Research Article

Development and Validation of RP-HPLC Method for the Determination of Itraconazole in Bulk and Capsule Dosage Form

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Accepted on: 17-02-2015; **Finalized on:** 31-03-2015.

ABSTRACT

A simple, accurate, precise and reproducible RP-HPLC method was developed for the estimation of Itraconazole in the bulk drug and in capsule dosage form. The objective was achieved under optimized chromatographic conditions on HPLC system with Enable C-18G column (250 × 4.6 mm, 5µm particle size) using mobile phase composed of acetonitrile and glacial acetic acid 0.1% w/v in the ratio of 50:50 v/v. The separation was achieved using an isocratic elution mode with flow rate of 1ml/min at room temperature. The effluent was monitored at 264 nm using UV detector. The retention time of itraconazole was found to be 3.44 minutes and the linear over a concentration range of 10-60 µg/ml with r^2 = 0.996. The developed method was validated as per ICH guide lines. The proposed method was found to be specific, accurate, precise and robust. Hence this method was conveniently and easily applied for routine analysis of Itraconazole in bulk drug and capsule dosage form.

Keywords: Itraconazole, RP-HPLC, ICH guidelines.

INTRODUCTION

Itraconazole[1](ITZ) is antifungal drug. It is a white to almost white powder, chemically4-[4-[4-[4-[[cis-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1RS)-1methylpropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one].with molecular formula $C_{35}H_{38}Cl_2N_8O_4$, molecular weight 706 g/mol and chemical structure was shown in Figure 1. Itraconazole is an orally active triazole antifungal agent, which demonstrates broad spectrum activity against a number of fungal species including dermatophytes, Malassesia furfur, Candida species, Aspergillus species, and Histoplasma capsulatum var. Capsulatum[2]. The mechanism of action of itraconazole relates to its binding of fungal cytochrome P-450 with resultant inhibition of ergosterol synthesis, an essential element of the cell membrane in propagating the growth of fungal and yeast colonies and perturbation of membrane bound enzyme function and membrane permeability[3]. Itraconazole is metabolized[4-6] via CYP3A4 enzymatic system to form primarily three active metabolites viz. hydroxy itraconazole, keto-itraconazole and N-desalkylitraconazole. Itraconazole and its metabolites are potent inhibitors of CYP3A4 isozyme and have been used as a tool to confirm the drug-drug interaction potential of a number of substrates such as simvastatin, lidocaine, tacrolimus, sirolimus etc.

Literature survey revealed that various analytical methods have been reported for the analysis of Itraconazole which include UV spectrophotometric methods[7-8], Visible spectrophotometric method[9], Reverse Phase High Performance Liquid Chromatography[10-15], LCMS[16-18], Ultra Pressure Liquid Chromatography[19], HPTLC[20] methods. The present study describes the development and validation of simple, economical, specific, accurate, precise RP-HPLC method for the determination of Itraconazole in pharmaceutical dosage form.

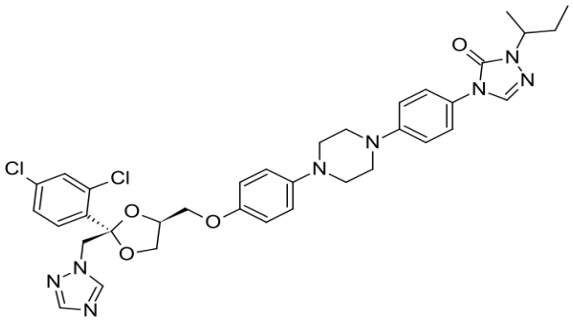

Figure 1: Chemical structure of Itracanazole

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure sample of Itraconazole with purities greater than 99 % was obtained as gift sample from Mylan Laboratories Hyderabad, India and Capsule formulation [SPORANOX] was procured from APOLLO Pharmacy, Visakapatnam, India with labelled amount 100 mg of Itraconazole. Acetonitrile (HPLC grade), water (HPLC grade), acetic acid (AR Grade) and were obtained from Merck India. 0.2 µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Enable Make C18G (250 X

4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "LC solutions" software. An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH) and UV-Visible Spectrophotometer (Elico SL 210, software-Spectral Treats) were used in this study.

Method

Selection of Wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Itraconazole. Suitable wavelength selected was 264 nm.

Chromatographic Conditions

The developed method uses a reverse phase C18 column, Enable Make C18G (250 X 4.6 mm; 5 µ), mobile phase consisting of acetonitrile and 0.1% w/v acetic acid in the proportion of 50:50 v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20 µl for every injection. The detection wavelength was set at 264 nm.

Preparation of 0.1% w/v acetic acid

Accurately measured 0.1 ml acetic acid was transferred into a 100 ml of volumetric flask and volume was made up to the mark with HPLC grade water. The solution was sonicated for 15 min and filtered through 0.2 µm membrane filter.

Preparation of Mobile Phase

The mobile phase was prepared by mixing acetonitrile and 0.1 % w/v acetic acid in the ratio of 50:50 v/v and later it was sonicated for 10 minutes for the removal of air bubbles.

Preparation of working standard solution

10 mg of Itraconazole was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 2 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as working standard solution (100 µg/ml).

Preparation of stock and working sample solution

Ten capsules were weighed separately and the average weight was determined. The capsule content equivalent to 100 mg of itraconazole was transferred to a 100 ml volumetric flask and dissolved in little portion of mobile phase then volume was made up to the mark with mobile phase. The resulting solution was sonicated for 3 minutes, followed by filtration through 0.2 µ nylon membrane filter to get sample stock solution of 1mg/ml. 1 ml of the above stock solution was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of working standard of 100 µg/ml. From this suitable aliquot was prepared and injected. From the calibration curve the concentration was determined.

RESULTS AND DISCUSSION

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Itraconazole at 3.7 min. Figure 2 represents standard solution (100 µg/ml). The total run time is 5 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (*Rt*), number of theoretical plates (*N*) and peak Asymmetric factor were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

In order to test the applicability of the developed method to a commercial formulation, "SPORANOX" was chromatographed at working concentration (100 µg/ml). The sample peak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

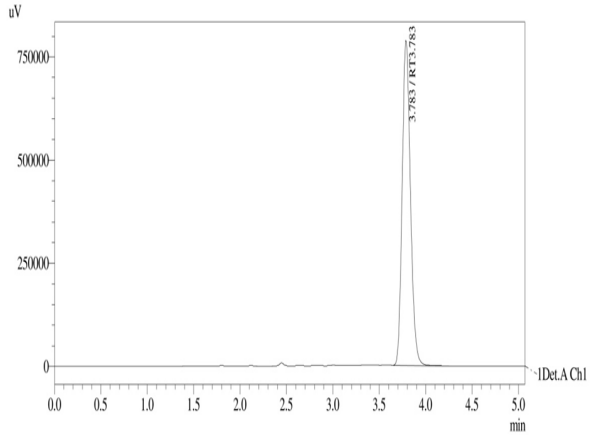

Figure 2: Chromatogram of Standard Itraconazole solution

Table 1: Results from system suitability studies

Property	Values ± SD*	%RSD	Required Limits
Retention time (t_R)	3.78 ± 0.0066	0.26	RSD<1%
Theoretical plates (N)	7491 ± 14.88	0.56	N>2000
Tailing factor (T)	1.231 ± 0.0223	1.421	T<2

*Average of six determinations

Method validation[21]

Validation of the analytical method is the process that establishes by laboratory studies in which the

performance characteristics of the method meet the requirements for the intended analytical application.

RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures.

The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, robustness, and ruggedness, limit of detection (LOD) and limit of quantitation (LOQ).

Specificity

Specificity was checked for the interference of excipients in the analysis of sample solution and was determined by injecting sample solution with added excipients under optimized chromatographic conditions to demonstrate separation of Itraconazole from excipients.

There is no interference of excipient peak on the peak of itraconazole indicating the high specificity of method.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies.

Intra-day Precision

In the intraday studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. %RSD was calculated and was found to be less than 2%.

Inter-day Precision

In the inter-day variation studies, six injections of standard solution were injected at different days. % RSD was calculated and was found to be less than 2%.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%).

At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 2.

The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 2: Results from recovery studies

Sample	Area	Sample amount (µg/ml)	Standard added (µg/ml)	Standard recovered* (µg/ml)	%Recovery ± SD*	%RSD
80%	1174038.4	30	24	23.96	99.83 ± 0.03	0.03
100%	1467548	30	30	29.89	99.63 ± 0.043	0.0431
120%	17610576	30	36	35.92	99.77 ± 0.032	0.032

*Average of three determinations

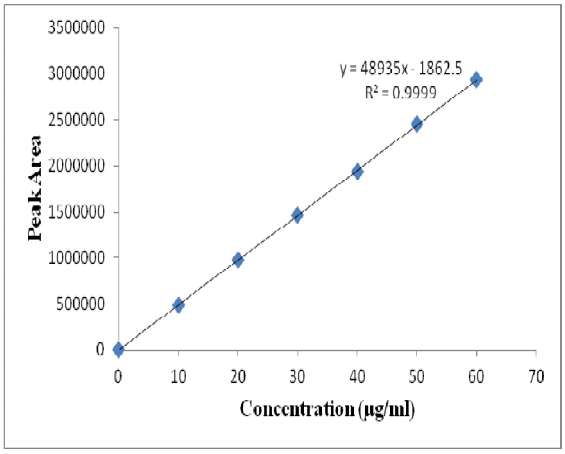

Figure 3: Linearity plot of Itraconazole

Table 3: Characteristic parameters of Itraconazole

Parameters	
Calibration range (µg/ml)	10-60
Detection Wavelength(nm)	264
Mobile phase (Acetonitrile: 0.1% acetic acid) (V/V)	50:50
Regression equation (Y)	48395x-1862.5
Retention Time(min)	3.78
Slope (b)	48395
Intercept (a)	-1862.5
Correlation coefficient (r^2)	0.999
LOD (µg/ml)	0.4389
LOQ (µg/ml)	1.341

Table 4: Robustness studies of Itraconazole

S. No	Condition	Modification	Mean Peak area ± SD*	Mean Rt ± SD*	Mean %RSD (for Peak Area)
1	Flow rate (ml/min)	0.9	1465321±4654	4.213	0.317
		1.1	1398754±4876	3.298	0.347
2	Wavelength (nm)	262	1432876±4321	3.543	0.301
		266	1457642±4562	3.653	0.312

*Average of three determinations

Table 5: Ruggedness studies of Itraconazole

S. No	Injection Number	Analyst-1			Analyst-2		
		Peak Area	Retention time (min)	Theoretical plates (N)	Peak Area	Retention time (min)	Theoretical plates (N)
1	1	1465324	3.753	7453	1468761	3.721	7665
2	2	1467854	3.767	7551	1466532	3.738	7442
	AVG	1466589	3.76	7502	1467646	3.729	7603
	SD	1788.9	0.0098	69.29	1576.1	0.012	86.97
	%RSD	0.12	0.26	0.92	0.10	0.32	1.14

Table 6: Assay studies of Itraconazole

Sample	Label Claim (mg)	Standard Area*	Sample Area*	Amount found* (mg)	(%) Recovery ± SD*
SPORANOX	100	1467035	1466811	99.98	99.98± 0.03

*Average of three determinations

Linearity

Standard solutions of Itraconazole at different concentrations were prepared. Calibration curve was constructed by plotting the concentration of drug versus corresponding peak area. The results show an excellent correlation between peak area and concentration of drug within the concentration range (10-60 µg/ml) for the drug and the results are given in Table 3.

The correlation coefficient of Itraconazole is greater than 0.99, which meet the method validation acceptance criteria and hence the method is said to be linear. The linearity plot was shown in Figure 3.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in wave length and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results were shown in Table 4.

Ruggedness

It was checked by determining precision on same instrument, but by a different analyst. Results of reproducibility are shown in Table 5.

Sensitivity

The sensitivity of measurement of Itraconazole by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD).

The limit of detection (LOD) and limit of quantitation (LOQ) was found to be 0.4389 µg/ml and 1.341 µg/ml.

Estimation Of Itraconazole In Pharmaceutical Dosage Form

The proposed method was successfully applied for the estimation of itraconazole in capsules. The assay results was shown in Table 6.

CONCLUSION

A high performance liquid chromatography method for the quantitative estimation of Itraconazole in bulk and capsule dosage form has been developed as per the requirements of present era. The method was validated and found to be applicable for the routine analysis of Itraconazole in capsule dosage forms without interference from the excipients. Statistical results and low % RSD values indicate that the method is precise, accurate, robust, specific, and can be used across a wide range of concentrations.

Considering already proposed methods in literature, advantages of this new proposed method and rapid results (retention time 3.44 mins), quick analysis time (run time 5 mins), economic mobile phase, user friendly and convenient approach. All these key features proposed that this method can be considered as advantageous over other methods.

Acknowledgement: The authors are grateful to Dr. L. Rathaiah, Chairman of Lavu educational society for providing necessary facilities to carry out the above research work.

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Source of Support: Nil, **Conflict of Interest:** None.