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'A NOVEL ANALYTICAL METHOD DEVELOPMENT BY HPLC, HPTLC AND UV TECHNIQUE FOR ACTIVE PHARMACEUTICAL INGRADIENTS IN FORMULATIONS'

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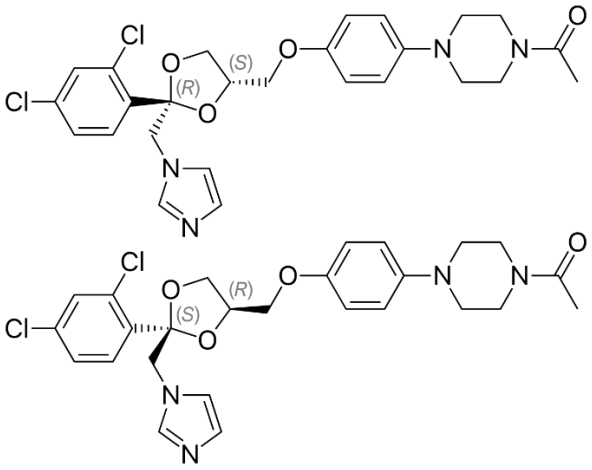
ABSTRACT

In this study, the author has developed a HPTLC method for the estimation of Chlorthalidone in Commercial brand CTD-12.5 of Chlorthalidone. The values obtained suggested that the proposed HPTLC method was simple, precise, rapid and robust for determination of ketoconazole. The mobile phase was simple to prepare and economical. The authors then validated the method as per ICH guidelines and correlated the obtained values with standard values. Satisfactory result were obtained.

KEYWORDS: HPTLC method, Chlorthalidone, CTD-12.5.

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

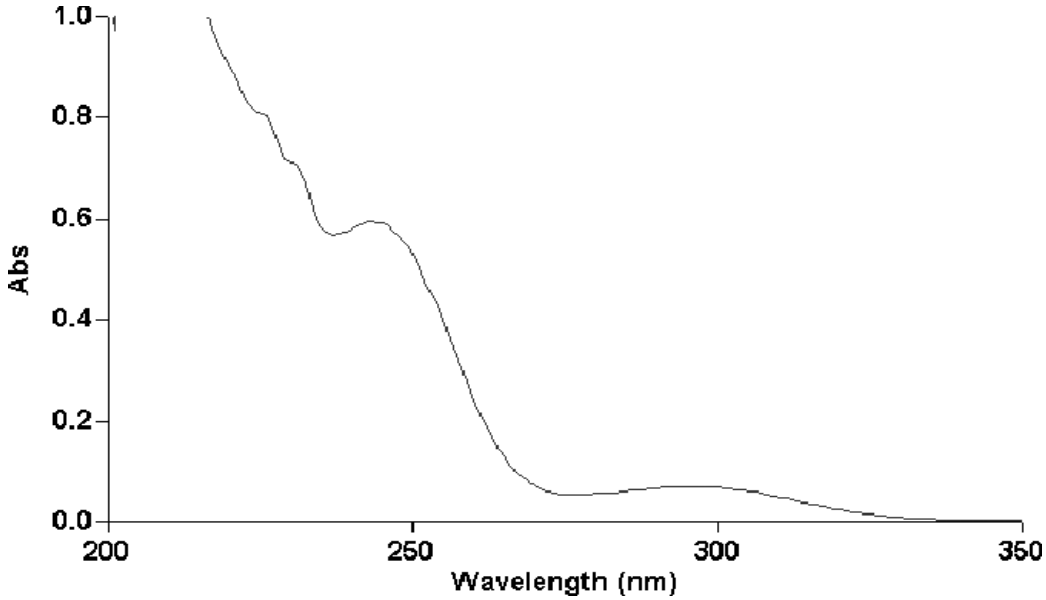
MATERIAL AND METHOD

Preparation of standard stock solution

Standard stock solution of Ketoconazole was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, working standard solution was prepared containing 100 µg/ml of Ketoconazole.

Selection of analytical wavelength

From the standard stock solution further dilutions were made using methanol and scanned over the range of 200 – 400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 246 nm.



Selection of mobile phase and chromatographic conditions

Chromatographic separation studies were carried out on the working standard solution of Ketoconazole 100 µg/ml. Initially, trials were carried out using various solvents in various proportions on normal TLC plates to obtain the desired R_f and shape for drug peak. After few trials, n-Hexane: Chloroform (6.5:5.5 v/v) was chosen as the mobile phase, which gave acceptable peak parameters. Other chromatographic conditions like chamber saturation time, run length, sample application volume were optimized.

Preparation of sample solution

A tablet containing 200 mg of Ketoconazole (KETOCIP) was weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with methanol to get concentration (1000 µg/ml) and was sonicated for 10 min. Solution was filtered and 1 ml of filtrate diluted to 10 ml with methanol. 4 µl of the resultant solution was applied on TLC plate to get concentration of 400 ng/ban.

Densitogram

Solution of Ketoconazole (100 µg/ml) was prepared. 4 µl (400 ng/band) of solution was applied on pre-activated TLC plate with the help of Hamilton syringe (100 µl), using Linomat 5 sample applicator. The development chamber was saturated with mobile phase for 10 min. The spotted plate was placed in the saturated chamber and developed up to 80 mm distance. The plate was dried and was scanned over 90 mm distance at 246 nm. The retention factor was found to be 0.24 ± 1.5.

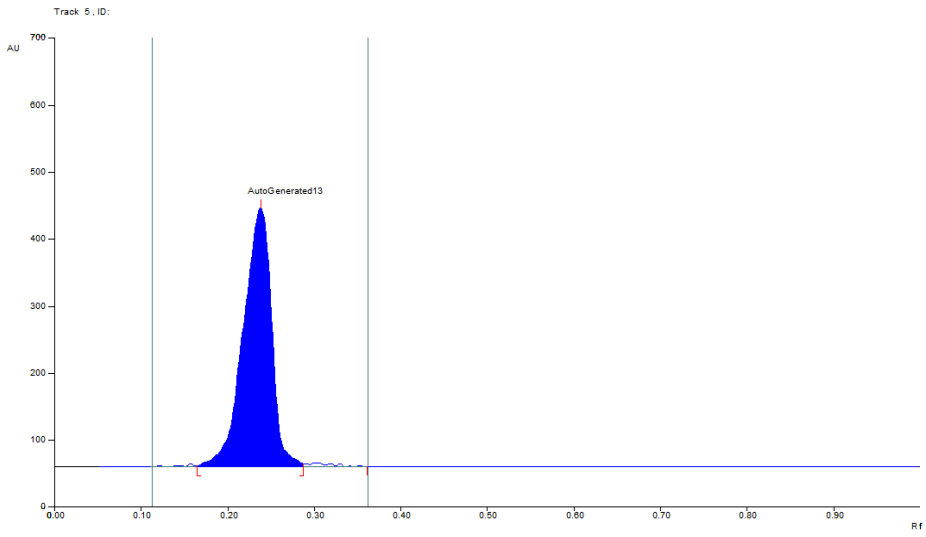


Fig. 2: Densitogram of standard solution of Ketoconazole (400 ng/band).

Table 2: System suitability parameters for Ketoconazole.

Summary of chromatographic parameters selected

Chromatographic parameter are summarized in Table 3.

Drug	Conc. (ng/band)	Rf	Area	Asymmetry
Ketoconazole	1000	0.43± 0.07	8447.7	0.86

Table No 3: Chromatographic Parameters.

1	Stationary phase	TLC aluminium plate precoated with silica gel 60 F_{254}
2	Mobile phase	n-Hexane: Chloroform(6.5:4.5v/v)
3	Detection Wavelength	246nm
4	Saturation time	10 min
5	Band width	6 mm

2. Validation of Analytical Method

The method was validated as per ICH Q2 (R1) guidelines.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.996, indicating the non-interference of any other peak of degradation product or impurity.

Linearity

From the 100 µg/ml solution of Ketoconazole, five replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing six concentrations over the concentration range of 200-1200 ng/band for Ketoconazole. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 3. The results found to be linear with regression equation of y = 9.1174x + 4941.3.

Table 3: Linearity study of Ketoconazole.

Replicate	Concentrations of Ketoconazole (ng/band)					
	200	400	600	800	1000	1200
1	5749.2	8871.6	11008.5	13079.3	14222.9	15176.4
2	5525	9204.9	11028.5	13039.9	14226	15091
3	5574.7	9008.8	11418.1	12718.6	13950.1	14872
4	5776.1	8936.3	11216.6	13053.6	14130.7	15039.5
5	5722.2	8799.1	11258	13017.4	14548.3	14440
Average	5669.44	8964.14	11185.94	12981.76	14215.6	14923.78
SD	112.205	155.352	170.516	148.811	217.111	292.359
% RSD	1.979	1.733	1.524	1.146	1.527	1.959

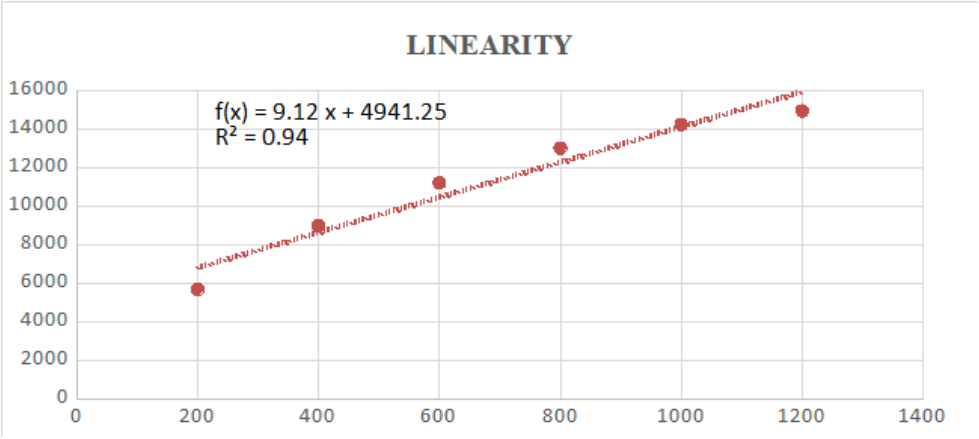


Fig 3: Calibration curve of Ketoconazole reference standard.

Range

Ketoconazole = 200-1200 ng/ban**.**

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 3 replicates of 3 concentrations were analyzed on the same day and percentage RSD were calculated. For the inter day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For intraday precision and inter-day precision results obtained are shown in Table 4.

Table 4: Intraday and Intraday variation studies data for Ketoconazole.

Concentration (µg/ml)	Intra-day Precision			Inter-day Precision		
	Average area	% Recovery	% RSD	Average area	% Recovery	% RSD
400	99.44904	1.22153	0.012289	8568.167	397.7962	1.229
800	100.3819	1.848947	0.018419	10432.63	602.2916	1.842
1200	100.7149	1.181856	0.011735	12287.37	805.7195	1.173

Range

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

LOD and LOQ are calculated from the formula: -

LOD= 3.3 σ / S

LOQ = 10 σ / S

Where, σ = standard deviation of Y intercept

S = slope of the calibration curve.

LOD = 33.381 ng/ band

LOQ = 101.153 ng/band**.**

Assay

KETOCIP (200 mg) tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was applied and area was recorded. Basic concentration of sample chosen was 400 ng/band from tablet solution. Concentration and % recovery were determined from linear equation. Assay results obtained are shown in Table 5.

Table 8: Assay of marketed formulation.

Sr. No.	Peak area	Amount recovered (ng/band)	% recovery
1	9.1174	403.372	100.843
2	9.1174	394.169	98.542
3	9.1174	395.749	98.934
4	9.1174	398.662	99.656
5	9.1174	399.533	99.883
6	9.1174	404.084	101.021
Mean			99.814
SD			0.993
%RSD			0.995

Accuracy

To check accuracy of the method, recovery studies were carried out by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 1000 ng/band. % recovery was determined from linear equation. Accuracy results obtained are shown in Table 9.

Table 9: Accuracy studies of Ketoconazole.

Level	Amount of sample taken (ng/band)	Amount of standard spiked (ng/band)	Area	Amount recovered (ng/band)	% recovery (Mean ±%RSD)
50%	1000	500	1066.8	101.007	1.196
100%	1000	1000	12916.4	98.371	1.325
150%	1000	1500	13989.90	99.222	0.660

8. Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which chamber saturation time was altered. Time was also changed from spotting to development and development to scanning and the effects on the area were noted. It was found that method is robust. The results obtained are shown in Table10.

Table 10: Robustness study.

Sr. No.	Parameters	Variation	%RSD
1.	Time from application to development	0 min	1.909
		30 min	0.494
		60 min	1.624
2.	Time from development to scanning	0 min	1.774
		30 min	0.455
		60 min	1.954
3.	Saturation Time	15 min	1.951
		13 min	1.183
		17 min	2.009

Summary of validation study

Table 11: Summary of Validation Parameters.

Sr. No.	Validation parameters	Ketoconazole
1.	Linearity equation R^2 Range	y = 9.1174x + 4941.3 R^2 = 0.9442
2.	Precision	(%RSD)
	Intraday	1.416
	Intraday	1.842
3.	Assay	400 ppm
4.	Accuracy	600 ppm
	50	11246.1
	100	10666.8
	150	10650.6
5.	Limit of detection	40.638
6.	Limit of quantitation	123.145
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION

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

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