Research Article

Development and Validation of RP-HPLC Method for Quantitation of Itraconazole in Tablets Dosage Form

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ABSTRACT

A simple, selective, accurate and precise high performance liquid chromatographic (HPLC) method for estimation of Itraconazole in tablets was developed and validated. The determination was carried out using ODS Hypersil C18 column (250 X 4.6) mm 5µ, with a mobile phase consisting of a mixture of Acetonitrile and 0.2% Triethylamine in isocratic elution. The flow rate was kept at 1.0ml /min and the detection was carried out by UV Detector at 260 nm. The retention time of Itraconazole was about 4 minutes. The method was validated for specificity, limit of detection, limit of quantification, linearity, accuracy, precision and robustness following ICH guidelines. This method permits determination of Itraconazole in tablets with detection limit 0.3 ppm and quantification limit 1.0 ppm. The linear regression analysis data for the calibration curve in the range of 1.0 ppm to 100 ppm showed good linear relationship with coefficient of correlation value, $r^2 = 0.9972$. All the parameters of validation were under the limit as specifiy by the ICH guidelines. Hence, this method is suitable for determination of Itraconazole in pharmaceutical dosage form. The method was found to be specific, linear, precise, accurate, robust and because of these parameter, this method was found effective for routine analysis.

Keywords: HPLC, itraconazole, method development, method validation

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INTODUCTION

Itraconazole invented in 1984, is a triazole antifungal agent prescribed to patients with fungal infections. The drug may be given orally or intravenously [1].

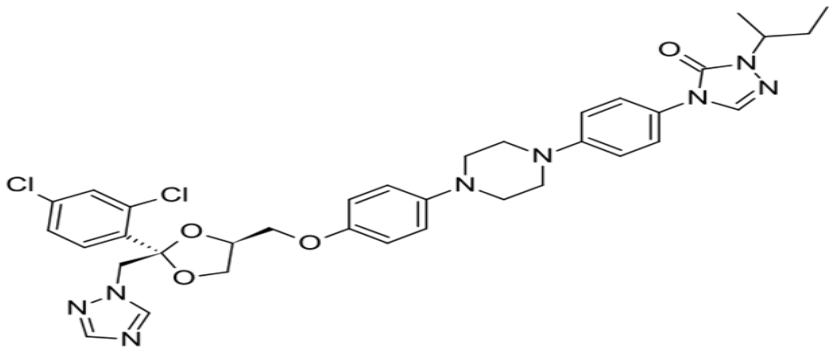

Figure 1: Chemical Structure Itraconazole

Itraconazole has a broader spectrum of activity than fluconazole (but not as broad as voriconazole or posaconazole). In particular, it is active against Aspergillus, which fluconazole is not. It is also licensed for use in blastomycosis, sporotrichosis, histoplasmosis, and onychomycosis [1]. Itraconazole is over 99% protein-bound and has virtually no penetration intocerebrospinal fluid. Therefore, it should never be used to treat meningitis or other central nervous system infections [2].

Itraconazole has also recently been explored as an anticancer agent for patients with basal cell carcinoma, non-small cell lung cancer, and prostate cancer. [2]

I choose this drug for my study purpose because finish form of this drug not mention in pharmacopoeia.

The objective of this mehod development to quantitation itraconazole in tablet formulation in short interval of time at short retention time which was cost effective and time saving. The method was found to be specific, linear, precise, accurate, robust and because of these parameter, this method was found effective for routine analysis.

MATERIALS AND METHOD

Apparatus:

The apparatus was Agilent Technologies 1260 Infinity AT with Quartnery pump with Auto Sampler injector HPLC. C-18 (250x 4.6) mm, 5µm particle size used as a column.

UV-VIS Spectrophotometer Jassco 550.

Chemicals, Reagents and Solvents:

Itraconazole tablets and Itraconazole working reference standard procured from Arvind Remedies Pharmaceutical Ltd.

Triethylamine (GR grade)

Acetonitrile (HPLC grade)

Methanol (GR grade)

Dilute Ortho Phosphoric Acid

Water (MiliQ)

Methods:

Chromatographic conditions

The quantification determination was carried out on a Agilent system equipped with UV detector.The analytical column was ODS Hypersil C-18 (250 ^{x} 4.6mm, 5µ). Mobile phase consisted of Acetonitrile: Buffer (90:10), Buffer was 0.2% solution of triethylamine, whose pH was adjusted 3 with the help of orthophosphoric acid. Mobile phase was mixed, filtered through 0.45µ membrane filter and then degassed using sonication. Mobile phase was also used as diluents. The flow rate was 1.0 ml/min and runtime was 10 minutes. The column was maintained at 45ºC temperature, UV detection was measured at 260nm and 10µl sample was injected [3].

UV-VIS Spectrophotometer Jassco 550, was used to determine maxima of λ for itraconazole.

PREPARATION OF STANDARD STOCK SOLUTION

STANDARD STOCK SOLUTION

Weight accurately 20mg working standard of itraconazole and transfer to 20ml volumetic flask. Add about 5-10 ml of Acetonirile and sonicate it for 20 minutes and then make up the volume with Acetonitrile till mark and filter through 0.45µ Millipore filter.

WORKING STANDARD SOLUTION

Pipette out 0.25ml of stock solution in 10 ml volumetric flask, and make up its volume with the mobile phase till its mark.

PREPARATION OF SAMPLE SOLUTION:

Average weight of twenty tablets was determined. Crush all twenty tablets and pass through 100 no. mesh sieve. A portion of powder was weight containing itraconazole equivalent to 50 mg and transfer to a 50ml volumetric flask add about 20-30 ml of Acetonitrile and sonicate for 20 minutes, then make up the volume with Acetonitirile till mark and filter through 0.45µ Millipore filter. After that take 0.25 ml of the prepared solution in 10 ml volumetric flask, the make up its volume till its mark with mobile phase.

METHOD VALIDATION

a. Specificity / Selectivity.

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc [4].

The specificity of the method was studied by degradation of Itaconazole W.R.S. by heating for 24 hours and spiked (degrade std.) to the sample matrix. There was no interference in the HPLC results by the matrices ingredients in both samples, which indicates that the method is specified. (**Table 1**) shows all the results for specificity.

b. Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value [4].

Detection limit of the method was determined through the signal-to-noise ratio (3:1) was calculated by comparing measured signals of ten replicates injection

of known low concentration of analyte (W.R.S).The detection limit of Itraconazole by using this method is obtained 0.3 ppm. (**Figure 2**) shows the LOD chromatogram.

Table 1: Specificity results for Itraconazole

Inj. No.	Concentration of Analyte (Std.)	Response of Std. Analyte	Retention Time	Concentration of Analyte	Response of Sample	Retention Time
	(ppm)	(Area)	(min)	Sample (ppm)	Analyte (Area)	(min)
1	100	50733146	3.907	25	12451031	3.907
2	100	50658713	3.907	25	12412968	3.907
3	100	44350164	3.907	25	12413992	3.903
4	100	44305471	3.907			
5	100	44182663	3.907			
6	100	44165229	3.940			
After degradation				**Sample + Degrade Std.**		
1	100	24902220	3.903	5 ml+ 5ml	18431438	3.903
2	100	24948648	3.907	5 ml+ 5ml	18458702	3.903
3	100	25063100	3.903	5 ml+ 5ml	18435012	3.903
4	100	24998655	3.903			
5	100	25000036	3.903			
6	100	25044326	3.907			

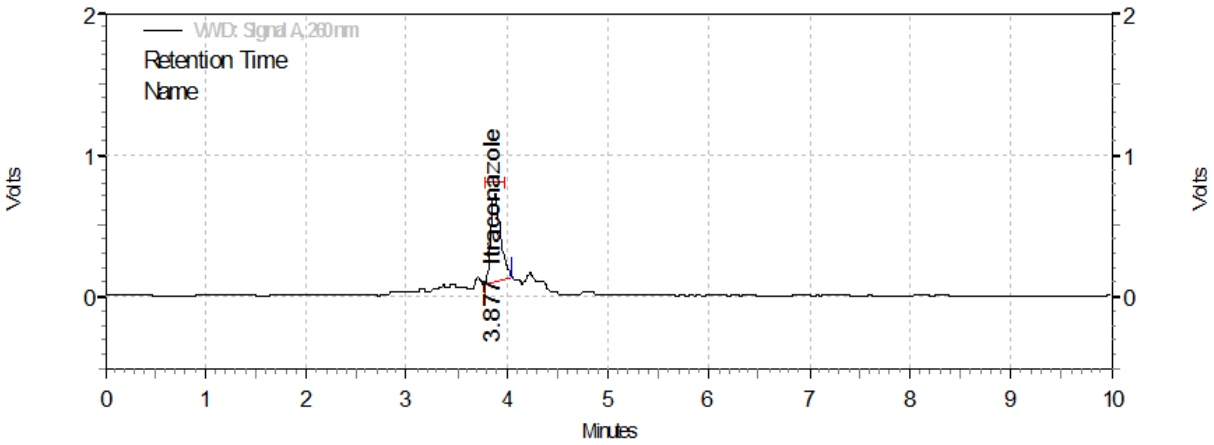

Figure 2: Chromatogram for LOD

c. Quantification Limit

The quantification limit of an individual analytical procedure is the minimum level in a sample at which the analyte can be quantified with acceptable accuracy and precision[4].

Quantification limit of the method was determined through the signal-to-noise ratio (10:1) was calculated by comparing measured signals of ten replicates of known concentration of analyte (W.R.S). The quantification limit of Itraconazole by using this method is obtained 1.0 ppm. (**Figure 3**) shows the chromatogram of LOQ.

d. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample[5].

The linearity of the method was determined of the standard solution of concentration between LOQ 1.0 ppm to 100 ppm . The linearity response for the itraconazole was determined by Ten injections of 1.0 ppm and six injection of 1ppm, 5ppm, 10ppm, 25ppm, 50 ppm and 5ppm . The linear regression data for the calibration curves

indicate that the response is linear over the concentration rage with coefficient of correlation, r^2 value as 0.9972. (**Table 2**) shows the results for linearity, and (**Figure 4**) shows calibration curve for linearity.

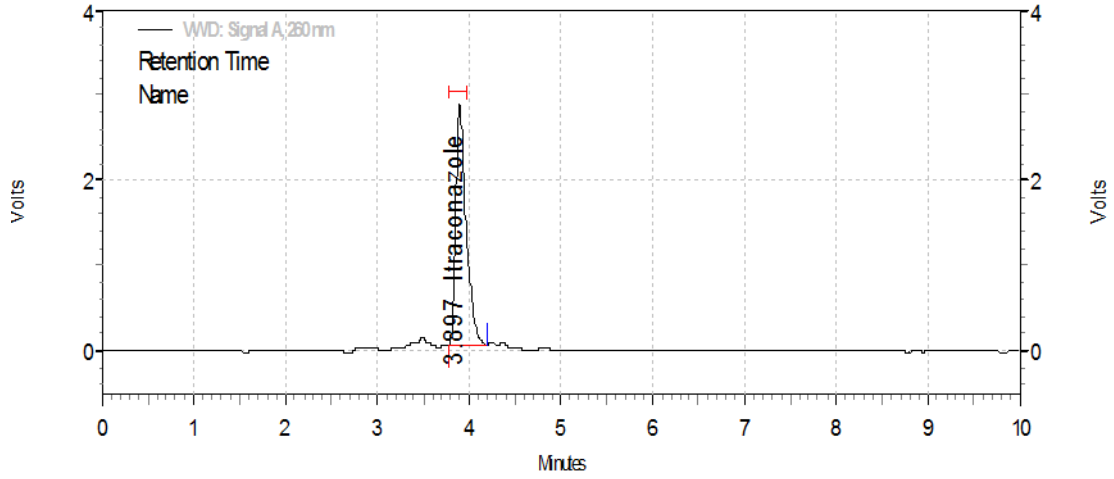

Figure 3: Chromatogram for LOQ

Table 2: Results for Linearity

Concentration	Response Area
1	373660
5	2205521
10	4530871
25	12559642
50	23076222
100	43030547

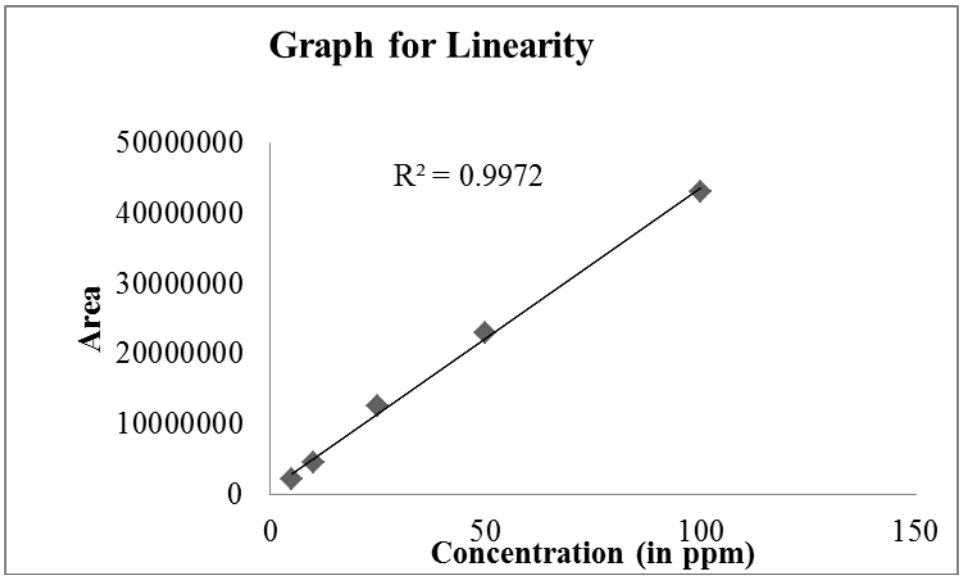

Figure 4: Calibration curve for Linearity

e. Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity[5].

The coefficient of correlation, r^2 value as 0.9972 obtained from linearity test showed

that, the method range has been 1.0 ppm to 100 ppm.

f. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[5].

The accuracy of the method was determined by spiking working standard at different concentration levels 80%, 100% and 120% of target concentration of Itraconazole. The resulting solutions were assayed in triplicate. The vales obtained for recovery 92.61%, 102.93% & 104.33% which are within limit and showed the accuracy of the method.

g. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility [6].

In this method precision was measured in terms of repeatability of application and measurement data. Repeatability of standard solution was carried out using six replicates Itraconazole standard solution.

Repeatability of the sample measurement was carried out in ten different sample preparations from a same homogenous sample.

The RSD for repeatability of Result is 0.308 %. This shows that the method is precise as relative standard deviation is below 5.0%. (**Table 3**) shows result for precision.

Table 3: Result for precision

Sample No.	Result (mg)
1	99.64
2	99.77
3	99.40
4	99.10
5	99.08
6	99.03
7	98.88
8	98.84
9	99.27
10	99.25
Average	99.23
STDEV	0.306104357
%RSD	0.308493083
Limit	NMT 5.0%

h. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [7].

Robustness of the method was determined by assay analyzing the same sample at ideal chromatographic condition by decrease of flow rate . The results of normal operating conditions (ideal) against changed conditions has no significant difference.

Hence, the robustness of the method is established to the extent of variations applied to the experimental conditions.

RESULT AND DISCUSSION

For achieving good chromatographic conditions of itraconazole several mobile phases, flow rates, columns,wavelenghts was analysed. After analysing itraconazole on various mobile phase, a mobile phase of pH 3 which is a mixture of Acetonirile:0.2% and Triethylamine (90:10) was preferred because of great response of peak without tailing and fronting. Flow rate investigation done from range of 0.8ml/min to 1.3ml/min then flow rate of 1ml/min on column C-18 was preferred good, because at this chromatographic conditions we were able to get great response of Itraconazole peak. The retention Time was found at about 4 min. The run time of sample was 10 min. (**Figure 5**) shows general

chromatogram of standard of itraconazole and (**Table 4**) and shows various chromatographic parameters and results of various validation parameters respectively.

Table 4: Various Chromatographic parameters

Elution	Isocratic
Column	ODS Hypersil C-18
Mobile Phase	Acetonitrile & 0.2% triethylamine (90:10)
Flow Rate	1.0 ml/min
Injection Volume	10µl
Temperature (Oven)	45ºC
Detection	UV
Run Time	10 minutes
Retention Time	About 4 min.

Table 5: Results of various Validation Parameter

Summary of Method Validation Parameters					
S.No.	**Parameters**	**Observed Critera**	**Obtained Value**	**Aceptance Criteria**	**Remark**
1	Specificity	Satisfactory	Satisfactory	Satisfactory	Satisfactory
2	LOD (0.3 ppm))	S/N ratio	3.541	NLT :- 3	Satisfactory
3	LOQ (1.0 ppm)	S/N ratio	10.966	NLT :- 10	Satisfactory
4	Linearity	R2 Value	0.9972	NLT: -0.995	Satisfactory
5	Accuracy By Recovery at 99.54 mg	80 % Revcovery	92.61%	80.0% to 120.0%	Satisfactory
		100 % Revcovery	102.93%	80.0% to 120.0%	Satisfactory
		120 % Revcovery	104.33%	80.0% to 120.0%	Satisfactory
6	Precesion By Repetability	RSD of Std. Response	1.119%	NMT:-2.0%	Satisfactory
		Result in mg	99.23mg	NA	NA
		RSD of Results	0.308%	NMT:-5.0%	Satisfactory
7	Robustness	Differenc betw. results	0.23%	NMT:-2.0%	Satisfactory

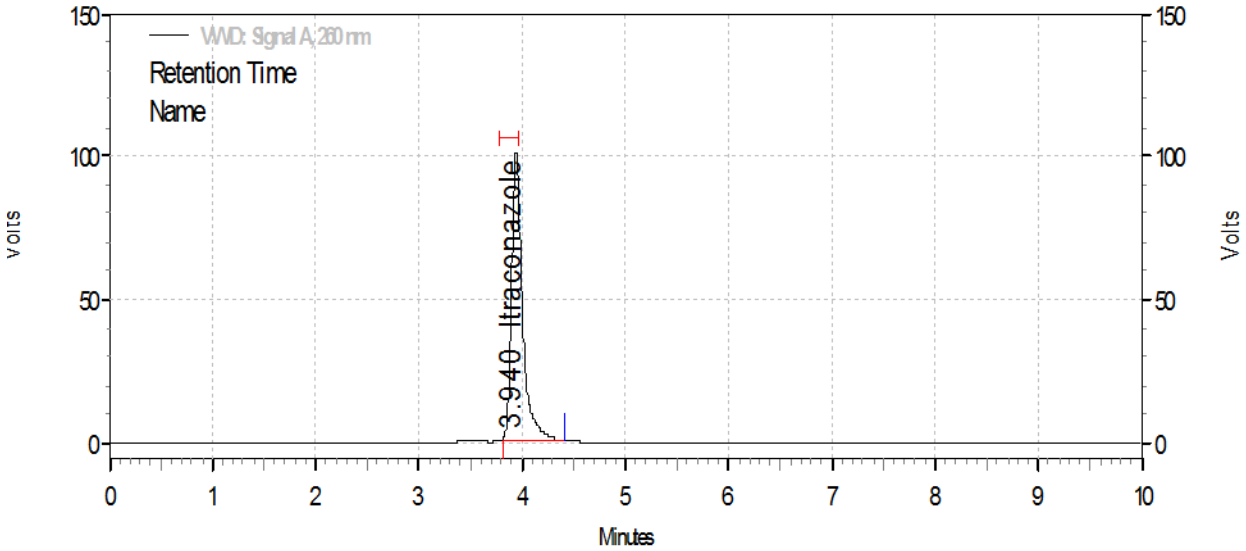

Figure 5: Chromatogram for itraconazole

CONCLUSION

It is concluded that the above describe method for estimation of itraconazole in tablet dosage form is appropriate because the result I get after the validation is under the described limit which provided by ICH guideline for validation. The method is found to be specific, linear, precise, accurate, robust and because all the parameter of this method is found effective for routine analysis.

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