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Method Development and Validation of Ketoconazole by HPLC

Patil Rohit*[1], Tamboli Ashpak[2], Shinde Monika[3]
Department of Pharmaceutical Chemistry, Sahyadri Collage of Pharmacy
Methwade, Sangola, Solapur, Maharashtra, India.413307.

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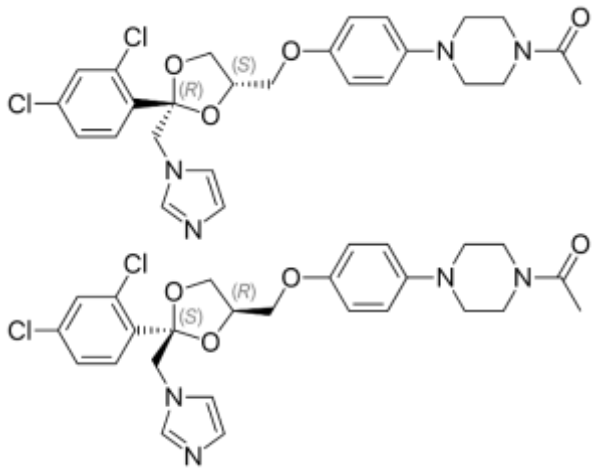
ABSTRCT:- A simple accurate rapid and precise RP-HPLC method has been developed and validated for determination of ketoconazole. The RP-HPLC separation was achieved in mobile phase water 0.1% Triethylamine at flow rate of 1 ml/min at ambient temperature. The runtime of slide is 10 min for ketoconazole.50%-0.1% Trimethylamine are used as dilute for validation of ketoconazole Agilent Zorbax SB-Aq (250x4.6mm, µ) column are used.

KEYWORDS:-Ketoconazole,0.1% Triethylamine, HPLC, Validation Reverse-phase HPLC method, ICH guidelines.

I. INTRODUCTION:-

Ketoconazole was discovered in 1967 at Janssen Pharmaceutical. Ketoconazole is chemically Cis-1 acety-4-[4-[2-(2,4-dichlorophenyl)-2H-imidazolylmethyl)-1,3-dioxolan-4-y1] methoxy]phenyl]-piperazine. Ketoconazole was the only systemic antifungal available for almost decade. Ketoconazole was introduced as the prototypical medication of the imidazole group of antifungals. Ketoconazole is practically insoluble in water, sparingly soluble in strong acid and soluble strong base. It is imidazole derivative with molecular weight 531.44.



Chemical structure of ketoconazole

Molecular formula-$C_{26}H_{28}Cl_2N_4O_4$
Molecular Weigh- 531.43g/mole

II. MATERIAL & METHOD:-

Ketoconazole was obtained from Vamsi Labs Ltd Solapur as a gift sample. Ketoconazole tablet was used as pharmaceutical dosage form for the study.it was purchase from local pharmacy,Atpadi

Chromatographic Condition:-

An Agilent Zorbax bonus RP-(250mm x 4.6mm,5, µ) column was used for the chromatographic separation under suitable condition. The mobile phase consisting of 0.1% triethylamine: ACN as a ratio 30:70 with the flow rate 1ml/min and run time was 10min. detection of the drug was Carried at 230nm.

Preparation of standard solution:-

1. **Standard stock solution-I(SSS-I)**

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Initially prepare a standard stock solution (SSS-I) of by adding 10 mg of ketoconazole in 10ml volumetric flask & add 5ml of diluent, mix for 2 min and make the volume to 10ml with diluent (Conc. Of ketoconazole 100 µg/ml)

2. Then add 1.0 ml of SSS-I in 10 ml volumetric flask and add 5ml diluent and vertex and make up the volume with diluent. (Conc. Of ketoconazole 100 µg/ml)

Chromatographic conditions

Column Temprature	30^0C
Flow rate	1ml/min
Mobile Phase	0.1%Triethylamine:CAN(30:70)
Runtime	10 minutes
Injection Volume	10 µl
Wavelength	230nm
Diluent	50%-0.1%Triethylamine:50%-CAN
Column	Agilent Zorbax SB-Aq (250 x 4.6 mm,5µ)

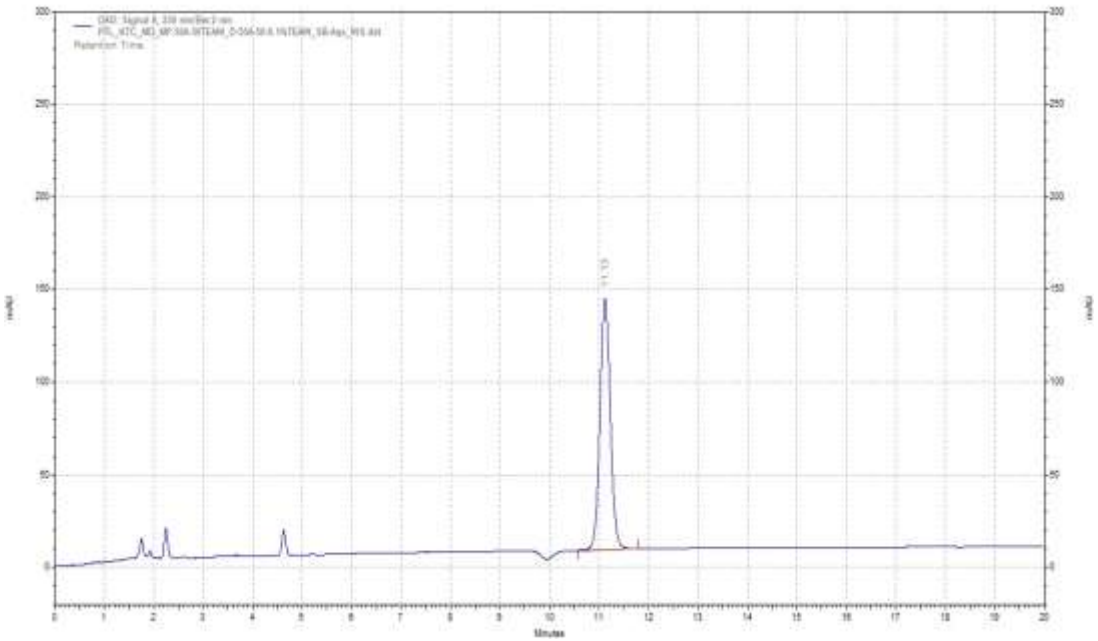

Fig.1 Chromatograph of Standard Ketoconazole

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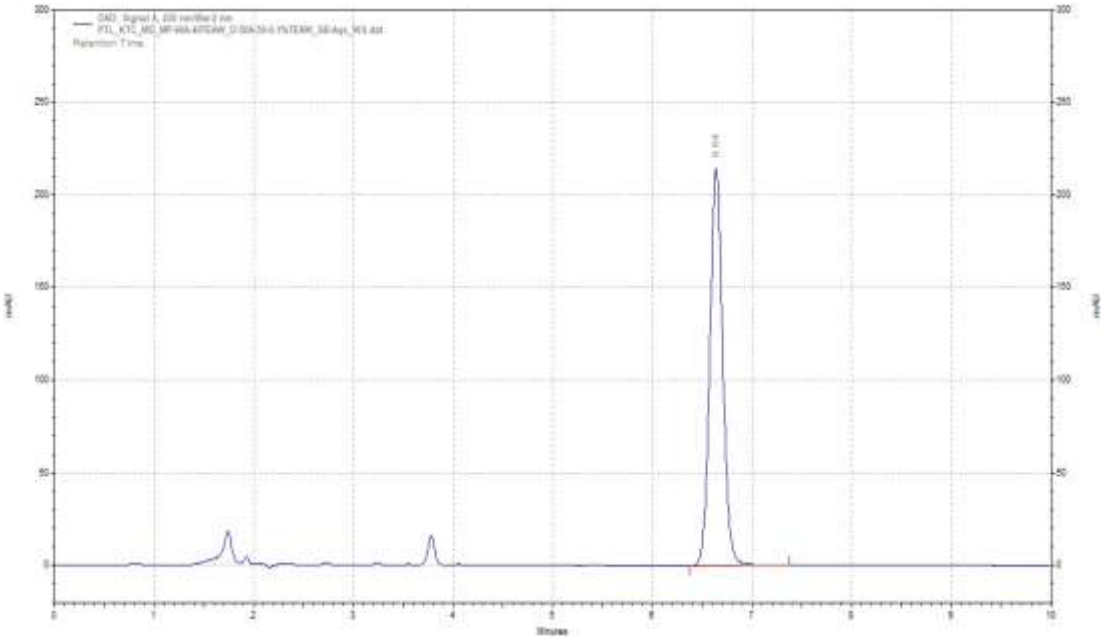

Fig2. Chromatograph of Sample Of Ketoconazole

Tablet Sample Preparation For Assay:-

a. Tablet sample solution (TSS):

1. 10 Tablets were weighted and average weight was calculated and tablet were crushed in mortar an pestle
2. Powder weight equivalent to 20mg ketoconazole was weighted into 10ml volumetric flask and add 5ml diluent, sonicated for 10 min and make the volume up to 10ml with diluent (Conc. Of ketoconazole =2000 µg/ml)
3. Pipette out 0.5ml from above solution in a 10ml volumetric flask and dilute it up to

The mark with diluent (Conc. Of ketoconazole 100 µg/ml

Selection of wavelength:-

The sample was scanned from 200-400 mm with PDA detector. The Wavelength selected for analysis choose was 230 nm of basis of appropriate intensity of ketoconazole.

Method Validation :

Linearity :

1. 5 sample of varying concentration ranging from 80-120 were made.
2. The concentration are given below :
3. The sample preparation are given as below :
4. X ml of Chlorthalidonewere added to 10ml diluents to make up the concentrations given above.

Table No:2

X ml Of SSI	Diluted
0.8	10
0.9	10
1	10
1.1	10
1.2	10

Accuracy:

1. Samples were prepared of 80% , 100% and 120% concentration by spiking the same amount of concentration given above table for Chlorthalidone.
2. Samples were injected in duplicate to calculate % RSD.
3. % Recovery was also calculated.

System Suitability :

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1. A single sample was prepared as described and 5 injections were made from same sample and checked for system suitability .

Precision:

The precision of the method was done by system precision. The percentage RSD value was found to be within the limit . The percentage RSD value for peak area ratio of Chlorthalidone obtained, thus the result showing that equipment used for the work.In that precision method mainly 5 samples are formed .

Limit of Detection (LOD) and Limit of Quantification (LOQ) :

1. It Was calculated for both drugs by using ANOVA technique
2. Formula : 3.3 ×Std. Error of Intercept

LOD = Coefficients of X Variable 1

LOQ =10 × Std. Error of Intercept

Coefficients of X variable 1

III. RESULT AND DISCUSSION :

Assay: Assay was found to be 101.87% of Chlorthalidone in below table .

Table No : 3

Sample	Area	Assay
WS	3880918	-
DP	3872522	99.78

Linearity :

5 Sample of varying concentration ranging from 80-120were made. The Concentration are given below :

Table No : 4

% Level	Ketoconazole Conc (µg/ml)	Area
80	80	2306514
90	90	3104748
100	100	3889328
110	110	4640133
120	120	5432558

Precision:

The precision of the Chlorthalidone method was found to be good with % RSD less than 2, Indicate the method was precise and the results presented below table .

Table No : 5

Sample ID	Area
Rep 1	3889328
Rep 2	3881561
Rep 3	3878016
Rep 4	3885097

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Rep 5	3870589
Average	3880918
STDEV	7136.446
RSD	0.18

Accuracy:
In accuracy study percentage recovery range of Chlorthalidone 100.56% to 100.100.50% . The range of % RSD is 0.07 % to 0.11% .

Table No : 6

Sample ID	**Reps**	**Spiked Conc(µg/ml)**	**Area**	**AmtRecovered(µg/ml)**	**% Recovery**	**Average**	**STDEV**	**RSD**
80%	Rep 1	79.976	4642278	80.42	100.56	100.51	0.072909	0.07
	Rep 2	79.976	4637518	80.34	100.46			
100%	Rep 1	99.97	5773415	100.02	100.05	100.11	0.085641	0.09
	Rep 2	99.97	5780404	100.14	100.17			
120%	Rep 1	119.964	6948968	120.38	100.35	100.43	0.108057	0.11

System Suitability:

Table No :7

Sample ID	RT	TP	Asymmetry
Rep 1	4.93	11010	1.04
Rep 2	4.93	11151	1.09
Rep 3	4.93	11000	1.09
Rep 4	4.93	10981	1.08
Rep 5	4.93	10856	1.07
Average	4.93		
STDEV	0		

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LOD & LOQ:
LOD & LOQ of Ketoconazole is 1.94µg/ml & 5.88 µg/ml.

Table No: 8

LOD	1.94	µg/ml
LOQ	5.88	µg/ml

IV. CONCLUSION:

It includes that the developed method is simple, accurate and precise and suitable for the routine analysis,The developed methods were validated as per ICH guidelines and were found to be within limit.

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