IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A Research On: Development & Validation Of Stability Indicating RP HPLC Method For Estimation Of Isavuconazole In Pharmaceutical Dosage Form

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Abstract:-

Analytic method validation that an analytical methodology is accurate, specific Reproducible Over the specified range an analytical will be analysed

RP-HPLC has Non polar (hydrophobic) stationary phase 4 an aqueous, moderately polar mobile phase, Isavuconazole is a triazole antifungal agent for treatment of isavuconazole Invasive aspergillosis.

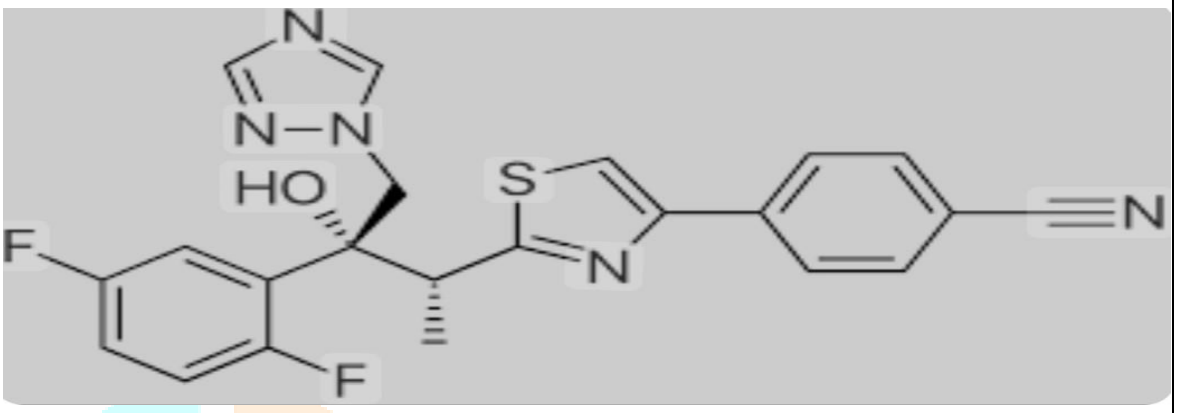
Validation of the method was carried out as per ICH guidelines. Linearity was established for Isavuconazole is in the range of 1.25-3.75µg/ml. The correlation coefficient of Isavuconazole was found to be 0.99. Percentage Recovery of Isavuconazole in formulations was found to be in the range of 98.0-102.0%. Due to its simplicity, rapidness and high precision, the method was Successfully applied to the estimation of Isavuconazole.

Keywords:- Isavuconazole, Reverse phase, HPLC, method development, method Validation.

Introduction:

Isavuconazole is a novel broad spectrum triazole anti-fungi drug. Isavuconazole is similar to other antifungal azoles which involves the inhibition of Cytochrome p450 dependent enzyme lanosterol.

Drug profile:

Name of drug	Isavuconazole	
Structure		
Chemical name	4-[2-[(2R,3R)-3-(2,5-Difluro phenyl)-3-hydroxy-4 (1H-1,2,4-triazol - 1 – y|) butane -2 – yl [triazole - 4 - yl] benzonitrile	
Molecular formula	C22H17F2N5O5	
Molecular weight	437.47 g/mol	
Solubility	Soluble in DMSO,ethanol,1 dimethyl formamide	
Boiling point	676 °c to 678°c	

High performance liquid Chromatography (HPLC):

HPLC is a laboratory technique used to separate, identify, and quantify the components of a mixture. It is a type of chromatography that uses a liquid mobile phase to separate the components of a mixture based on their interactions with a stationary phase. HPLC is a advanced techniques of column Liquid chromatography. HPLC recognized from traditional liquid chromatography because operational pressure are fundamentally higher.

Types of HPLC

1. Reversed-Phase HPLC (RP-HPLC): The most common type of HPLC, which uses a non-polar stationary phase and a polar mobile phase.

2. Normal-Phase HPLC (NP-HPLC): Uses a polar stationary phase and a non-polar mobile phase.

3. Size-Exclusion Chromatography (SEC): Separates molecules based on their size.

4. Ion-Exchange Chromatography (IEC): Separates molecules based on their charge.

Reverse phase -high performance liquid Chromatography (RP-HPLC):

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) is a type of liquid chromatography that uses a non-polar stationary phase and a polar mobile phase to separate and analyze the components of a mixture.

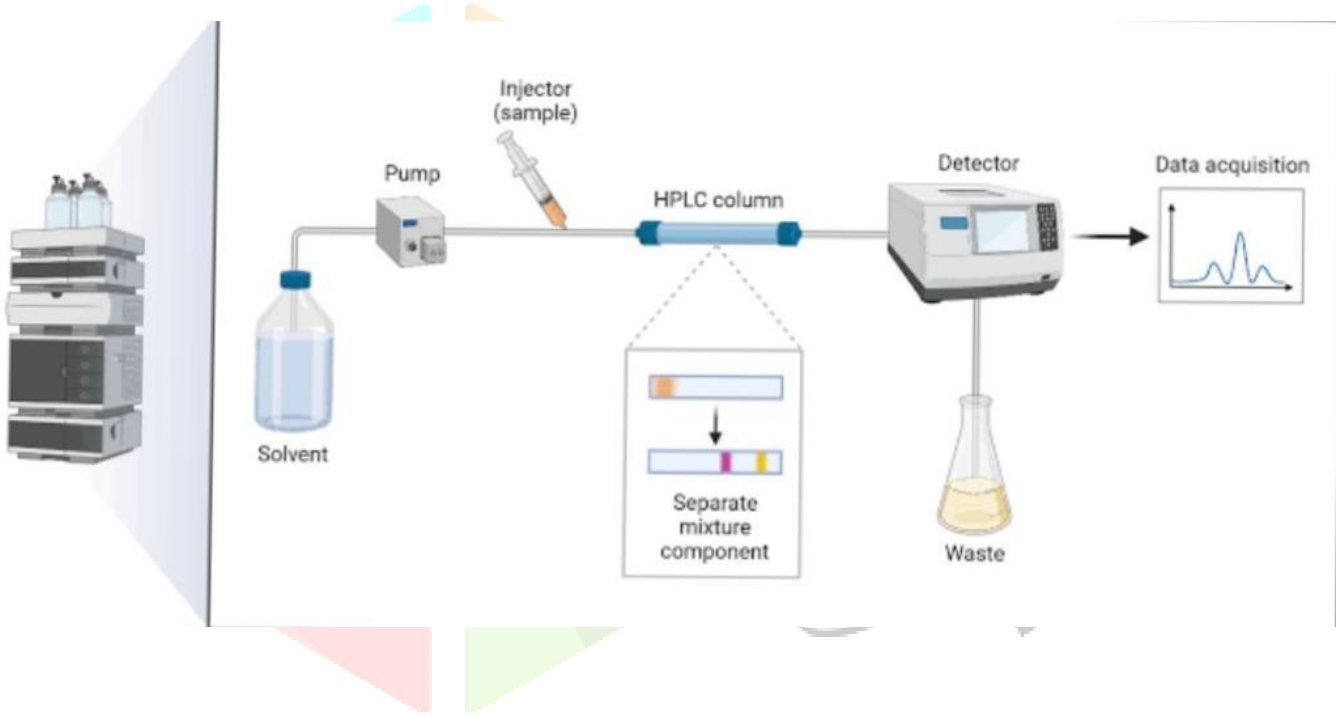
- Advantages of RP-HPLC

1. High Sensitivity: RP-HPLC can detect very small amounts of a substance.

2. High Selectivity: RP-HPLC can separate and identify multiple components of a mixture.

3. High Accuracy: RP-HPLC can provide accurate and precise results.

Instrumentation of RP-HPLC:



1.Mobile phase / solvent Reservoir:

The reservoir holds the mobile phase is other no more than a glass bottle often the reagent bottle that holds our HPLC solvent can used as a reservoir. Solvent is delivered from the reservoir to the pump by means of tension Using tubing called inlet line to the pump.

2.Solvent delivery system:

The solvent delivery system is described like a deliver System of continuous pulse free flow of mobile phase to the HPLC regardless of the system back pressure.

3. Pump:

The role of the pump is to a force a liquid through the liquid chromatographic at a specific flow rate expressed in ml (min Nomal flow rate in HPLC in the 2 to 2 ml/min range

4. Injector:

The injector serves to introduce the liquid sample into the flow stream of the mobile phase. Typical sample volumes are 5 to 20 lid.

5. Column:

Considered the heart of chromatograph. The column's Stationary phase separate the sample components of Interest Using Various physical & chemical parameters.

The small particle inside the column are what cause the high back pressure at normal flow rate.

6. Detector:

A detector detect the individual molecule that comout (elute) from the column.

7. Computer :-

Frequency called the data system the Computer not only controls all the module of the HPLC instrument but it taker the signal from the detector.

- EQUIPMENT AND APPARATUS USED:

i. Single pan balance (Metler Toledo)
ii. Control Dynamics pH meter (Metler Toledo)
iii. HPLC MAKE WATERS 2695
iv. UV Visible Detector (Photo Diode Aride 2998)
v. Chromatographic data Software: Empower 2
vi. YMC Pack pro (250x4.6) mm, 5µ, C18
vii. INERTSIL (150x4.6) mm, 5µ,
viii. Vacuum filter pump, (NUPORE filtration systems, 0.45 µ, 47mm)
ix. Mobile phase reservoir
x. Ultra-sonicator

- Reagents used:
-

i. Methanol HPLC grade (RANKEM)
ii. Potassium dihydrogen Phosphate, Laboratory reagent (STANDARD REAGENT)
iii. Ammonium acetate (STANDARD REAGENT)
iv. Water (HPLC GRADE WAT

- Calibration :-

Calibration plots were constructed by analysis of appropriate working solutions concentration 1.25,1.87, 2.5, 3.12, 3.75 ug/ml of Isavuconazole in the mobile phase & ploting concentration agains peak area response for each injection.

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: 300C

Volume: 8 µl

Detector: PDA

Standard preparation:

Accurately weigh & transfer 100 my of Isavuconazol in 100 ml of volumetric flask & make up the volume with HPLC grade water & sonicated to dissolve it completely & make volume up to the mark with the same solvent.

From the above solution take 2.5 ml into 25 ml of Volumetric flask & dilute it with solvent Up to mark.

Sample preparation:

Acccurately weight &transfer 100 my of Isavuconazol in 100 ml of volumetric flask & add 20ml of methanol & Sonicate 20 minutes to dissove it completely & make up the volume with HPLC grade water

From the above solution take 2.5ml into 25ml of Volumentric flask & dilute it with solvent up to the mask.

Result and Discussion

Method development optimization

Column chemistry, solvent selectivity [solvent type), solvent strength [volume fraction of organic solvent (s)in the mobile phase], additive, detection wavelength and flow rate were varied to determine the chromatographic condition giving the best separation.

The mobile phase condition were optimized, so there was no interference with the time isavuconazole peak from solvent or excipient peaks. Other criteria for example the time reavired for analysis, assay sensitivity, solvent noise & use of the same solvent system for example of the drug from formulation material during drug analysis, were also considered.

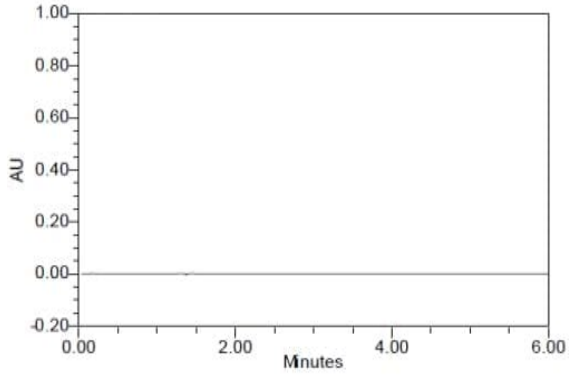


Fig.2 Chromatogram for blank interference

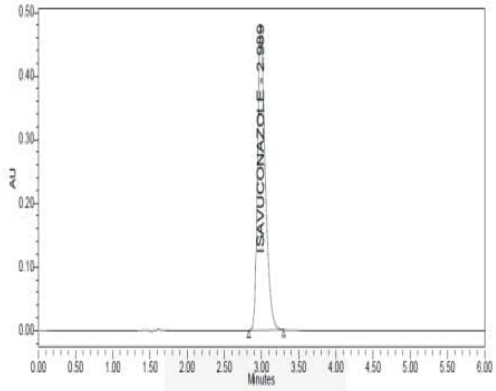


Fig.3 Chromatogram for standard

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.

Method validation

Linearity: The Using the linearity of the method was tested using the calibration solutions described above plot of concentration against response were linear in the range of 1.25-3.75ug/ml.

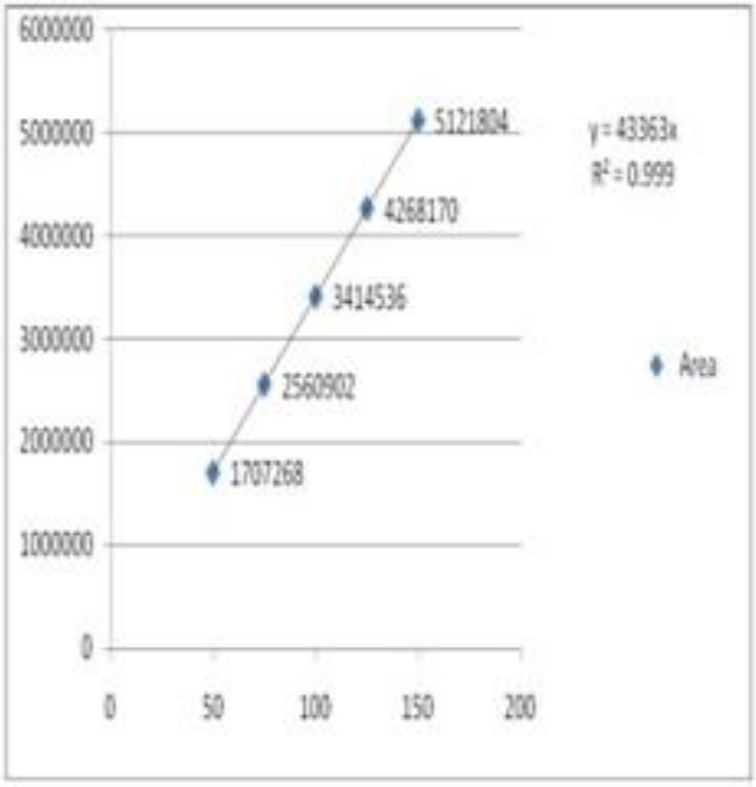


Fig 4: Linearity curve of Isavuconazole by RP-HPLC method

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 flask with amounts of Isavuconazole equivalent to 50, 100&150% of the standard concentration of Isavuconazole as in the analytical method.

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 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	

Specificity: The specificity of the method war tested by chromatography of a mixture of commonly used tablet excipients.

For example:- starch, lactose & magnesium sterate & Comparing the chromatography with that Obtained from a mixture of drug & the same additive.

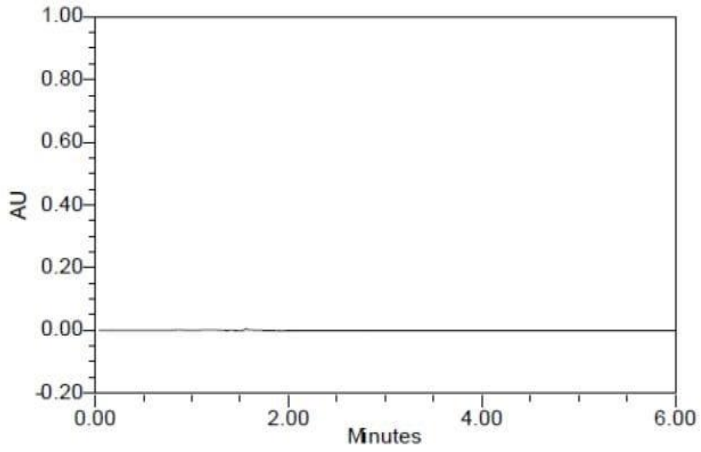


Fig 5. Chromatogram obtained from placebo

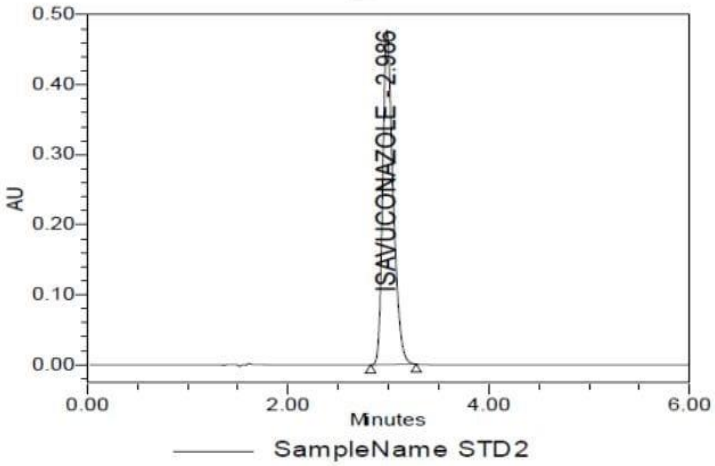


Fig 6. Chromatogram obtained from capsule sample.

Conclusion

The RP-HPLC method for analysis of Isavuconazole in formulation is very simple, sensitive, and acrurate.

The run time is 5 min only; So many sample can also be proccessed and analyzed in a short period of time.

The procedure described is suitable for the routine estimation of Isavuconazole in pharmaceutical formulation.

Reference

1. A Text Book of Pharmaceutical Analysis by David. G. Watson, 1999, 2nd edition.

2. High-Performance Liquid Chromatography.

3. Cresemba (Isavuconazole sulfate) Prescribing Information" (PDF). Northbrook, IL: Astellas Pharma US, Inc.

4.http://www.thelancet.com/journals/lancet/article/PIIS0140-6736%2815%2901218-0/abstrac t

5. http://www.aspergillus.org.uk/media/92811

6. http://cid.oxfordjournals.org/content/54/suppl_1/S23.full

7. Rogatsky E. Modern high performance liquid chromatography and HPLC 2016 International Symposium. JChromatogr Sep Tech.2016

8. Joseph C. Touchstone (2006), History of Chromatography, Journal of liquid chromatography, volume 8 (16), 1647-1665. https://doi.org/10.1080/10826079308021679.

9. Bergh J. J., Breytenbach, J. C. Stability-indicating High-performance Liquid-chromatographic Analysis of Trimethoprim in Pharmaceuticals. J. Chromatogr. 1987; 387: 528-531.

10. ICH Harmonised Tripartite Guideline validation of analytical procedures: text and methodology Q2(R1)

11. International Conference on Harmonization, " Q2B: Validation of Analytical Procedures: Methodology; Availability & quot; Federal Register 62(96), 27463-27467 (1997).

12. Analytical and Bioanalytical Chemistry August 2012, 513-523.

13. Journal of Chromatography B: Biomedical Sciences and Applications Volumes 887-888, Pages 1-142 (1 March 2012)