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Review Article

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Different HPLC analysis method of itraconazole

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ABSTRACT

The present review article deals with the method developed for itraconazole by HPLC method. A wide variety of columns, mobile phase combinations, degassers used for the study. Different flow rates where adjusted for mobile phase combinations. In some methods forced degradation studies was also carried out and the methods developed were validated according to the ICH guidelines. After studying different research articles it came to light that HPLC method development of itraconazole has been done in different dosage forms of itraconazole and still there is huge potential for new methods to be developed with different mobile phase and column combinations. These new method data are required by regulatory agencies.

Keywords: Itraconazole, Linearity, Antifungal

INTRODUCTION

Itraconazole is a (1-(butan-2-yl)-4-{4-[4-(4- {[2R,4S)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4 triazole- 1- ylmethyl) 1, 3 – dioxolan – 4 -yl] methoxy}phenyl) piperazin -1-yl] phenyl} 4, 4-dihydro-1H-1,2,4- triazole-5-one) is member of the drug class known as anti-fungal. It is used for the inhibition of fungal cytochrome p450 enzyme “lanosterol 4 demethylase”, used in the conversion of lanosterol to ergosterol, which is a main sterol in fungal cell membrane, thus inhibits replication and promotes cell death in case of the yeast cells transformation into hypothetically invasive hyphae.

Literature survey revealed that very few methods have been reported for the analysis of Itraconazole which include UV spectroscopy, Reverse Phase High Performance Liquid Chromatography, Ultra Pressure Liquid Chromatography, LCMS, HPTLC methods. The present article deals with existing methods developed for itraconazole by HPLC.

Sarvani Paruchuri et al. developed a simple, economic, selective, precise and accurate reverse phase high performance liquid chromatography method for analysis of Itraconazole and Related Substances and validated according to ICH guidelines. Itraconazole was well separated using Thermo Hypersil BDS C18, 150mm X 4.6 mm, 5µm column for assay quantification in isocratic mode with mobile phase comprising of buffer: Acetonitrile (65:35) with a flow rate of 1.5ml/min and Thermo Hypersil BDS C18, 100 mm x 4.6 mm, 3 µm column for Related substances quantification in gradient mode with mobile phase comprising of 0.08M tetra butyl ammonium hydrogen sulphate: Acetonitrile with a flow rate of 1.5ml/min.

The retention time was found out to be 6.2min and % assay was found to be 99.9%. The percentage recovery was found to be 99.6 to 101.2%. Proposed method was validated for precision, accuracy, linearity, range, specificity and robustness. The drug was subjected to forced degradation and stability studies.[1]

Important parameter found in the study are given in Figure1,Table1-3.

Figure1. Linearity parameter

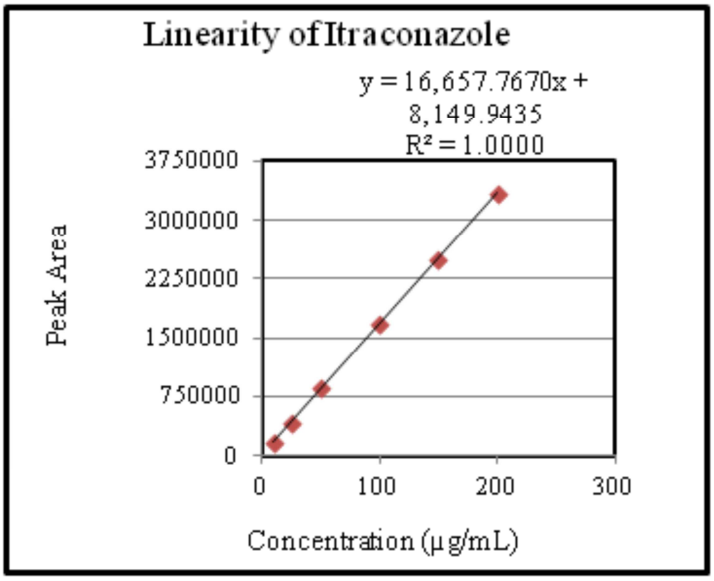

Table.1.Validation Parameters

Parameters	Assay
Linearity	10-200mcg/ml
Precision (%RSD)	0.2
Accuracy	100.2
Assay	99.9
Ruggedness(%RSD)	0.33

Table.2.Optimised Chromatographic Conditions

Parameters	Assay
Column	Thermohypersil BDS C18 150X4.6mm, 5µm
Mobile phase	Buffer: ACN (65:35)
Flow rate	1.5ml/min
Wave length	UV at 225nm
Injection volume	10µl
Column temperature	25^0C
Run Time	12min

Table.3.Linearity Data

Related substances Mcg/ml	Area	Rt
10	168080	6.14
25	424817	6.13
50	849375	6.13
100	1675595	6.14
150	2502949	6.13
200	3339989	6.13

M. Salomi, et al. Developed a validated RP-HPLC method for the estimation of Itraconazole in pure and pharmaceutical dosage form. In this a simple, fast and precise RP–HPLC method was developed for the

quantification of Itraconazole in pure and pharmaceutical dosage form. The quantification was carried out using Dionex C18 4.6 X 250mm, 5µm enhanced polar selectivity column and mobile phase comprised of methanol and pH 7.5 potassium dihydrogen phosphate in the ratio of 40:60 and degassed under ultrasonication. The flow rate was 1.5ml/min and the effluent was monitored at 306nm.

The retention time of Itraconazole was found to be 5.2 min. The method was validated in terms of linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation in accordance with ICH guidelines. Linearity of Itraconazole was in the range of 200-600 µg/mL. The percentage recoveries of Itraconazole were 99.33% to 99.66% from the capsule formulation. The proposed method is suitable for determination of Itraconazole in pharmaceutical dosage form.[2]

Important parameter found in the study are given in Table4-6.

Table 4: Optimized chromatographic parameters
Optimized Chromatographic parameters

Elution	Isocratic
Mobile phase	methanol and pH 7.5 KH_2PO_4 (40:50)
Column	Dionex C18column
Flow rate	1.5ml/min
Detection	306nm
Injection volume	10µl
Retention time	5.278 min
Run time	7 min

Table 5: System suitability parameters
Parameters Values

Retention time	5.278 min
Theoretical plates	8609.000
Tailing factor	1.138

Table 6: Linearity results for Itraconazole

S.No	Concentration (µg/ml)	Area AU (n=6)
1	200	3564715
2	300	5346123
3	400	7179256
4	500	8907315
5	600	10694359

Kumudhavalli *et al.*, developed a simple, specific, accurate and precise reverse phase high performance liquid chromatographic method and validated for the estimation of Itraconazole in capsule dosage form. An inertsil C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing Tetrabutyl ammonium hydrogen sulphate buffer solution and Acetonitrile in the ratio of 40:60v/v was used. The flow rate was 1.5ml/min and effluents were monitored at 225nm. The retention time for Itraconazole was 5.617min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.85µg/ml and 2.60µg/ml respectively and recovery of Itraconazole from capsule formulation was found to be 98.3 to 100.3%.The system suitability parameters such as theoretical plates and tailing factor were found to be 2927.43 and 1.08 The proposed method was successfully applied for the quantitative determination of Itraconazole in capsule formulation.[3]Important parameters are given in table 7 and table 8

Table 7: Validation and System Suitability Parameters

Linearity range (µg/ml)	50 – 200 µg/ml
Correlation coefficient	0.9993
Retention time (min)	5.61
Tailing factor	1.08
Limit of detection (µg/ml)	0.85 µg/ml
Limit of quantification (µg/ml)	2.60 µg/m
Precision (RSD %)	0.27%

Table 8: Result of Robustness Study

Factor	Level	Mean*	%RSD
Flow rate (ml/min)	1.4ml/min	1803429	0.1
	1.6ml/min	1803429	0.1
Mobile phase ratio	8:62	1651399	0.5
	42:58	1682078	0.4
Column temperature	25	1676695	0.6
	35°C	1664691	0.7

Azevedo *et al*. developed HPLC-FLD Method for Itraconazole Quantification in Poly Lactic-co-glycolic Acid Nanoparticles, Plasma and Tissue. In this a high performance liquid chromatography (HPLC) procedure using fluorometric detection was developed for determination of itraconazole in polymeric poly(lactic-co-glycolic acid) nanoparticles, plasma and tissue. Linearity, limits of detection and quantification, recovery, precision, selectivity and stability were established. The developed method was tested in itraconazole detection and quantification of biodistribution of nanoparticles administered intraperitoneally to Balb/C female mice. This study developed an analytical method for HPLC with fluorometric detection for quantification of itraconazole in polymeric nanoparticles, tissue and plasma, which is sensitive, low cost, viable for routine usage and with potential for application in itraconazole biodistribution and pharmacokinetics studies.[4]

Using the developed analytical method, ITZ retention time was 7.6 min and internal standard retention time was 9.1 min with 11 min for each run.

The chromatographic runs were completed at 11 min, a short run when compared to most methods for ITZ quantification in HPLC, resulting in less waste, in accordance with the principles of green analytical chemistry.

Important parameters found are given in table 9

Table 9 Linear regression parameter and limits of detection and quantification for itraconazole (area *vs.* Concentration)

Calibration curves	Range of concentration / (µg mL–1)	Slope	Intercept	r2	LOD / (µg mL–1)	LOQ / (µg mL–1)
Mobile phase	10-0.20	720801	–4366.1	0.9992	0.151	0.459
Mobile phase	0.2-0.01	780523	297.6	0.9999	0.003	0.008
Kideny	10-0.02	707156	14640	0.9996	0.001	0.004
Liver	10-0.02	642671	5640	0.9994	0.007	0.020
Lung	10-0.02	691500	11502	0.9999	0.009	0.026
Spleen	10-0.02	646530	12203	0.9999	0.020	0.061
Plasma	10-0.02	653569	12560	0.9998	0.039	0.117

Andelija Malenovic *et al*. Chemometrically assisted optimized and validated an RP-HPLC method for the analysis of itraconazole and its impurities. In this chemometrically assisted optimization and validation of the RP-HPLC method intended for the quantitative analysis of itraconazole and its impurities in pharmaceutical dosage forms was carried out. To reach the desired chromatographic resolution with a limited number of experiments in a minimum amount of time, Box Behnken design was used to simultaneously optimize some important chromatographic parameters, such as the acetonitrile content in the mobile phase, pH of the aqueous phase and the column temperature. Separation between itraconazole and impurity F was identified as critical and selected as a response during the optimization.

The set optimal mobile phase composition was acetonitrile/water pH 2.5 adjusted with o-phosphoric acid (50:50, V/V). Separations were performed on a Zorbax Eclipse XDB-C18, 4.6 ×150 mm, 5 m particle size column with the flow rate 1 mL min^{-1}, column temperature set at 30°C and UV detection at 256 nm. The established method was then subjected to method validation and the required validation parameters were tested.[5]

Important parameters found are given in table 10 and table 11

Table10. Important parameters for linearity

Parameter	Itraconazole	Impurity B	Impurity F
a	42.63	89.78	59.91
b	41.92	1.05	0.56
R	0.9999	1.0000	1.0000
t_b	0.617	3.083	1.726

Table 11. Important parameters for accuracy

Concentration 1 (µg mL^{-1})	160	0.8	0.8
Recovery 1 (%)	101.2	98.5	94.3
Concentration 2 (µg mL^{-1})	200	1.0	1.0
Recovery 2 (%)	104.3	99.9	96.7
Concentration 3 (µg mL^{-1})	240	1.2	1.2
Recovery 3 (%)	105.1	95.0	97.6

Durisehvar Ozer Unal *et al.* developed method for the determination of ITR and metabolite from plasma by LC-MS/MS system, which is reliable and robust. The method uses simple and economical liquid-liquid extraction of the drugs from human plasma. The lowest standard 1.0 ng/mL for ITR and 2.0 ng/mL for HITR on the calibration curve was identified as the lower limit of quantification (LOQ) with a precision of less than or equal to 20 %.

The calibration curve was prepared from seven spiked plasma samples within the range of 1.0-600.00 ng/mL, including LOQ for ITR and 2.0-600.00 ng/mL, including LOQ for HITR.

The standard calibration curves was linear over the concentration range from 1.0-600 ng/mL with mean r^2=0.99509 for ITR and r^2=0.99252 for HITR respectively. The LOQ was 1.0 ng/mL for ITR and 2.0 ng/mL for HITR. The calibration curve had a regression equation of y=0.04118x -0.00102 for ITR, y=0.00534x-0.00129 and for HITR respectively . The limit of detection of developed method is as low as LC-MS/MS system by liquid-liquid extraction procedure. The validation results were included specificity, accuracy, extraction recovery, linearity and range. The assay can be applied easily successfully to the pharmacokinetic and bioequivalence studies.[7]

Important parameters found are given in table 12-14

Table 12 intra-day coefficient of variation and relative error% for determination of HITR

Sample	Conc. of HITR (ng/mL)	Mean conc. of HITR (ng/mL)	Relative Error%	SD	CV%	n
QC1	2	1.98	98.14	0.13	6.73	18
QC2	6	5.62	100.55	0.47	8.30	18
QC3	30	26.73	89.73	1.29	4.84	18
QC4	480	489.02	102.25	31.73	6.49	18
QC5	600	577.57	101.79	45.02	7.80	18

Table 13. Intra-day coefficient of variation and relative error % for determination of ITR

Sample	Conc. of ITR (ng/mL)	Mean conc. of ITR (ng/ mL)	Relative Error%	SD	CV%	n
QC1	1	0.98	98.88	0.05	5.47	18
QC2	3	3.02	93.71	0.16	5.36	18
QC3	30	26.92	89.11	1.02	3.78	18
QC4	480	490.80	101.88	17.43	3.55	18
QC5	600	610.71	96.26	21.95	3.59	18

Table 14. The Intra And Inter Day CV% Value

	Itraconazole (1-600 ng/mL)		Hydoxy-ITR (2-600 ng/mL)	
	Min.	Max.	Min.	Max.
Inter-day CV% Range(n=6)	2.43	5.53	1.48	9.79
Intra-day CV% Range(n=18)	3.55	5.47	4.84	8.30

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