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A Study Of Method Development, Validation and Forced Degradation Studies of Clotrimazole by Using UV Spectrophotometry

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ABSTRACT

To develop a simple, precise, accurate, and stability indicating a UV-method for estimation of Clotrimazole. In bulk and formulated dosage form. The method was under subjected to stress degradation at different conditions recommended by the International Conference on Harmonization (ICH). The drug samples are generated and used for the degradation studies. The λ_{max} of the Clotrimazole was found to be 220 nm. The linearity of calibration curve (Absorbance Vs Concentration) in pure solution was checked over the concentration ranges of about 5-30µg/ml for Clotrimazole respectively, with the correlation coefficient higher than 0.999. The regression equation of the curve was Y = 0.0168x + 0.0041. The % RSD was found to be within the limit as per ICH guidelines. The obtained percentage recovery of Clotrimazole was found to be within the limit 100% ± SD. The proposed method can be successfully applied for the method development, validation and stress degradation studies of Clotrimazole. The percentage degradation limit should be 5-20%. The drug Clotrimazole was found to be within the limit.

Keywords: UV Spectroscopy, Clotrimazole, Forced degradation, validation

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INTRODUCTION

Clotrimazole is chemically known as 1-(2-chlorophenyl) biphenyl methyl)-1H-imidazole (shown in fig no: 1).it is used in Anti-fungal infection. It works to kill individual Candida or fungal cells by altering the permeability of the fungal cell wall. It binds to phospholipids in the cell membrane and inhibits the bio synthesis of ergo sterol and other sterols required for cell membrane production.

Pharmacokinetic fundamentals of vaginal treatment with Clotrimazole, after the vaginal treatment with Clotrimazole the small fraction absorbed into the systemic circulation between 3% and 10% of the dose is subjected to metabolism and excretion as after oral or intra venous administration.[1-3]

Extensive literature survey was carried out which revealed that a few reports on spectrophotometric methods are also available. Till date there is no report available on Clotrimazole in forced degradation studies using UV Spectrophotometry The specific aim of the research was to develop a UV method for the forced degradation studies of Clotrimazole, in bulk and formulated dosage form and to validate the proposed methods in accordance with ICH guidelines for the intended analytical application [4-12].

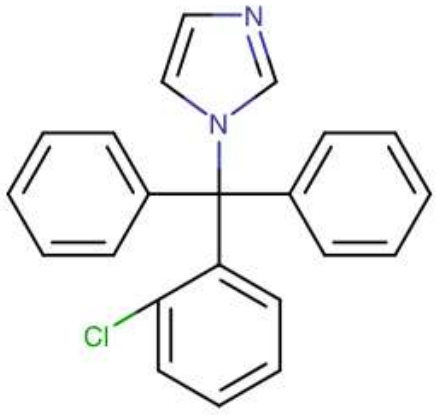

Figure 1 Structure of Clotrimazole

MATERIALS AND METHOD

Instrumentation:

A UV-VISIBLE spectrophotometer (LAB India) equipped with UV detector 1.0 cm matching quartz cells was used.

Chemicals and reagents

The Standard Clotrimazole was obtained from Saimirra inno Pharm Pvt Ltd, Chennai, Tamilnadu, India. Clotrimazole tablet (candid-v3) was obtained from Glenmark pharmaceuticals Ltd, Hydrochloric acid (AR Grade) was obtained from Himedia laboratories (India) Pvt Ltd.

METHOD DEVELOPMENT BY UV SPECTROSCOPIC METHOD:

Solvent of selection:

The solubility of Clotrimazole was determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility was carried out in polar and non-polar solvents. From the solubility data 0.1 N Hcl was selected as solvent for the analysis of Clotrimazole.

Determination of λ max:

The quantity containing 100mg of Clotrimazole were taken in 100ml standard flask and volume was made up to the mark with 0.1 N Hcl to obtain 1000µg/ml .from which 10 ml of solution was taken from above standard flask, and diluted to 100ml and made up to volume to obtain 100µg/ml. From the 100µg/ml stock solution, 1 ml was taken and transferred into 10 ml standard flash, and the volume was made up to the mark with 0.1N Hcl to obtain 10µg/ml concentration of Clotrimazole respectively. The above solution was s canned over range of 200-400nm. It is shown by figure: 2

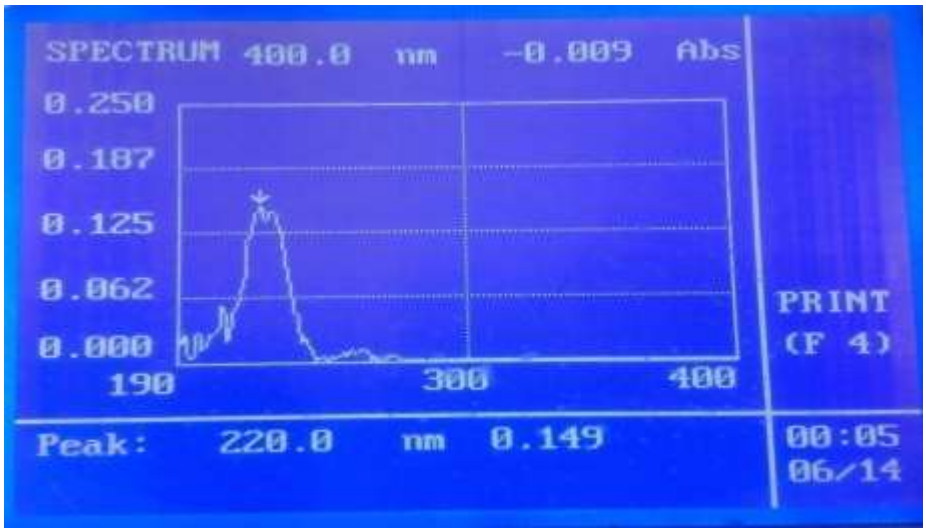

Figure 2 Spectrum of Clotrimazole

Assay of Clotrimazole:

Standard preparation:

The quantity containing 100mg of Clotrimazole was taken into 100ml clean, dry standard flask, 0.1N Hcl was added and the volume was made up to the mark to obtain 1000µg/ml. From the 1000µg/ml stock solution 0.1ml was taken into10ml standard flask and diluted up to the mark with 0.1N Hcl ml to obtain 10µg/ml respectively.

Sample preparation:

10 Tablets were weighed and powder it, a powder equivalent to 100mg of Clotrimazole was taken into 100ml clean, dry standard flask and volume was made up to the mark with 0.1N Hcl to obtain

1000µg/ml. From the 1000µg/ml stock solution 10ml was taken into 100ml standard flask and diluted up to the mark with 0.1N Hcl to obtain 100µg/ml. From the above solution to pipette out 1ml of solution into 10 ml clean, standard flask and volume made up to the mark with solvent to form 10µg/ml concentration respectively.

VALIDATION OF UV SPECTROSCOPY:

Linearity studies:

The linearity of calibration curve (Absorbance Vs Concentration) in pure solution was checked over the concentration ranges of about 5-30µg/ml for Clotrimazole respectively and the results for linearity values of Clotrimazole were shown in the following Table no: 1 & Figure: 3.

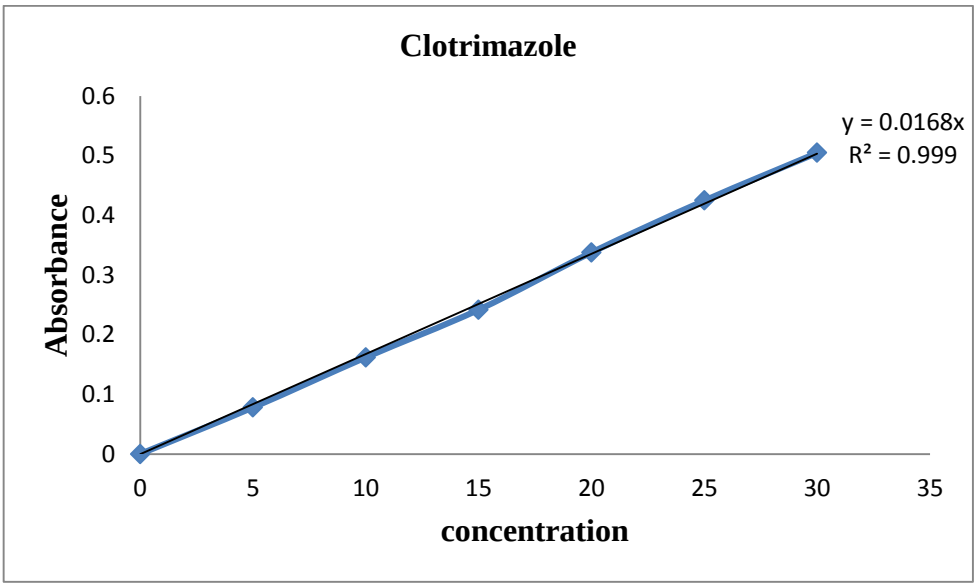

Figure: 3 Calibration curve of Clotrimazole

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

The percentage recovery of Clotrimazole was found to be 100.29%, 99.80%, and 100.97% from 80%, 100% and 120% sample solutions respectively. The obtained percentage recovery of Clotrimazole was found to be within the limit. This indicates the proposed method was more accurate. It shown in table: 2.

Precision:

Precision was determined by using the method to assay sample for a sufficient number of times to obtain statistically valid results. The precision then expressed in term of relative standard deviation. Acceptance criteria for the precision of the method should not be more than 2%. The results for intraday were shown in the table: 3.

Ruggedness: (Intermediate precision)

Ruggedness of the method was confirmed by the analysis of formulation was done by using different analysts. The amount and % RSD was calculated. The readings were tabulated in table 4.

Robustness:

Robustness of the method was confirmed by deliberate change in the flow rate, wave length and mobile phase composition was made to evaluate the impact on proposed method. The sample were analysed in six replicates and % RSD was calculated. The readings were tabulated in Table 5.

Degradation studies:

All stress decomposition studies were performed at an initial drug concentration of 1000µg/ml.

Degradation studies of Clotrimazole in Acidic condition:

To pipette out 1ml of stock solution (1000µg/ml) concentration of Clotrimazole, added 1ml of acidic medium 0.1NHCl was added in 10 ml of volumetric standard flask, the volume made up to the mark with 0.1N NaOH. The solution was heated at 60°C for a period of 4hrs. In a different time intervals the sample aliquots was withdrawn at 2hr and 4hr, then neutralized with 2ml of 0.1N NaOH. For the blank, 0.5ml solution of 0.1N HCl and 0.5ml solution of 0.1N NaOH was used.

Degradation studies of Clotrimazole in Alkaline condition:

To pipette out 1ml of the stock solution (1000µg/ml) concentration of Clotrimazole, added 1ml of alkaline medium 0.1N NaOH was added in a 10ml of volumetric standard flask, the volume made up to the mark with 0.1HCl .the solution was heated at 60°c for a period of 4hrs.in a different time intervals the sample aliquots was withdrawn at 2 and 4hr, and then neutralized with 2ml of 0.1N HCl. For the blank, 0.5ml solution of 0.1N HCl and 0.5ml solution of 0.1N NaOH was used.

Degradation studies of Clotrimazole in Oxidation condition:

To pipette out 1ml of the stock solution (1000µg/ml) concentration of Clotrimazole, added 1ml of 3% v/v solution of hydrogen peroxide (oxidizing medium). The volume made up to the mark with 0.1NaOH.then the solution was analyzed without heat at 0, 2 and 4hrs, didn't find out the degradation. Further went for heated at 60°c for a period of 4hrs.in a different time intervals the sample aliquots were withdrawn at 2 and 4hr. 0.1N NaOH used as a blank.

Degradation studies of Clotrimazole in Thermal condition:

Clotrimazole sample was taken in a petriplate and exposed to dry hot air oven at 0 for 2days of 1mm thickness in a petridish. 10mg of the sample was diluted with 0.1N NaOH in order to make the volume up to 10ml. From this solution; dilutions were carried out to achieve the concentration for the analysis.

Table: 1 Linearity data for Clotrimazole

Concentration (µgm/ml)	Absorbance of Clotrimazole	Statistical analysis of Clotrimazole
5	0.078	
10	0.162	Slope = 0.016
15	0.242	Correlation
20	0.338	co-efficient = 0.999
25	0.425	
30	0.505	

Table: 2 Accuracy for Clotrimazole by UV method

Level	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount found (µg/ml)	Amount recovered	% Recovery	SD	%RSD
80%	10.07	8.045	18.115	8.025	100.29		
100%	10.05	10.150	210.2	10.278	99.80	0.587566	0.005855
120%	10.03	12.145	22.175	12.557	100.97		

***n = 3**

Table: 3 Intraday analysis of Clotrimazole by UV method

S. No	Clotrimazole	
	15 (µg/ml)	20 (µg/ml)
	0.217	0.354
	0.215	0.352
	0.219	0.349
Average	0.217	0.351667
S.D	0.002	0.002517
%RSD	0.921659	0.715624

***Mean of six observations**

Table: 4 Ruggedness of Clotrimazole (different analysts)

S. No.	Clotrimazole			
	Analysts 1		Analysts 2	
	15 (µg/ml)	20 (µg/ml)	15 (µg/ml)	20 (µg/ml)
	0.215	0.362	0.218	0.326
	0.217	0..357	0.216	0.328
	0.219	0.359	0.214	0.331
Average	0.217	0.359333	0.216	0.328333
SD	0.002	0.002517	0.002	0.002517
%RSD	0.921659	0.700356	0.925926	0.766481

*mean of six observations

Table 5 Robustness of Clotrimazole

S. No.	Clotrimazole 218nm (15µg/ml)	222nm (15µg/ml)
	0.278	0.293
	0.276	0.298
	0.281	0.297
Avg	0.278333	0.296
SD	0.002517	0.002646
%RSD	0.904172	0.893835

Table: 6 Stress degradation studies for the determination of Clotrimazole

S.no	Stress condition	Time	Percentage of degraded	Percentage of recovered
1.	0.1N Hcl	2hrs	10.45	89.55
		4hrs	16.76	83.24
2.	0.1N NaOH	2hrs	9.46	90.54
		4hrs	17.47	82.53
3.	3% H_2O_2	2hrs	9.73	90.27
		4hrs	15.7	84.3
4.	Thermal	48hrs	0	100

RESULTS AND DISCUSSION:

In the present work, we have developed a newer, simple, accurate and cost effective UV Spectrophotometric method for the effective determination of Clotrimazole in bulk and formulated Tablet dosage form. Detection of λ max of Clotrimazole was found to be 220nm respectively. The percentage purity of Clotrimazole was found to be 99.30%w/v. The calibration plot for Clotrimazole was observed to be linear in the range of 5-30µg/ml and the correlation co efficient was found to be 0.999 respectively. In precision study it was found that %RSD was less than 2% which indicated that the proposed method has good reproducibility. In accuracy study the % recovery of Clotrimazole in bulk drug samples were ranged 100.29%, 99.80%, and 100.97% which indicate that the method was accurate. Ruggedness study found that %RSD was less than 2%. This indicates that the proposed method was accurate. LOD & LOQ, the limit of detection (LOD) of Clotrimazole was found to be 30.59185µg/ml respectively; LOQ of Clotrimazole was found to be 92.70258µg/ml respectively.

CONCLUSION:

A simple, precise and accurate method was developed by UV Spectroscopy method has been developed for analysing of Clotrimazole in fixed-dose combination of formulated tablets. The method was validated for linearity, precision, accuracy, ruggedness, robustness and LOD & LOQ. The present analytical method can be used for its intended purpose.

REFERENCE:

1. Indian Pharmacopoeia., (2014) Published by the Indian Pharmacopoeia commission 7(2), (pp.181, 400, 1442).
2. A.V.Kasture., K.R.Mahadik., S.G.Wadodkar, H.N.More., (2013). *Pharmaceutical Analysis Instrumental Methods,* 21(1), Nirali Prakashan Pvt Ltd (pp.19.3-20.1), Pune.
3. K.D. Tripathi., Essential of medical pharmacology. (2006). Published by Jaypee brothers medical publisher (Pvt) ltd, (sixth edition), (pp.792-793).
4. Beckett A.H,Stanlake J.B., (2002). *Practical pharmaceutical chemistry*. 2(2), CBS publishers and distributors (pp.275-278), New Delhi.
5. Chatwal, G.R. & Anand, K.S. (2008). *Instrumental Methods of Chemical Analysis*. (5th edition), Himalaya publishing house (pp 2.117- 2.154), New Delhi.
6. Adnan Manassra, Mustafa Khamis, Magdyel-Dakiky, Zuhair Abdel-qader, Fuad Al-rimawi., (2010). Simultaneous HPLC analysis of Betamethasone and Clotrimazole in cream formulation. *Pharmaceutica analytica acta*, 1(2), ISSN: 2153-2435, (pp.1-3), www.Pharm Anal Acta.
7. Bharath S A, Arshad M. D, Venugopal Darak, K Kalyan Chakravathy., (2011). Development and validation of simultaneous spectrophotometric estimation of clotrimazole and tinidazole in tablet dosage form. *Journal of analytical chemistry*, 1(2), (pp.13-17), ISSN 2229 – 6867, www.ijpijournals.com.
8. Code Q2A (1994). *Text on validation of an analytical procedure*. ICH Harmonised Tripartite Guidelines. ISSN: 0975-413 (pp.1-5), Geneva Switzerland.
9. FDA Guidance for Industry. Analytical procedures and method validation (draft guidance), August 2000, 19(1), (PP. 74-79).
10. FDA Guidance for Industry. INDs for Phase 2 and 3 Studies – chemistry, manufacturing, and Controls information. May 2003, 68(97), (PP. 1- 2)
11. Gurdeep R. Chatwal., Sham K. Anand., (2011). *Instrumental methods of chemical analysis* (fifth edition), Himalaya publishing house Pvt Ltd, (pp.1.6, 2.150, 2.170, 2.169, 2.172).
12. Useni Reddy Mallu K. Hussain Reddy, Varaprasad Bobbarala, Somasekhar Penumajji., (2011). Determination of Beclomethasone dipropionate, Clotrimazole, Chloramphenicol and Lidocaine in pharmaceutical formulations using a novel RP-HPLC method.

International Journal of Pharma and Bio Sciences, 2(3), ISSN: 0975-6299, (pp.453-462), www.ijpbs.net.