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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF POSACONAZOLE IN API AND TABLET FORMULATION

Gurumurthy.Telugu* Dr.P.Venkata Suresh
Department of Pharmaceutical Analysis & Quality Assurance,
Creative Educational Society's College of Pharmacy, Kurnool, Andhra Pradesh

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Abstract:
The objective of the present work is Method Development and Validation for the estimation of Posaconazole in Bulk and Formulation by RP-HPLC. A simple, rapid, accurate, precise, robust and reproducible RP-HPLC method was developed for the estimation of Posaconazole. The method was developed by using Inertsil ODS-2V column, with Mobile phase comprising of ACN: Water in the ratio of (90:10v/v) at a flow rate of 1ml/min and the effluents were monitored at 262nm using PDA detector. Chromatograms are eluted at retention time of 3.98min The R^2 was found to be 0.9998. The accuracy was carried out and results were within 98-102% and the %Relative Standard Deviation was found to be <2%. Detection Limit and Quantitation Limit were found to be 0.26µg/ml and 0.80µg/ml respectively. The proposed method was successfully applied to posaconazole bulk quantification, showing the method is useful for determination of the drug in routine analysis

Keywords: *Simple, RP-HPLC, Chromatographic separation, Posaconazole, ICH guidelines,*

Corresponding author:
Gurumurthy.Telugu,
Department of Pharmaceutical Analysis & Quality Assurance,
Creative Educational Society's College of Pharmacy,
Kurnool, Andhra Pradesh

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1. INTRODUCTION:

Posaconazole is a synthetic systemic triazole antifungal agent. It is used to treat invasive infections in severely immunocompromised patients those with acquired immunodeficiency syndrome and haemopoietic stem-cell transplant recipients [1, 2]. Posaconazole is 4-{4-[4-(4-{[(3*R*,5*R*)-5-(2,4-difluorophenyl)-5-(1*H*-1,2,4-triazol-1-yl methyl) -tetrahydrofuran-3-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-2-[(1*S*,2*S*)-1-ethyl-2-hydroxy-propyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (Fig. 1)[3-4], is a triazole antifungal drug, approved by the Food Drug Administration in 2006 and characterized for the broader spectra of action between triazole, besides the less potential of interactions. It is the 1st azoles agent to prove activity upon the zygomycetes, a difficult-to-treat family that involve *Mucor* and *Rhizopus* species. According to review of literature, it was known that analytical methods like HPLC and UPLC methods are available for the determination of Posaconazole as alone or in composite with other antifungal drugs in plasma and serum [5-10]. So an attempt was made to develop a simple, precise, sensitive, rapid and accurate method for the Posaconazole detection using an economical mobile phase which is ecofriendly and validate the method by using RP-HPLC.

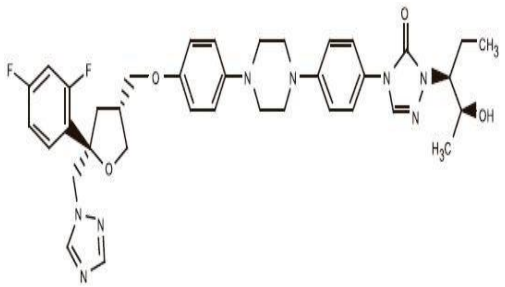

Fig 1: Structure of Posaconazole

2. MATERIAL AND METHOD

2.1 Sample

Posaconazole drug is obtained from Mylan Labs and. Tablets of Posaconazole purchased at local market. All secondary standards were standardized against primary standards

2.2 Solvents &Reagents

Hplc grade , Acetonitrile and Water was purchased from Fischer Scientific chemicals. All other reagents were of analytical grade, and their solutions were prepared with purified water (HPLC grade)

2.3 Instrumentation & analytical conditions

HPLC experiments were performed on an Shimadzu technologies consists of binary pump, an automatic injector and diode array detector, LC solution software was employed for data acquisition. Optimum chromatographic separation of Posaconazole have been achieved with in 3.98 min employing an Inertsil ODS-2V column as stationary phase using a mobile phase composed of ACN: Water (90:10v/v), flow rate of 1ml/min and detection at 262nm.The experiment were conducted at 30ºC and the injection volume was 20µl for standard and samples.

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2.4 Preparation of Standard solution

Weigh accurately about 10mg of Posaconazole standard drug and is transferred into 10ml of volumetric flask. To it add a little quantity of Mobile phase for the drug to dissolve and after complete dissolution of the drug make up to the mark with Mobile phase (1000 µg/ml). From the above primary stock solution (1000µg/ml), pipette out 1ml of the solution and transfer it into another volumetric flask and make up to the mark with Mobile phase (100µg/ml). From the above standard solution, several working standard solutions are prepared by serial dilution technique.

2.5 Preparation of Sample solution

Weigh 5 tablets and find out the average weight of each tablet and powder it .Accurately weigh the powdered tablet equivalent to 5mg of Posaconazole and transferred into 25ml conical flask, to it add little quantity of mobile phase and degassed it for complete dissolution of the drug and make up to the mark with mobile phase(200µg/ml) primary stock, from primary stock solution, pipette out 0.5µl of the solution into 10ml volumetric flask and make up to the mark with mobile phase (100µg/ml)-secondary stock

3. RESULT AND DISCUSSION:

3.1 HPLC method development

Chromatographic parameters were optimized to obtain a simple, rapid, and method for The estimation of Posaconazole in bulk and combined dosage form some internal parameters were evaluated such as chromatographic column, mobile phase composition temperature was adjusted to obtained a reasonable run time, good symmetry, suitable theoretical plates and acceptable resolution the chromatogram of optimized working standard solution of Posaconazole is shown in the Table-1

Table- 1 Optimized chromatographic condition

S.NO	Parameter	Method
1	Column Specification	C18 Inertsil ODS-2V column (250×4.6, 5µm)
2	Mobile Phase	ACN: WATER (90:10 v/v)
3	Temperature	30ºC
4	Flow Rate	1ml/min
5	Injection Volume	20µl
6	Detection Wavelength	262nm
7	Type of Elution	Isocratic
8	Detector	PDA detector

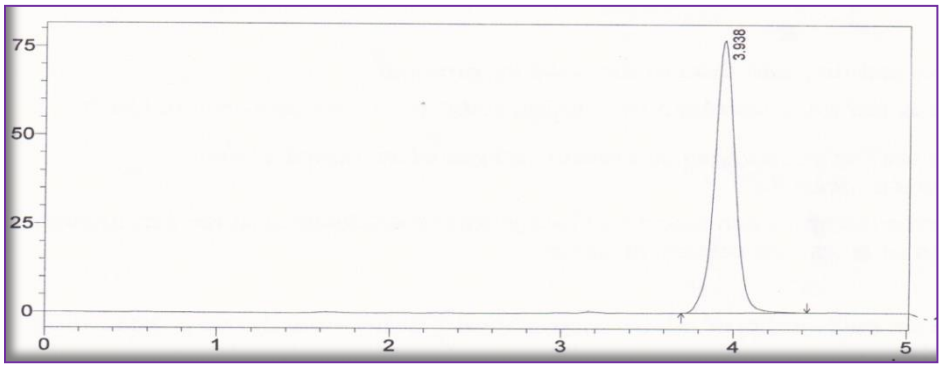

Figure-1 Optimized chromatogram of Posaconazole

3.2 Analytical method validation

3.2.1 Specificity:

Chromatograms for blank and Posaconazole were generated individually to ensure the identity of analyte under study.

3.2.2 System suitability:

Table-2 System suitability data

S.no	**Parameter**	**Acceptance criteria**	**Result**
2	Plate count	NLT 2000	5341
3	Tailing factor	NMT 2.0	1.16
4	Resolution	NLT 2	-
5	%RSD	NMT 2.0	0.34

3.2.3 Linearity:

The Posaconazole was chromatographed using the Mobile Phase; the linearity of peak area response versus concentrations was studied from 10-50µg/ml for Posaconazole a linear response was observed over the examined concentration range. The correlation coefficient found to be 0.9998

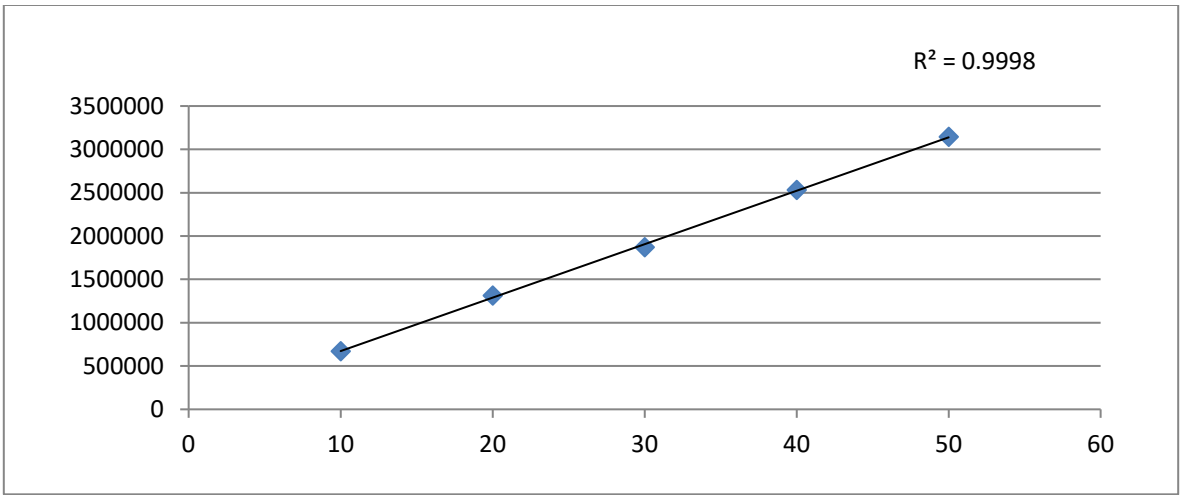

Figure-2 Calibration curve of Posaconazole

3.2.4 Precision:

Precision was determined as repeatability and intermediate precision by analyzing the samples in accordance with ICH guidelines. Determinations were performed on the same day as well as on consequent days each stage of precision was investigated by 3sequential replicates of injections of 30µg/ml the precision was expressed as the relative standard deviation (RSD)

Table-3 Precision data

S.no	Concentration (µg/ml)	Amount Found (µg/ml)	Percentage %	Average %	S. D	%RSD
1	30	29.4	98%	98.4%	1.12	1.23
2		28.9	96.3%			
3		29.6	98.6%			
4		29.9	99.6%			
5		29.5	98.3%			
6		29.9	99.6%			

3.2.5 Accuracy:

The study of recovery of Posaconazole was evaluated in triplicate at 3 concentrations level i.e. 50%, 100%, 150%, of working concentrations of the sample. The percentages of recoveries were calculated.

Table-4 Accuracy data

%Concentration (at specific conc level)	Amount present(µg/ml)	Amount added	Amount Recovered	% Recovery	Mean recovery
50%	30	15	14.9	99.3	99.56
100%		30	30.2	100.6	
150%		45	44.5	98.8	

3.2.6 Robustness:

Robustness was carried by varying three parameters from the optimized chromatographic conditions such as flow rate (±0.2ml/min), wave length (±2nm), and column temperature (+2C)

Table-5 Robustness data

Parameter	Normal	Variation
Wavelength Variation	262nm	260nm
		264nm
Column Oven Temperature Variation	30ºC	28ºC
		32ºC
Flow Rate Variation	1ml/min	0.8ml/min
		1.2ml/min

3.2.7 Limit of detection &Limit of quantification:

The sensitivity of the method was estimated in terms of limit of detection and limit of quantification. LOD =3.3×ASD/S and LOQ=10×ASD/S, where "ASD" is the average standard deviation and "S" slope of the line.

Table-6: LOD and LOQ data

Drug name	LOD	LOQ
Posaconazole	0.26 µg/ml	0.80 µg/ml

CONCLUSION:

In the present investigation, the chromatographic method developed for Posaconazole is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies

Significance:

1. Posaconazole is an antifungal drug which is used in many conditions of infections and its method development for its detection from bulk and marketed preparations would help greatly for its rapid separation, testing and detection.

2. Acetonitrile (ACN) as one component of mobile phase performs dual functions of separation as well as column preservation.

3. Water as second component is comparatively much better than phosphate buffer for column life Usage

4. The use of 90 parts of ACN in mobile phase will yield better column life, results in lesser expenses and overall ultimately profit.

5. The sensitivity of proposed method can be proved by lowest values of LOD and LOQ as obtained by the method.

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