted 100 mg Itraconazole was dissolved in 100ml Methanol. This solution was used as standard stock solution.

Preparation of diluent:

Methanol of HPLC grade were selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

Procedure:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containing ITR was injected in different combinations of solvents, to get a stable peak with good peak characters. Each solution was filtered through Membrane filter (size 0.15µ). To achieve peaks with good symmetry various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Chromatographic Parameters:

Column:	C18 (Phenomenex) /4.6 x 250 mm
Flow Rate: Wavelength: Injection volume:	1.0ml/ min 264 nm 20µl
Column oven Temperature: Run Time:	Ambient (250C) 10 minutes
Mobile Phase:	ACN : Water (70:30 % v/v)

volumetric flask and makeup the volume upto the mark with HPLC water.

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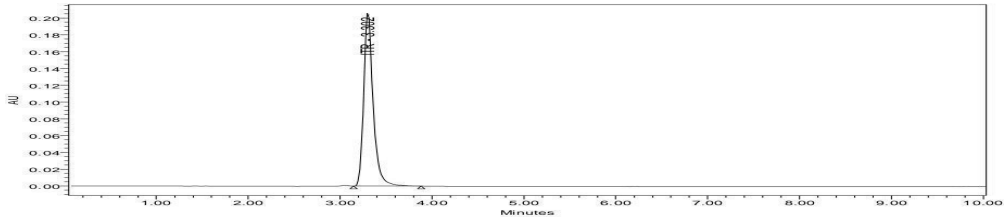

Fig. No. 9: Separation of ITR in selected mobile phase showing retention time at 3.30 min.

System suitability studies

System suitability is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from 5 replicate injections of standard solutions.

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. Standard working solution of ITR was injected five times under optimized chromatographic conditions. System suitability parameters were recorded and reported.

B) Procedure:

Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20 □L std. drug solution was injected which was made in five replicates and the system suitability parameters were recorded.

Table No. 11: Result of System suitability test

Sr. No	Peak area ITR	Retention Time ITR	Symmetry ITR	No. of theoretical Plates ITR
1	149820	3.32	1.20	7450
2	150212	3.45	1.10	7520
3	148950	3.28	1.25	7605
4	150780	3.50	1.00	7552
5	149600	3.40	1.20	7485
Mean	149872	3.39	1.14	7522
S. D	683.16	0.09	0.1	57.64
%R.S.D.	0.46	2.67	8.44	0.68

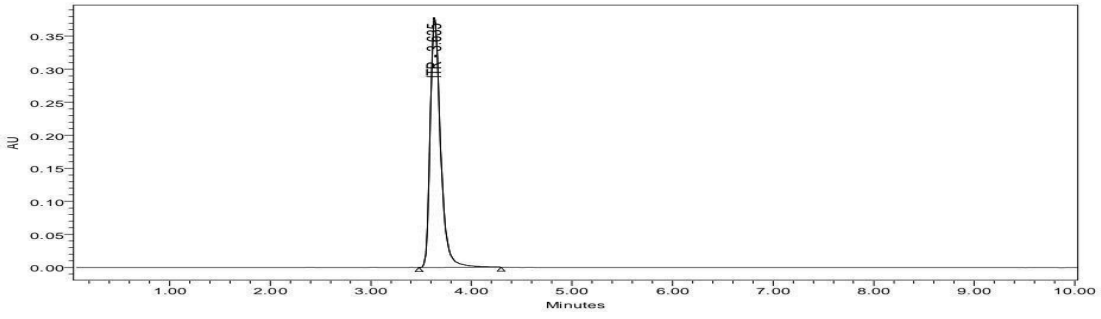

Fig. No 10: Separation of ITR in selected mobile phase showing retention time at 3.63 min.

Application of proposed method for estimation of ITR in Solid Dosage: Standard stock solution:

a) Preparation of standard solutions:

Itraconazole Tartrate standard stock solution: Accurately weighed quantity of 100 mg ITR was dissolved in Methanol and volume was made up to 100 ml mark by same to obtain 1000 µg/ml stock solution.

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Itraconazole standard working solution: Pipette out 1 ml from standard stock solution and dilute it with 10 ml methanol to obtain 100 µg/ml of ITR.

Sample solution preparation:

Entire content of Istapor (100mg) was transferred to a 100 ml volumetric flask, the volume was made upto the mark with methanol, the resultant concentration was 1000 µg/ml. The whole content was centrifuged at 5000 rpm for 10 min followed by passing through 0.45 µ membrane filter. 1 ml of resultant was transferred to a 10 ml volumetric flask and the volume was made upto the mark with methanol, the concentration of working sample solution was 100 µg/ml.Procedure:

Equal volume (20□L) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response

i.e. peak area of major peaks were measured. The content of ITR was calculated by comparing a sample peak with that of standard.

Amount of drug in tablet was calculated using following formula-

$$\% \text{ Estimation} = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times 100$$

Where,

At = Area count for sample solution.

As = Area count for standard solution.

Ds = Dilution factor for standard.

Dt = Dilution factor for sample.

Ws = Weight of standard (mg)

Wt = Weight of sample (mg)

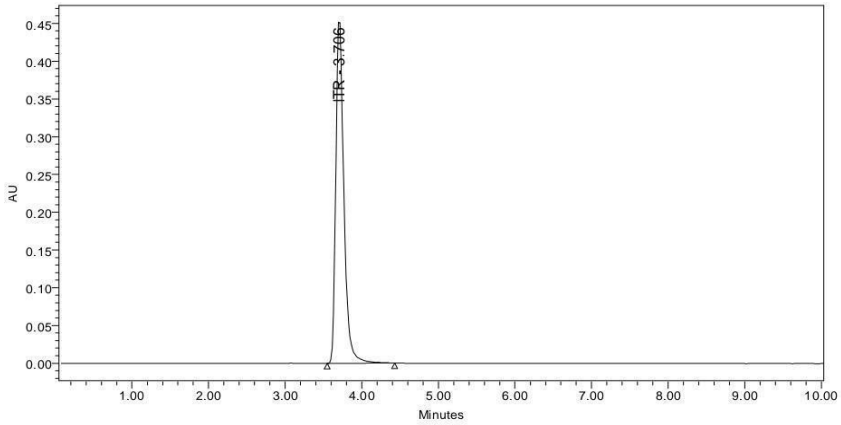

Fig. No.11: Chromatogram of ITR marketed formulation showing retention time 3.70 min.

Validation parameters:

1. Accuracy
2. Robustness
3. Linearity and range
4. Specificity
5. Placebo Interference study

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Accuracy:

The accuracy samples were prepared by spiking the standard into the pre- analyzed formulation sample at different concentrations (80%,100% and 120%) and injected each in triplicate. The resultant mix was injected and recovery of standard spiked was calculated.

The % Recovery was then calculated by using formula

% Recovery = A-B x 100/ C

Where- A = Total amount of drug estimated.

B = Amount of drug found on pre analyzed basis.

C = Amount of pure drug added.

Calculate the amount recovered, % recovery, average recovery, % RSD of triplicate sample preparation, overall recovery and overall % RSD. Record the observation into the following table.

Table No 12: Accuracy studies by standard addition method

	ITR		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt recovered (µg/ml)	79.44	99.31	119.20
	80.15	100.24	120.36
	79.88	99.85	119.20
% Recovery	98.33	99.33	99.44
	98.33	98.66	98.88
	99.16	99.33	100.00
Mean % recovery	98.60	99.10	99.44
% RSD	0.48	0.39	0.56

Acceptance criteria:

1) The % RSD for the triplicate at each spike level shall be NMT 2.0.

2) The overall % RSD for % recovery for all spike levels shall be NMT 2.0.

3) The % recovery at each spike level shall be NLT 98.0 and NMT 102.0 of the added amount.

Robustness:

Effect of Variation in flow rate of mobile phase by ± 10%:

Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system at –10% flow rate (0.9mL/min) and +10% flow rate (1.1mL/min) when compared with the Test method flow rate.

Procedure: Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measured the peak response for the major peaks.

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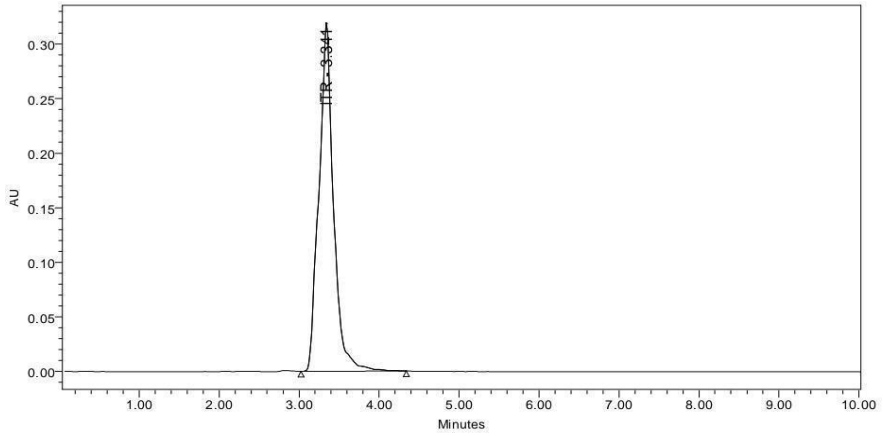
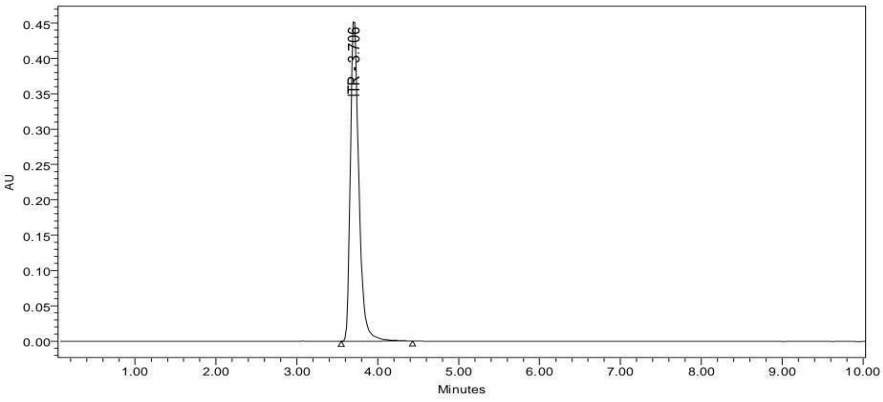


Flow 0.9 ml/min



1.1 ml/min

Fig. No. 12: Chromatograms of Change in Flow Rate

Checked the system suitability and recorded the results in the table.

Table No. 13: System suitability of change in Flow Rate

Sr. No.	System Suitability parameter		Observations for flow rate			Limits
			Unchanged	0.9 ml	1.1 ml	
1	The % RSD of peak area response for five replicate injections	ITR	1.027	0.92	0.85	NMT 2.0
2	Theoretical plates	ITR	7813.50	7870.30	7892.20	NLT 2000
3	Tailing factor	ITR	1.28	1.91	1.10	NMT 2.0
4	Retention Time (Min)	ITR	3.54	3.34	3.70	

Observation: The allowable variation in flow rate of the method is from 0.9ml/min to 1.1 ml/min

Acceptance criteria: All the system suitability parameters shall pass

Change in organic composition of mobile phase + 10% (ACN:Water) System suitability dilution was prepared and injected into the HPLC system at –10% and + 10 % ACN (Organic phase) compared with the optimized method mobile phase concentration.

Procedure:

Injected standard solution into the HPLC system in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks. Check the system suitability and record the results in the table10% (ACN : Water 60:40)

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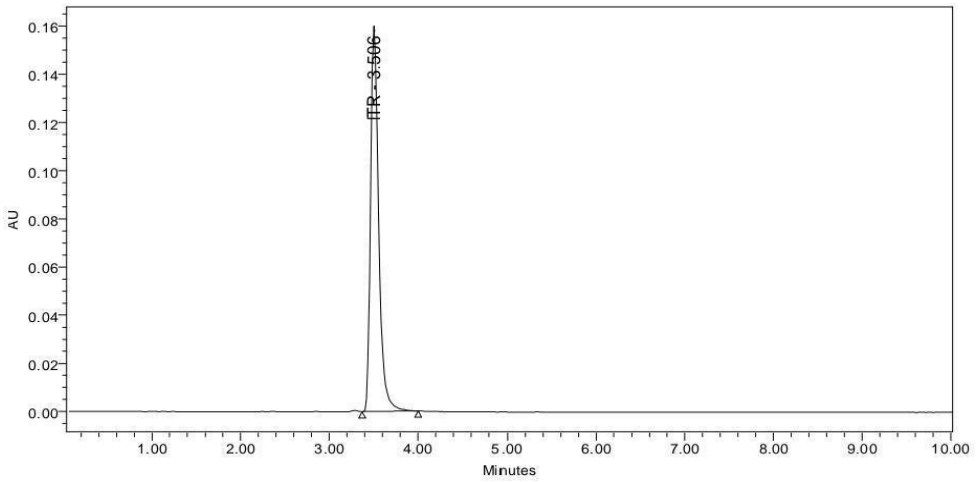

+10% (ACN : Water 80:20)

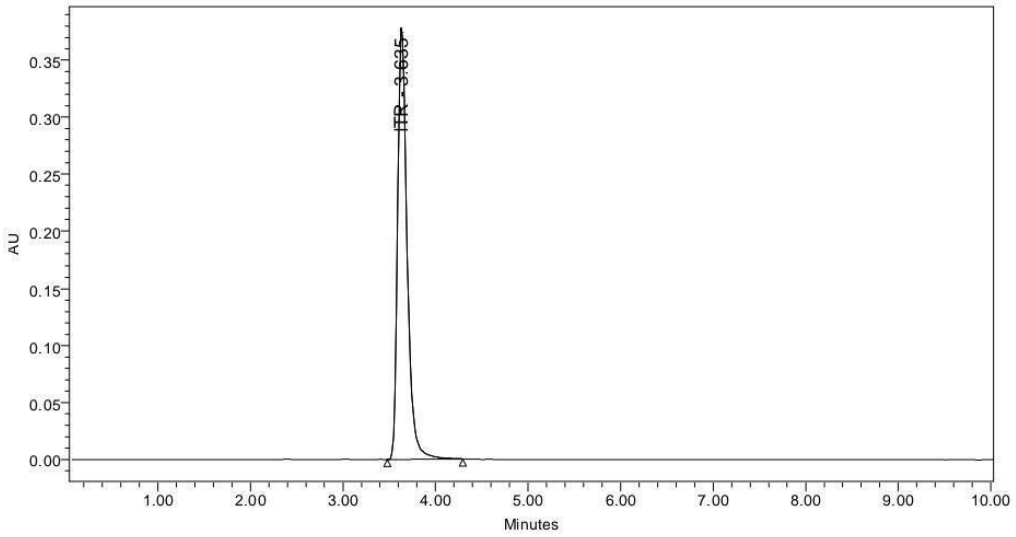

Fig. No. 13: Chromatograms of Change Organic Composition of mobile Phase Table No. 14: System suitability of change in Organic Composition

Sr. No.	System Suitability parameter		Observations			Limits
			Unchanged	- 10%	+ 10%	
1	The % RSD of peak area response for five replicate injections	ITR	1.017	0.655	0.046	NMT 2.0
2	Theoretical plates	ITR	7797.20	7820.30	7850.20	NLT 2000
3	Tailing factor	ITR	1.28	1.166	1.08	NMT 2.0
4	Retention Time (Min)	ITR	3.20	3.50	3.63	

Observation: The allowable variation in ACN composition of method is from 90% to 110%. Acceptance criteria: 1. All the system suitability parameters shall pass.

Effect of Variation in Wavelength by ± 2 units:

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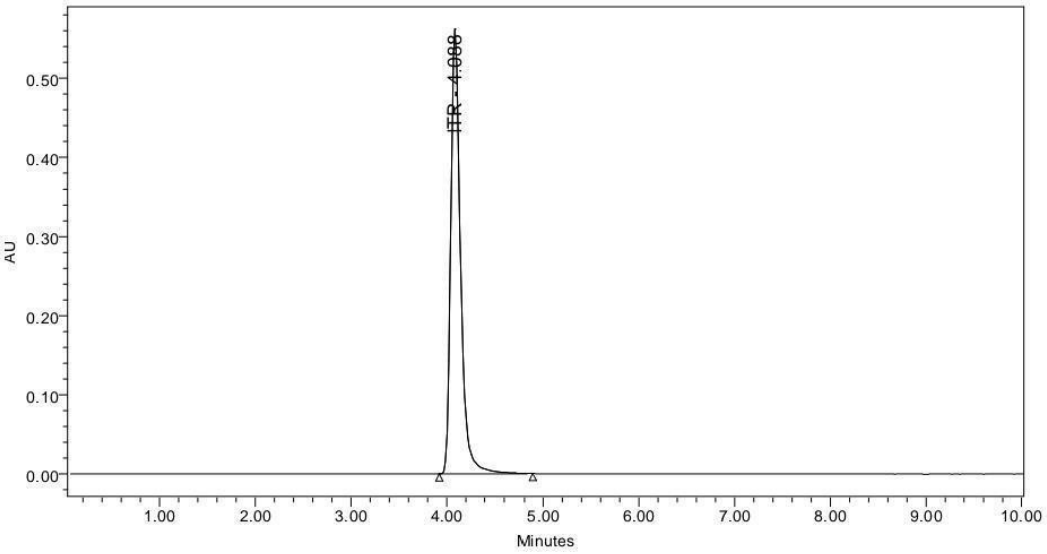
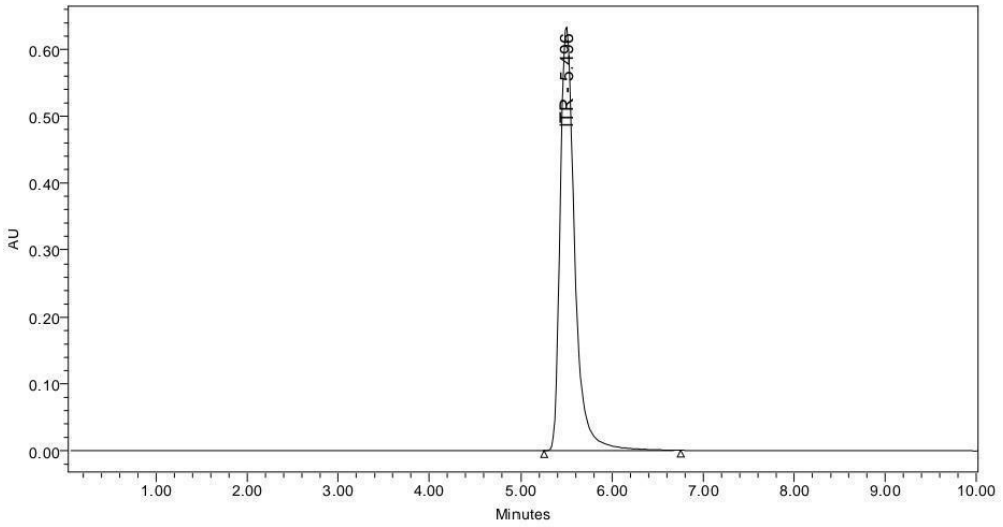
Prepared the system suitability solution (Standard Preparation) and inject into the HPLC system. Measure the peak area response at different wavelengths at flow rate 1 ml/min.

Procedure:

Injected standard solution into the HPLC System in normal conditions and followed by the robust conditions. Measure the peak response for the major peaks.



At 262 nm wavelength



At 266 nm wavelength

Fig. No. 14: Chromatograms of Change in wavelength.

Table No. 15: System suitability of change in wavelength

Sr. No.	System Suitability parameter		Observations for wavelength			Limits
			Unchanged	262nm	266nm	
1	The % RSD of peak area response for five	ITR	1.017	0.3638	0.141	NMT 2.0

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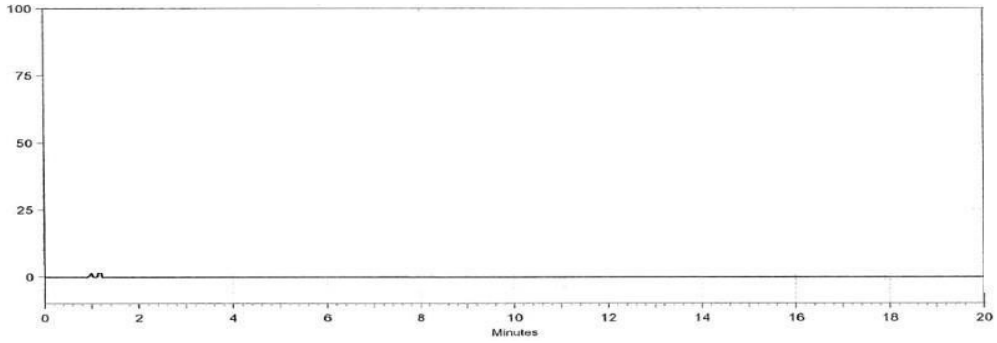
	replicate injections					
2	Theoretical plates	ITR	7735.50	7760.30	7787.20	NLT 2000
3	Tailing factor	ITR	1.06	1.00	0.94	NMT 2.0
4	Retention Time (Min)	ITR	4.08	4.0	5.4	

Specificity:

Placebo Interference study:

Prepared the placebo solution by weighing equivalent amount of placebo present in the sample to be taken for assay preparation in triplicate, diluted it as per the test method and injected into the HPLC system. Evaluate the % interference from placebo and recorded the observation.

Sample matrix



Placebo Preparation

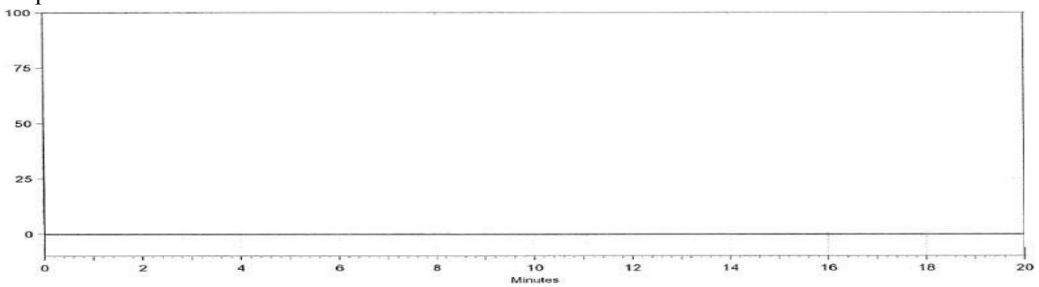


Fig. No. 15: Chromatograms of placebo interference study

Table No. 16 : Placebo Interference Acceptance criteria

Observation	Placebo prep.1	Placebo prep.2	Placebo prep.3
% Interference	No Interference	No Interference	No Interference

No interference should observe from placebo at the retention time of ITR.

Linearity and range:

Prepared the series of standard concentrations ranging from 50 % to 150 % of the targeted concentration of ITR. Each of the linearity dilution was injected into the HPLC system with optimized chromatographic parameters.

Procedure:

Separately inject standard preparation and linearity preparations into the HPLC system, record the chromatograms and measure the peak responses for ITR peaks.

The details of mean peak areas for linearity concentrations are presented in following table and plot the graph of concentration verses average area response for ITR, the correlation coefficient and equation of regression were recorded.

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Table No.17:- Observations of Linearity and range study for ITR

Sr. No.	% Level	ITR	
		Conc. (µg/ml)	Mean peak area
1	50	50	74850
2	80	80	119760
3	100	100	149820
4	120	120	179640
5	150	150	224550

Acceptance criteria: The correlation coefficient shall be NLT 0.99

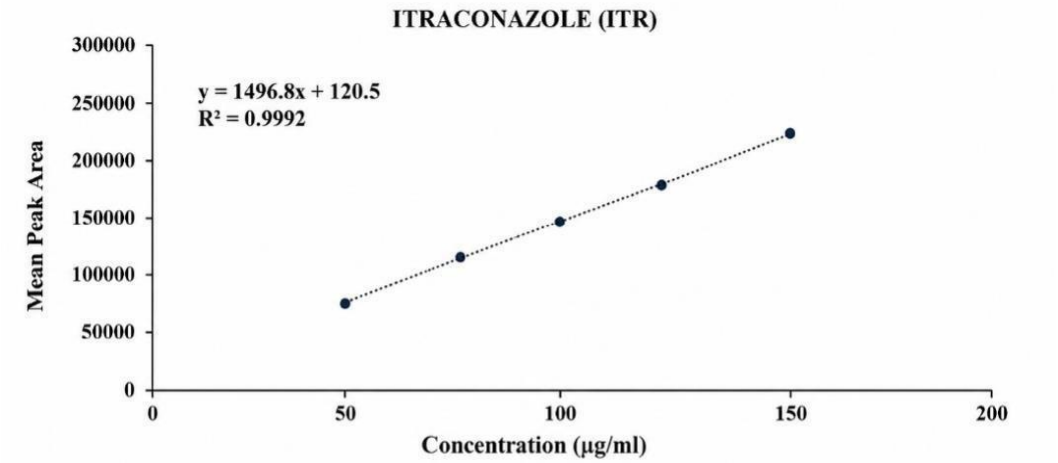

Fig. No. 16: Plot of linearity and range study for ITR

II. RESULT AND DISCUSSION

High Performance Liquid Chromatography, which is a highly sophisticated technique, is widely used for the determination of active pharmaceutical ingredients from their formulations. In the present study, an HPLC method was developed for the analysis of Itraconazole from its solid dosage form.

Itraconazole is a broad-spectrum antifungal drug belonging to the triazole class and is used for the treatment of systemic fungal infections. It is poorly soluble in water, therefore method development requires careful selection of solvent system and chromatographic conditions to obtain a sharp and well-resolved peak. In the present investigation, an attempt has been made to develop a simple, rapid, accurate, and precise HPLC method for estimation of Itraconazole from its formulation.

Pure Itraconazole standard was procured from a certified supplier, and the percentage purity of the drug was as stated by the supplier. The drug sample was prepared by suitable dissolution in a compatible solvent and further diluted with the mobile phase for analysis. The chromatographic separation was carried out using a reversed-phase C18 column, since this stationary phase provides good retention and separation for lipophilic compounds for Itraconazole.

A suitable mobile phase consisting of an organic solvent and buffer system was optimized to achieve proper peak symmetry, minimal tailing, and good resolution within a short retention time. Detection was performed at the selected wavelength corresponding to the maximum absorbance of Itraconazole in UV spectrum. The method was validated for linearity, accuracy, and robustness according to standard analytical requirements. The calibration curve showed good linearity over the selected concentration range, indicating proportional response of peak area with concentration. Accuracy was established through recovery studies, and the recovery values were found to be within acceptable limits. The method was also specific, as no interference was observed from excipients present in the solid formulation.

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Therefore, the developed HPLC method was found to be simple, economical, sensitive, and suitable for routine quality conc. Itraconazole analysis of in pharmaceutical dosage forms.

Table No. 18: Details of API

Drug	Supplied by	Quantity	Purity (Assay)
ITR	Arrow Chem Mumbai.	10 g	99.8 % w/w

These were not analyzed in our study and the % purity stated by the suppliers was taken as standard for comparison studies.

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multi component system. This technique was employed in the present investigation for estimation of ITR in Solid formulation. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

HPLC Column Selected:

HPLC Waters 600 system with C18 (Phenomenex) /4.6 x 250 mm column and PDA detector were used for the study. The standard and sample solution of ITR were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

Mobile Phase selected:

Mobile phase composed of water and ACN (30:70 % v/v). An isocratic program was developed contributing a total run time of 10 min. The wavelength 264 nm was selected for the evaluation of the chromatogram of drugs. The selection of the wavelength was based on the λ max obtained by scanning of standard solution. This system gave good resolution and optimum retention time with appropriate tailing factor (<2). The mean values of system suitability test result are depicted in Table below. The following chromatographic conditions were established by trial and error and were kept constant throughout the method.

Table No. 19: Chromatographic Parameters:

Column	C18 (Phenomenex) /4.6 x 250 mm
Flow Rate	1 ml/min
Wavelength	264 nm
Injection volume	20µl
Column oven Temperature	Ambient
Run Time	10 minutes
Mobile Phase	Mixture of ACN & water in ratio 70:30 % v/v

Mobile phase-preparation

Take HPLC WATER grade 1000 ml in volumetric flask, and Sonicate it around 15 min

Preparation of diluent:

Methanol of HPLC grade were selected as common solvent for preparation of stock solution and developing spectral characteristics of drugs, further dilutions from stock solutions were made in the Mobile phase.

Table No. 20: Summary of system suitability of Test results

	Peak area	Retention Time	Symmetry	No. of theoretical Plates
Sr. No	ITR	ITR	ITR	ITR

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3	148950	3.28	1.25	7605
4	150780	3.50	1.00	7552
5	149600	3.40	1.20	7485
Mean	149872	3.39	1.14	7522
S. D	683.16	0.09	0.1	57.64
%R.S.D.	0.46	2.67	8.44	0.68

Thus the results obtained for such method are given as follow:

After establishing the chromatographic conditions, Mix standard and marketed preparation solutions were prepared and analyzed by procedure described under experimental work. It gave accurate, reliable results and was extended for estimation of drugs in marketed tablet formulation.

Amount of drug in tablet was calculated using following formula:

$$\text{Assay (mg/ml)} = \frac{A}{A_s} \times \frac{D}{D_t} \times \frac{W_s}{W_t} \times \frac{p}{100} \times \text{Wt mg/ml of test sample}$$

% Label claim = Assay (mg/ml) × 100

Label claim in mg/ml

Where,

At = Area count for sample solution. As = Area count for standard solution. Ds = Dilution factor for standard.

Dt = Dilution factor for sample. P = Potency of drug

VALIDATION

Validation of these methods was performed as per the USP guidelines for these following parameters:

Linearity & Range:

The linearity of an analytical procedure is its ability, within a given range, to produce test results that are directly proportional to the concentration of the analyte in the sample. Linearity was established for itraconazole over five concentration levels in the range of 80% to 150% of the target concentration. A graph was plotted with concentration on the X-axis and mean peak area on the Y-axis. The R^2 value was found to be 0.999 for itraconazole. The results showed an excellent correlation between concentration and mean peak area within the selected range. Thus, the developed method can be considered linear, precise, accurate, specific, and suitable for routine quantitative analysis of itraconazole in solid dosage form.

Accuracy:

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results were well within the range 99-101%. Thus the method was found to be accurate.

Table No. 21: Result of Accuracy Studies

	ITR		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amt taken (µg/ml)	80	100	120
	80	100	120
	80	100	120

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Amt recovered (µg/ml)	79.44	99.31	119.20
	80.15	100.24	120.36
	79.88	99.85	119.20
% Recovery	98.33	99.33	99.44
	98.33	98.66	98.88
	99.16	99.33	100.00
Mean % recovery	98.60	99.10	99.44
% RSD	0.48	0.39	0.56

Robustness:

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected for each of the changes made to access the Robustness of proposed analytical method.

Following Parameters were covered under robustness parameter.

1. Effect of variation in flow rate of mobile phase by ± 10%
2. Organic phase composition (± 10%)
3. Change in Wavelength by ± 2 units

The results suggested all the system suitability parameters were within limits.

Specificity :

Is the ability of an analytical method to assess the analyte unequivocally in the presence of excipients, impurities, degradants, and other matrix components. It was evaluated for itraconazole by injecting the blank, placebo, and the control sample solution prepared as per the proposed method to check for any interference at the retention time of itraconazole. Thus, no interference was observed at the retention time of itraconazole, confirming that the developed method is specific for the drug in its formulation.

III. SUMMARY AND CONCLUSION

SUMMARY

Itraconazole is a broad-spectrum antifungal drug used for the treatment of fungal infections, and it is an important pharmaceutical compound in solid dosage form analysis. Literature survey shows that only a limited number of analytical methods have been reported for the estimation of itraconazole, especially by RP-HPLC in Solid dosage forms.

The present study was undertaken with the objective of developing a suitable, sensitive, simple, accurate, and precise analytical RP-HPLC method for the estimation of itraconazole in its pharmaceutical formulation. Since itraconazole is poorly soluble in water and highly lipophilic, careful selection of solvent system, stationary phase, and mobile phase composition is necessary to obtain proper extraction, good peak shape, and satisfactory chromatographic separation.

In the developed RP-HPLC method, the analyte was separated using a mobile phase composed of a suitable mixture of organic solvent and buffer, optimized to achieve good resolution and short retention time. An isocratic program was used, giving a practical and convenient run time for routine analysis. A C18 reversed-phase column was employed because it provides better retention and separation for lipophilic compounds like itraconazole. The detection wavelength was selected based on the UV absorption maximum of itraconazole to ensure maximum sensitivity.

The method was validated in terms of accuracy, linearity and range, robustness, specificity. The results showed that the method was sensitive, reliable, reproducible, rapid, and economical for quantitative analysis of itraconazole in solid dosage form.

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Conclusion

From the results of the study, it can be concluded that the present RP-HPLC technique was successfully used for the estimation of itraconazole in solid dosage form.

The method showed good reproducibility and was found to be accurate, specific, and sensitive. The analysis of itraconazole dosage form was successfully performed by the developed and validated RP-HPLC method.

The RP-HPLC method was also simple, accurate, reproducible, and economical, making it suitable for routine quality control analysis of itraconazole alone in pharmaceutical dosage forms.

No interference of additives or excipients was observed in the method, confirming its specificity for the drug. Further studies on other pharmaceutical formulations and biological samples may provide additional support for wider application of this analytical method.

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