IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
July 2016 Vol.:6, Issue:4

A Validated RP-HPLC Method for the Estimation of Isavuconazole in Bulk and Formulation

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Submission: 7 July 2016
Accepted: 12 July 2016
Published: 25 July 2016

Keywords: Isavuconazole, Reverse phase HPLC, Method development, Method Validation

ABSTRACT

A simple, rapid, precise and accurate RP-HPLC method was developed and validated for the estimation of Isavuconazole. An Inertsil$^{(R)}$ (150*4.6*5µ*C18) column kept at ambient temperature, using a mobile phase consisting of 0.1M Ammonium acetate: methanol (60:40) at a flow rate of 1.2 ml/min and UV detection at 251nm. The retention time of Isavuconazole was found to be 2.96 min. Validation of the method was carried out as per ICH guidelines. Linearity was established for Isavuconazole is in the range of 1.25-3.75µg/ml. The correlation coefficient of Isavuconazole was found to be 0.99. Percentage Recovery of Isavuconazole in formulations was found to be in the range of 98.0-102.0%. Due to its simplicity, rapidness and high precision, the method was successfully applied to the estimation of Isavuconazole.

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INTRODUCTION

Isavuconazole is a novel broad-spectrum triazole anti-fungal drug. It is administered as a prodrug Isavuconazonium sulfate (BAL8557; brand name **CRESEMBA**) used for the treatment of invasive aspergillosis and treatment of invasive mucormycosis in patients 18 years of age and older. It is available in the form of an oral tablet or an Intravenous (IV) injection.

Structure of Isavuconazole

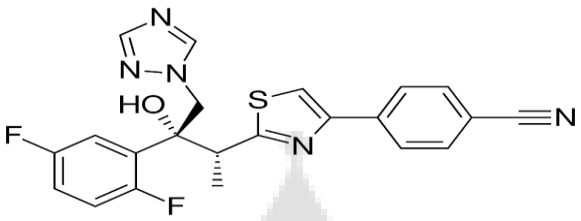

Fig 1. Molecular Structure of Isavuconazole

The mechanism of action of Isavuconazole is similar to other antifungal azoles which involve the inhibition of cytochrome P450 dependent enzyme lanosterol 14 α-demethylase, which converts lanosterol to ergosterol by demethylation. As a result, methylated sterol precursors accumulate and the supply of ergosterol within the fungal cell membrane depletes, weakening the membrane structure and function. Isavuconazole inhibition is not as potent in mammalian cell demethylation. In this paper, we describe a simple, accurate, sensitive and validated RP-HPLC method for analysis of Isavuconazole in tablet formulation. This method can be successfully used for quality-control analysis of drugs and for other analytical purpose.

EQUIPMENT AND APPARATUS USED:

- Single pan balance (Metler Toledo)
- Control Dynamics pH meter (Metler Toledo)
- HPLC MAKE WATERS 2695
- UV Visible Detector (Photo Diode Aride 2998)
- Chromatographic data Software: Empower 2
- YMC Pack pro (250x4.6) mm, 5µ, C18
- INERTSIL (150x4.6) mm, 5µ, C18

- Vacuum filter pump, (NUPORE filtration systems, 0.45 µ, 47mm)
- Mobile phase reservoir
- Ultra-sonicator

Reagents used:

- Methanol HPLC grade (RANKEM)
- Potassium dihydrogen Phosphate, Laboratory reagent (STANDARD REAGENT)
- Ammonium acetate (STANDARD REAGENT)
- Water (HPLC GRADE WATER)

Finalized Chromatographic Parameters:

Mobile Phase: 0.1M Ammonium Acetate: Methanol (60:40)

0.1M Ammonium acetate buffer preparation:

Weigh accurately 7.708g of Ammonium acetate in 1000ml beaker and makeup with HPLC graded water and sonicated it for 15 mins.

Chromatographic Conditions:

Column: Inertsil 150x4.6mm, 5µ, C18.

Flow Rate: 1.2ml/min

Temperature: 30^0c

Volume: 8 µl

Detector: PDA

Calibration

Calibration plots were constructed by analysis of appropriate working solutions concentration 1.25, 1.87, 2.5, 3.12, 3.75 µg/ ml of Isavuconazole in the mobile phase and plotting concentration against peak area response for each injection.

Standard preparation:

Accurately weigh and transfer 100 mg of Isavuconazole in 100ml of volumetric flask and make up the volume with HPLC grade water and sonicated to dissolve it completely and make volume up to the mark with the same solvent.[13] From the above solution take 2.5 ml into 25 ml of volumetric flask and dilute it with solvent up to the mark.

Sample preparation:

Accurately weigh and transfer 100mg of Isavuconazole in 100ml of volumetric flask and add 20ml of methanol and sonicate (20mins) to dissolve it completely and make up the volume with HPLC grade water. From the above solution take 2.5 ml into 25ml of volumetric flask and dilute it with solvent up to the mark.

RESULTS AND DISCUSSION

Method development and optimization

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), additive strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized, so there was no interference with the Isavuconazole peak from solvent or excipient peaks. Other criteria, for example the time required for analysis, assay sensitivity, solvent noise and use of the same solvent system for extraction of the drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase, the column was equilibrated by passage of at least twenty column volumes of the new mobile phase. To investigate the appropriate wavelength for determination of Isavuconazole, UV–visible spectra in the range 200–400 nm were acquired from a solution of the drug in the mobile phase. From the UV spectra obtained the wavelength selected for monitoring the drug was 254 nm. Solutions of the drug in the mobile phase were injected directly for HPLC analysis and the responses (peak area) were recorded at 245 nm. It was observed there was no interference from the mobile phase or baseline disturbance at 245 nm. Therefore, it was, concluded that 245 nm was the most appropriate wavelength for analysis of the substance with suitable sensitivity.

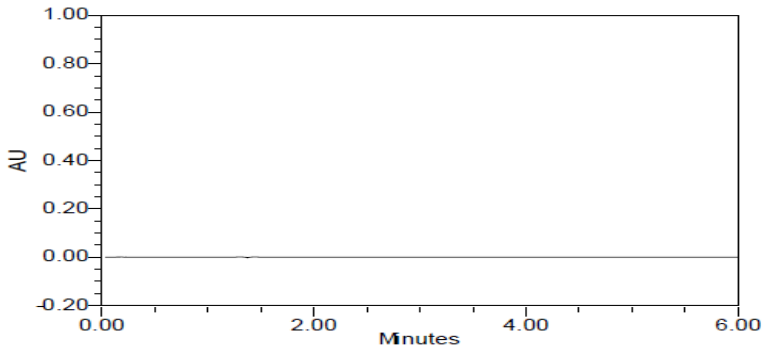

Fig.2 Chromatogram for blank interference

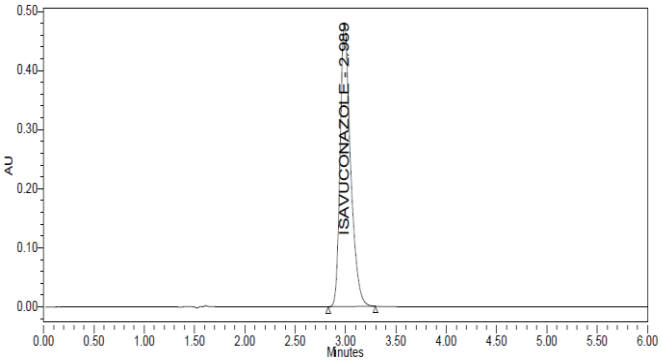

Fig.3 Chromatogram for standard

Method Validation

Linearity

The linearity of the method was tested using the calibration solutions described above. Plot of concentrations against responses were linear in the range of 1.25-3.75 µg mL-1 (Figure 4). The mean regression equation was *Y* = 43363x. The correlation coefficient was 0.9999.

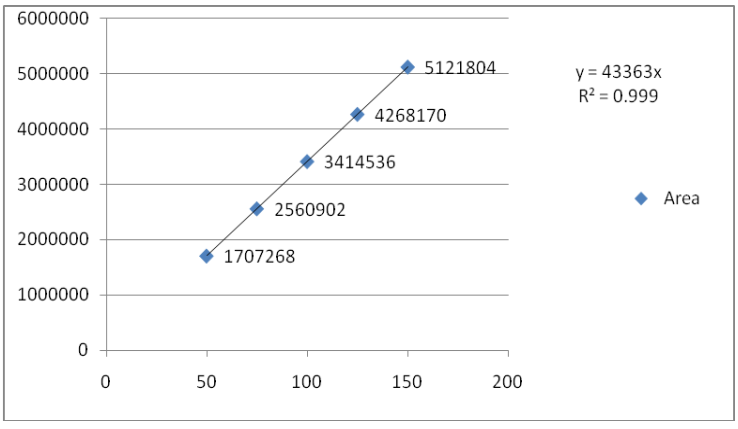

Fig 4: Linearity curve of Isavuconazole by RP-HPLC method

Limits of detection and quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal-to-noise ratio (SNR) is 3:1. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy. It is usually regarded as the amount for which the SNR is 10:1. Two types of solution, blank solution and solutions containing known, progressively decreasing concentrations of the analyte, were prepared and analyzed. LOD and LOQ were 2.2 and 9.5 µg /mL, respectively.

Accuracy

Recovery studies were performed in triplicate after spiking raw material in volumetric flasks with amounts of Isavuconazole equivalent to 50, 100 and 150% of the standard concentration of Isavuconazole as in the analytical method. The results obtained (Table 1) indicate that recovery were excellent, not less than 99% and that relative standard deviations also less than 2%.

Table 1. Accuracy of the method

Isavuconazole						
Spiked Level	**Sample Weight**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**% Mean**
50%	50.00	1716705	50.000	50.08	100	100
50%	50.00	1705812	50.000	49.76	100	
50%	50.00	1723288	50.000	50.27	101	
100%	100.00	3436993	100.000	100.26	100	100
100%	100.00	3410194	100.000	99.47	99	
100%	100.00	3423950	100.000	99.87	100	
150%	150.00	5133439	150.000	149.74	100	100
150%	150.00	5125778	150.000	149.52	100	
150%	150.00	5146371	150.000	150.12	100	

Precision

Intra-day precision was calculated from results obtained from five-fold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on five consecutive days. The results obtained are listed in Table 2.

Table 2. Intra-day and inter-day precision of the method

Peak Name: ISAVUCONAZOLE

	SampleName	Peak Name	RT	Area
1	PRECESION1	ISAVUCONAZOLE	2.971	3443020
2	PRECESION2	ISAVUCONAZOLE	2.966	3434298
3	PRECESION3	ISAVUCONAZOLE	2.965	3418377
4	PRECESION4	ISAVUCONAZOLE	2.967	3420220
5	PRECESION5	ISAVUCONAZOLE	2.963	3413309
6	PRECESION6	ISAVUCONAZOLE	2.964	3446310

Specificity

The specificity of the method was tested by chromatography of a mixture of commonly used tablet excipients, for example starch, lactose and magnesium stearate (blank placebo) and comparing the chromatogram with that obtained from a mixture of drug and the same additives (placebo). The chromatograms obtained (Figures 5 & 6) showed separation of the analyte from the excipients was complete, *i.e.* there was no interference from the excipients under the chromatographic conditions used for the analysis.

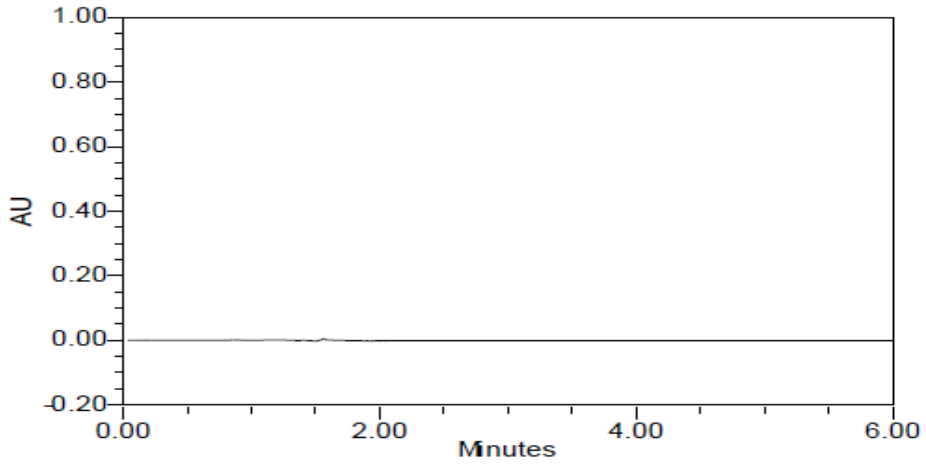

Fig 5. Chromatogram obtained from placebo

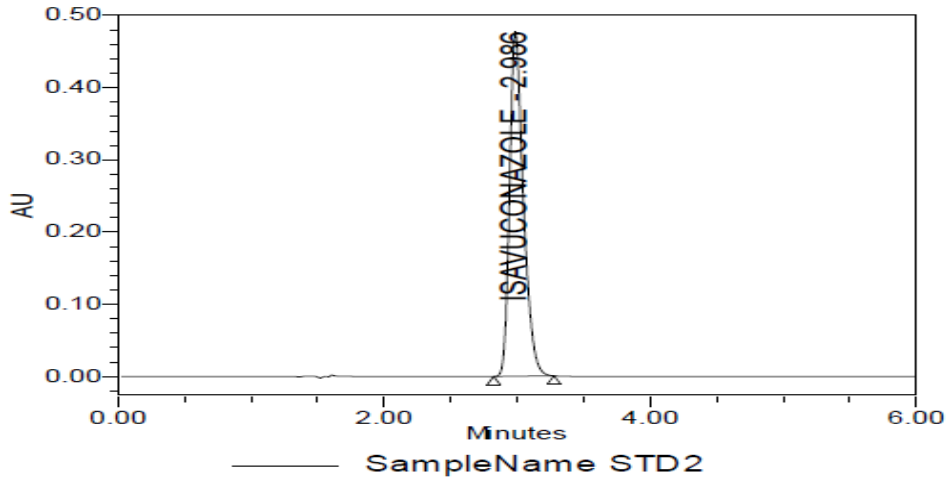

Fig 6. Chromatogram obtained from capsule sample.

CONCLUSION

This RP-HPLC method for analysis of Isavuconazole in formulations is very simple, sensitive, and accurate. The run time is 5 min only; so many samples can also be processed and analyzed in a short period of time. The procedure described is suitable for the routine estimation of Isavuconazole in pharmaceutical formulations.

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