Asian J. Pharm. Res. Vol 2, Issue 1, 66-69, 2012.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal
of
PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

METHOD DEVELOPMENT, VALIDATION AND STABILITY STUDY OF GRISEOFULVIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV SPECTROMETRIC METHOD

***Arun Kumar Dash and Debananda Mishra**

*****Department of Pharmaceutical Analysis and Quality Analysis,
Royal College of Pharmacy and Health Sciences, Berhampur, Odisha, India.

ABSTRACT

A simple method for the estimation of Griseofulvin in bulk and pharmaceutical dosage forms has been developed. Methanol was chosen as the solvent system. The λmax was found to be 293nm. The responses were linear in the range of 5-35µg/ml. The regression equation of the calibration graph and correlation coefficient were found to be y = 0.078x - 0.001 and 0.999 respectively. The %RSD values for both intraday and interday precision were less than 1%. The recovery of the drug from the sample was ranged between 99.62% and 100.49%. The proposed method was validated for precision, accuracy, intraday and interday assay. Commercial tablets containing 375mg and 250mg of Griseofulvin were analyzed by the proposed method and the results were well within the claimed limits. Further stability studies of Griseofulvin were carried out under acidic, alkaline, hydrolytic and photolytic conditions as per SIAM (Stability Indicating Assay Methods) as described by ICH.

Key words: Griseofulvin, UV spectrophotometry, Validation, Stability Indicating Assay Methods.

INTRODUCTION

Griseofulvin is an antifungal agent derived from the mold *Penicillium griseofulvum*. Chemically Griseofulvin is (2S,6'R)- 7-chloro- 2',4,6-trimethoxy- 6'-methyl- 3H,4'H-spiro [1-benzofuran- 2,1'-cyclohex[2]ene]-3,4'-dione. It binds to tubulin, interfering with microtubule function inhibiting mitosis. It is used in treatment of fungal infections of the skin (commonly known as ringworm) and nails of animals and humans. A literature survey reveals that there are various methods available for estimation of Griseofulvin like Fluorimetric [1], HPLC [2,3], HPTLC [3], LC-MS [4], GC [5]. However some of these methods are costlier, time consuming and are only for estimation of drugs from the biological fluids. Also the above methods are not validated for its performance under stress conditions thus rendering them unsuitable for stability studies. Thus an attempt was made to develop a new, simple, accurate and validated method for determination of Valsartan by UV spectrophotometric method along with its stability studies. The method was validated as per the procedures and acceptance criteria of ICH guidelines.

MATRIALS AND METHOD

Instruments

All absorbance measurements were done with Shimadzu 1700 double beam UV-Spectrophotometer (Japan) with 10mm matched quartz cell and Borosil glass wares were used for the study. All weighing were done on Sartorious BT 2245 electronic balance. All the chemicals and reagents used were of analytical grade (AR) procured from Merck.

Standard Stock Solution

The standard stock solution was prepared by dissolving 10 mg of drug in 10ml of methanol to get a concentration of 1000 mg/ml. It was appropriately diluted with methanol to get a concentration of 100µg/ml and was kept as the stock solution.

Corresponding Author :- **Arun Kumar Dash** Email:- arun.dash@live.com

Asian J. Pharm. Res. Vol 2, Issue 1, 66-69, 2012.

Determination of λmax

The standard solution of Griseofulvin (10 mg /ml) was scanned in the wavelength region of 200-400 nm and the λmax was found to be 293 nm.

Preparation of calibration curve

The stock solution of Griseofulvin was according diluted to obtain concentration in the range of 5-35µg/ml. The absorbances were observed against methanol as blank and the calibration curve was plotted between concentration (x-axis) and absorbance (y-axis).

Assay of tablet dosage form

10 tablets of brand Gris-OD (manufactured by Dr. Reddy's Laboratories, Hyderabad) containing 375mg of Griseofulvin were weighed, average weight determined and finely crushed to powder. An accurate weight equivalent to 10mg of the drug was transferred to 100ml volumetric flask. The drug was extracted 4 times by adding solvent in potions, 20 ml each time and the volume was made upto the mark by using solvent. It was then diluted (within the linearity range), absorbances of the sample solution were recorded at determined λmax and the concentration of the drug in sample was found out. Similarly, the assay of Grisovin (manufactured by Glaxo Smithlkine Pharmaceuticals, Mumbai) containing 250mg of Griseofulvin was carried out.

VALIDATION

1. ***Accuracy*:** The accuracy of the proposed method was tested by recovery studies at 80%, 100%, and 120% by adding a known amount of pure drug to the pre-analyzed formulation of concentration 30µg/ml.

2. *Precision*: The precision of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (30µg/ml) within the Beer's range and finding out the absorbance by the proposed method.

3. *Intraday Assay*: The intraday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (30µg/ml) within the Beer's range and finding out the absorbance by the proposed method at 3 different time period of the same day.

4. *Interday Assay*: The interday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (30µg/ml) within the Beer's range and finding out the absorbance by the proposed method on 3 different days.

5. *Robustness*: The robustness of the method was carried out by changing the solvent system to methanol and water in the ratio 92:8 and 85:15 respectively separately.

6. *Ruggedness*: In order to determine the ruggedness of the proposed method, the method was carried out by two analysts.

DEGRADATION STUDIES

Acid degradation: Accurately weighed 10 mg of the drug was taken in a 10 ml volumetric flask and few drops methanol were added to dissolve the drug .The volume was made up with freshly prepared 0.1N HCl. Then this solution was kept inside a water bath maintained at a temperature of 70°C. Samples were withdrawn in regular interval of 1 hour and the absorbance was measured at the determined λmax and recorded.

***Alkali Degradation*:** Accurately weighed 10 mg of the drug was taken in a 10 ml volumetric flask and minimum amount of methanol were added to dissolve the drug. The volume was made up freshly prepared 0.1N NaOH. This solution was kept inside a water bath maintained at a temperature of 70°C. The samples were withdrawn in regular interval of 1 hour and the absorbance was measured at the determined λmax and recorded.

***Oxidation Degradation*:** Accurately weighed 10 mg of the drug was taken in a 10 ml volumetric flask and few drops of methanol were added to dissolve the drug and the volume was made up with freshly prepared 3% H_2O_2. The samples were withdrawn in regular interval of 6 hour for 36 hours and absorbance were measured at the determined λmax and recorded.

RESULTS AND DISCUSSION

a. *Calibration Curve*: The absorbance for the different concentrations (5-35µg/ml) was recorded at 293nm. The regression equation of the calibration curve was found to be y=0.077x+0.007. The calibration curve is shown in figure 3.and represented in table 1.

b. Accuracy: The % recovery was found to be in the range of 99.62% and 100.49%.

c. Precision: The % RSD for precision was found to be 0.5118.

d. Intraday Assay: The % RSD for Intraday Assay was found to be 0.56839.

e. Interday Assay: The % RSD for Interday Assay was found to be 0.57766

Low values of % RSD indicate that the proposed method is accurate. The data is represented in table 2.

f. Robustness and Ruggedness: The %RSD for Robustness was found to be 0.811641 and 0.302458 for the proposed method by taking methanol and water in the ration 92:08 and 85:15 respectively while the %RSD for Ruggedness was found to be 0.217821 and 0.271501 when it was performed by two analysts separately. The data is represented in table no. 3.

g. Stability Results: The results obtained in various stabity testings are given in table no. 4

Asian J. Pharm. Res. Vol 2, Issue 1, 66-69, 2012.

Fig 1. Structure of Griseofulvin

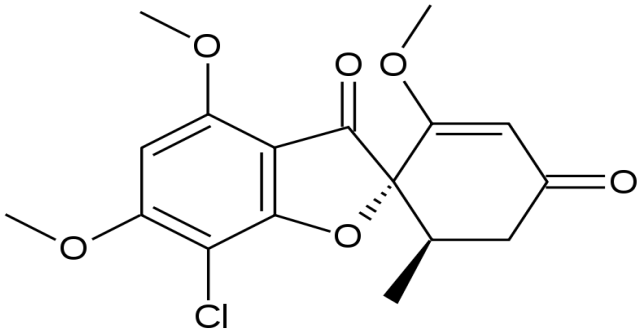

Fig 2. UV Spectrum of Griseofulvin

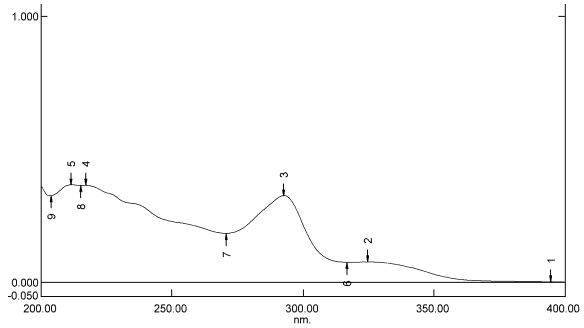

Fig 3. Calibration curve of Griseofulvin.

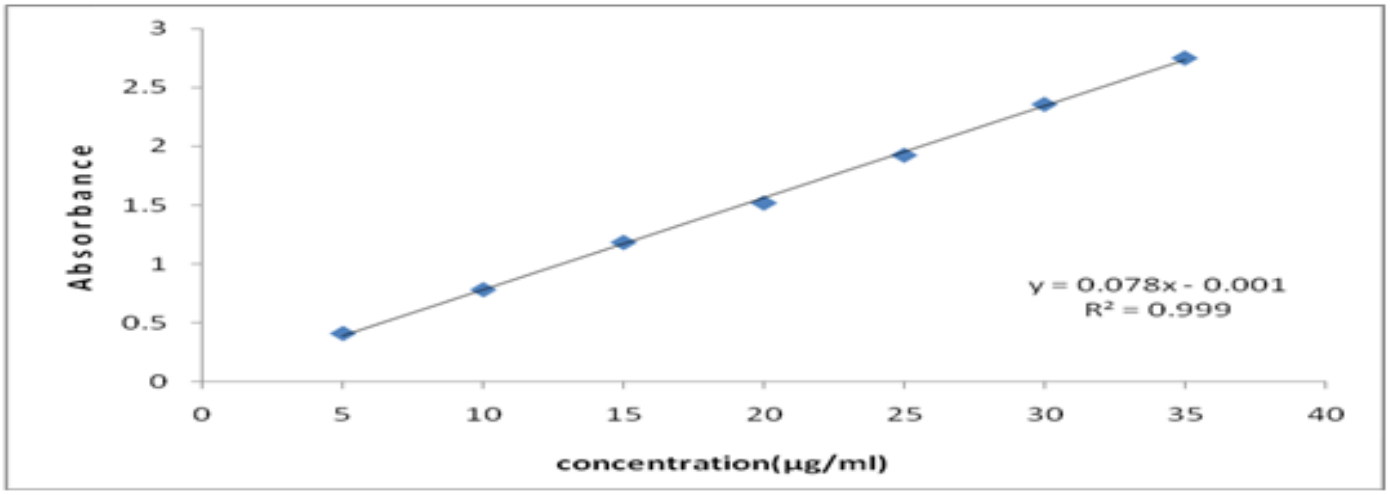

Table 1. Absorbance values for the calibration curve of Griseofulvin

Conc. Of drug (µg/ml)	Absorbance at 293 nm
05	0.407
10	0.780
15	1.182
20	1.518
25	1.925
30	2.358
35	2.752

Table 2. Results Showing Ruggedness of the Proposed Method

	INTRADAY	INTERDAY
Concentration (µg/ml)	30	30
Mean Absorbance*	2.355	2.357
% Relative Standard Dev.	0.56839	0.57766

* Represents the mean absorbance values of 6 replicates.

Asian J. Pharm. Res. Vol 2, Issue 1, 66-69, 2012.

Table 3. Results Showing Robustness and Ruggedness of the Proposed Method

	ROBUSTNESS		RUGGEDNESS	
	Methanol :Water (92:08)	Methanol :Water (92:08)	ANALYST-I	ANALYST-II
Conc(µg/ml)	30	30	30	30
*Mean Absorbance	2.356	2.345	2.358	2.352
% RSD	0.811641	0.302458	0.217821	0.271501

* Represents the mean absorbance values of 6 replicates

Table 4. Stability Study Results

Stress Condition	Time (in hours)	Absorbance after degradation	Concentration(µg/ml)		% Degraded
			Before	After	
Acid Hydrolysis (0.1N HCl)	4	1.968	30	25.21	15.96
Alkaline Hydrolysis (0.1N NaOH)	4	1.546	30	19.83	33.97
Oxidation (3% H_2O_2)	42	1.828	30	23.42	21.93

Table 5. Assay result of the marketed formulation by the proposed method

Formulation	Label claimed(mg)	Observed Amount	% Recovery
Gris-OD	375	372.46	99.32
Grisovin	250	246.53	98.61

CONCLUSION

The proposed method was found to be simple, precise and rapid for the determination of Valsartan from pure and its dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims. Thus the proposed method can be used as an alternative method to the reported ones for the routine analysis of the drug in bulk and pharmaceutical dosage forms and can also be used for dissolution or similar studies.

ACKNOWLEDGEMENT

We are thankful to the authorities of Department of Pharmaceutical Analysis and Quality Assurance, Royal College of Pharmacy and Health Sciences, Andhapasara Road, Berhampur for providing necessary requirements to carry out this research work.

REFERENCES

1. Bedford C, Child KJ, Tomich EG. Spectrophotofluorometric assay of Griseofulvin. *Nature*, 184, 1959, 364–5
2. Hackett LP, Dusci LJ. Determination of Griseofulvin in human serum using high-performance liquid chromatography. *J Chromatogr*, 155, 1978, 206–8
3. Kreuzig F. Determination of Griseofulvin in rat plasma by high-performance liquid chromatography and high-performance thin-layer chromatography. *J Chromatogr*, 163, 1979, 322–6.
4. Mistri HN, Jangid AG, Sanyal M, Shrivastav P. Electrospray ionization LC-MS/MS validated method to quantify Griseofulvin in human plasma and its application to bioequivalence study. *J Chromatogr B Analyt Technol Biomed Life Sci*., 850, 2007, 318–26.
5. Margosis M. Analysis of antibiotics by gas chromatograph-Griseofulvin. *J Chromatogr*., 70, 1972, 73–80.