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Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY FOR GRISEOFULVIN BY RP-HPLC IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To development and validation of a stability indicating reverse phase HPLC (RP-HPLC) method for the analysis of griseofulvin, an antifungal drug, and its assay method, in tablet dosage forms.

Methods: The proposed RP-HPLC method utilizes Shiseido C18, 250 mm × 4.6 mm i.d., 5µ column (at ambient temperature), isocratic run [using Methanol and Water (70:30) as mobile phase], at a flow rate of 1.0 ml/minute, and UV detection at 291 nm for analysis of griseofulvin. This method was selected after applying different chromatographic conditions, the chromatographic variables like flow rate, the composition of mobile phase and nature of stationary phase were studied.

Results: The reported method is linear over the range of 0.1-1.2µg/ml with a coefficient of correlation (r^2) value 0.9998, slope 274.9 and intercepts 19093. The precision study revealed that the percentage relative standard deviation was within the acceptable limit and the mean recovery was found to be between 98%-102%. Griseofulvin was exposed to acidic, alkaline, oxidative, thermal and photolytic stress conditions and the sample was taken at different time intervals. The stressed samples were analyzed by the proposed method. The proposed method can be used for routine analysis stability testing and assay of griseofulvin in quality control laboratories.

Conclusion: An economical, accurate, sensitive and precise HPLC method with ultraviolet detection was developed and fully validated for quality control analysis of griseofulvin in tablets. The proposed method is very rapid, where the total analytical run time is 5.16 minute.

Keywords: RP-HPLC, Method validation, Griseofulvin, Estimation

INTRODUCTION

Griseofulvin is an antifungal drug that is administered orally. It is used both in animals and in humans, to treat fungal infections of the skin (commonly known as ringworm) and nails. It is derived from the mold *Penicillium griseofulvum*. Griseofulvin (7-chloro-2', 4, 6-trimethoxy-6'-methyl-Gris-2'-en-3, 4'-Dione) is primarily used to treat dermatophyte infections in humans and animals. Griseofulvin is a poorly water-soluble drug, which displays a dissolution rate-limited absorption pattern in humans and animals. Hence, it is often used as a model drug to assess the influence of various physicochemical, physiological, and dosage form factors on the absorption kinetics and bioavailability of hydrophobic drugs. Its molecular formula is $C_{17}H_{17}ClO_6$. Its molecular weight is 352.766, and physical state is a yellowish white crystalline powder with slight peculiar odor. It is freely soluble in DMF, methanol, ethanol, practically insoluble in water. UV λ max is 291 nm in methanol [1]. The structure of griseofulvin shown in (fig. 1).

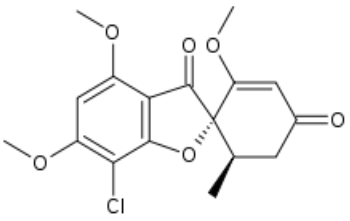

Fig. 1: Chemical structure of griseofulvin

Literature survey reveals that various analytical methods are present for estimation of griseofulvin with reversed-phase high-performance liquid chromatography (RP-HPLC) [2-5], but the present study is the first time report on stability indicating assay of griseofulvin in the presence of degradation products by HPLC.

In this method, isocratic elution method is selected for the analysis of griseofulvin because

It gave better baseline separation and peak shape, which is suitable for the routine analysis of griseofulvin. In view of above in the present study, we hereby report the development and validation of a stability indicating isocratic reverse-phase HPLC (RP-HPLC) method for analysis of griseofulvin in the presence of degradation products as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Griseofulvin was provided by Unichem Healthcare Ltd, Mumbai. Methanol was from merk Specialties Pvt. Ltd., Mumbai. Acetonitrile was from spectrochem Pvt. Ltd., Mumbai.

All chemicals were at least of analytical grade and used as received. Purified HPLC grades water

Was obtained by reverse osmosis and filtration through a milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

Instrumentation

A Cyber lab LC 100 plus (USA) HPLC system was utilized. Separation was carried out on a shiseido C18, 250 mm x 4.6 mm, 5µm particle size column (at ambient Temperature), and isocratic run under reverse phase partition chromatographic condition. The equipment was controlled by a PC with properly installed chromatographic software.

Chromatographic conditions

The mobile phase consisted of Methanol: Water (70:30) filtered through 0.45 µm nylon filter and degassed in ultrasonic bath prior to use. The sample solutions were also filtered using 0.45µm

membrane filters. The mobile phase was delivered isocratic at a flow rate 1.0 ml/min. The injection volume was a 20µl and the total run time was 5.16 min. The detection was carried out at 291 nm.

System suitability parameters

The system suitability test is an integral part of chromatographic methods and used to verify that the reproducibility of the chromatographic system is adequate for the analysis to be performed. An RP-HPLC method was developed keeping in mind the system suitability parameters i.e. retention time, asymmetry, theoretical plates, and percent relative standard deviation of six injections were evaluated.

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components that may be expected to be present. The specificity of the method was performed by injecting standard mix solution of griseofulvin, marketed formulation, and blank.

Calibration curve

Aliquots of the standard solution were diluted in a series of 10 ml volumetric flasks with the solvent to obtain the concentration range 0.1-1.2 µg/ml to for griseofulvin. Calibration curve was constructed by plotting an area under curve against concentration.

Forced degradation study

To evaluate, intrinsic stability griseofulvin was subjected to force degradation as per International Community on Harmonization (ICH) guidelines to get an idea of how drug substance or product degrades, degenerate and behaves under changing condition, which helps in developing stability indicating method of analysis.

Preparation of standard solution

A solution of griseofulvin was prepared by accurately weighed and transferred about 10 mg of griseofulvin reference/working standard into a clean 50 ml volumetric flask, then 25 ml of solvent was added and sonicate for five minutes to dissolve the griseofulvin, volume was made up to the mark with solvent this gave a solution of concentration 200µg/ml. From this solution, 5 ml was pipetted out and transferred to a 10 ml clean volumetric flask and the volume was made up to the mark with solvent this result in a stock solution of concentration 100µg/ml. From this stock solution, 0.1 ml solution was pipetted out and transferred to a 10 ml clean volumetric flask and volume was made up with solvent this result in a standard solution of concentration 1µg/ml.

Sample solution

A sample solution of griseofulvin was prepared by accurately weighed and transferred powdered tablet equivalent to 10 mg drug into a clean 50 ml volumetric flask, 25 ml of solvent was added and sonicate for five minutes to dissolve the griseofulvin, volume was made up to the mark with solvent, this give a solution of concentration 200µg/ml. From this solution, 5 ml was pipetted out and transferred to a 10 ml clean volumetric flask and the volume was made up to the mark with solvent this result in a stock solution of concentration 100µg/ml. From this stock solution, 0.1 ml solution was pipette out and transferred to a 10 ml clean volumetric flask and volume was made up with solvent this result in a standard solution of concentration 1µg/ml.

Blank solution

Methanol: Water (70:30) was used as a blank solution (Diluent).

Placebo solution

Methanol: Water (70:30) was used as a placebo Solution.

RESULTS AND DISCUSSION

Various mobile phases were tried by permutation and combinations and also by varying flow rate, column temperature and types of buffers with varying pH and solvents. The prepared different mobile phases were filtered through 0.45µm membrane filter paper prior to use. The mobile phase composition at a ratio of 70:30 (v/v) of methanol and water was found to be most suitable to obtain peak of griseofulvin at 5.16 min is well defined and free, from tailing.

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines as follows [6].

System suitability parameter

The optimized method developed resulted in the elution of griseofulvin at 5.16 min. (fig. 2) represents the typical chromatogram of standard griseofulvin. System suitability parameters were evaluated for six replicate injections of the standard at 10µg/ml.

Limit of detection and quantification

The detection limit (LOD) is the lowest amount of analyte in the sample, which can be detected but not necessarily quantified as an exact value. The quantification limit (LOQ) is the lowest amount of analyte in the sample, which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ are calculated as given in table 1.

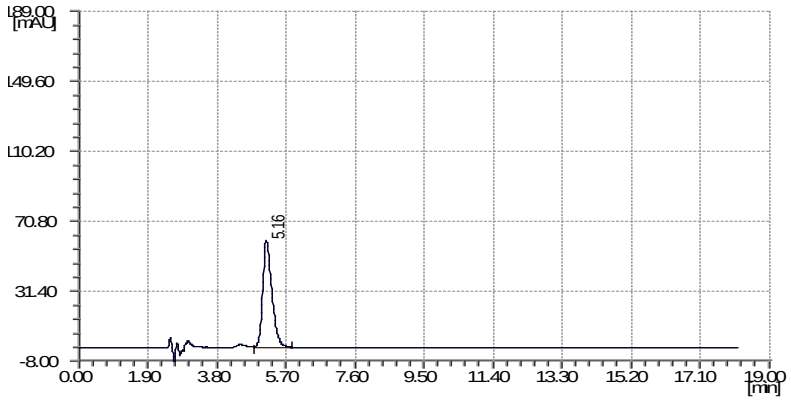

Fig. 2: Effect of methanol: water

Table 1: Linearity results, limit of detection (LOD) and limit of quantitation (LOQ)

Compound	r^2	Calibration curve equation	LOQ(%RSD)	LOD(%RSD)
Griseofulvin	0.9998	Y=19093X-274.92	1.1	0.7

Linearity

Linearity of method was evaluated by preparing a series of concentrations 0.1µg/ml, 0.5µg/ml, 0.8µg/ml, 0.9µg/ml, 1 µg/ml, 1.1µg/ml and 1.2µg/ml of griseofulvin. The solutions were analyzed in triplicate and measured the peak response of the analyte. Linearity curve was plotted; linear regression equation was found to be y= 19093x–274.92, with a correlation coefficient of 0.9998. Linearity data's are summarized and shown in table 1. The linearity curve and overlaid chromatograms are shown in (fig. 3) and (fig. 4).

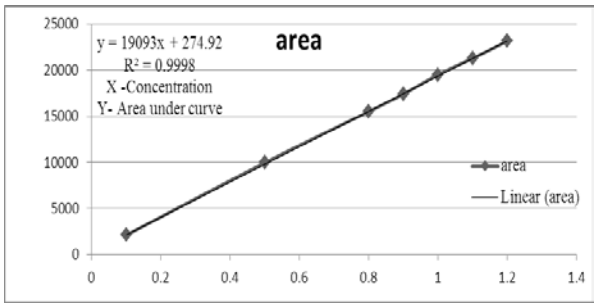

Fig. 3: Linearity curve of griseofulvin

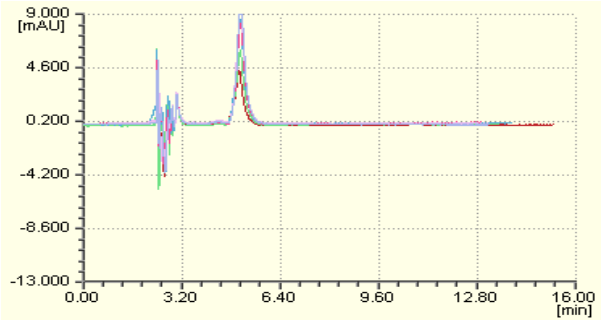

Fig. 4: Overlain chromatogram of griseofulvin

Precision

Three injections of same concentration were given on the same day, and these studies were also repeated on different days to determine inter-day precision. Assay µg/ml and RSD values obtained indicate a valid method. The result shown in table 2 indicates that the method is selective for the assay of griseofulvin without interference from the excipients used in these tablets. The results obtained for the evaluation of precision of the method are compiled in table 2.

Table 2: Intra-assay precision data of proposed RP-HPLC method (Method ruggedness)

Assay	Mean (%w/w)	SD (N=6)	RSD (%)
Assay-1	21590.91	203.05	0.94%
Assay-2	21635.98	345.71	1.5%
Intra Assay	21613.44	274.38	1.2%

Table 2: Inter-assay precision data of proposed RP-HPLC method

Assay	Mean (%w/w)	SD (N=6)	RSD (%)
Assay-1	21191.98	328.82	1.5%
Assay-2	21181.48	376.48	1.7%
Intra Assay	21186.73	352.65	1.6%

Accuracy

The accuracy of the method was carried out by recovery studies. A known concentration of griseofulvin was analyzed at three different levels (80%, 100% and 120%). Each solution was prepared in triplicate and analyzed in triplicate after suitable dilution. The recovery data obtained at each level were within 2%. The average recovery yield at three different levels of 80%, 100%, and 120% were found to be 100.3, 99.67 and 99.45 respectively. Since the results obtained were within the acceptable range 98.0 to 102.0%, the method was deemed to be accurate. The accuracy results are summarized in table 3.

Table 3: Accuracy of griseofulvin

Parameters	% Taken	Mass taken (mg/1tab.)	Mass found (mg/1tab.)	% Recovery
		8.1	8.00	100
	80	8.0	7.99	99.8
		7.9	8.00	101.2
		10.1	9.99	99
	100	10.0	10.00	100
		10.0	10.00	100.01
		12.0	11.99	99.99
	120	12.1	12.00	99.18
		12.1	12.00	99.19
X				99.81
SD				0.662
%RSD				0.663

Specificity (forced degradation studies)

Griseofulvin was allowed to hydrolyze in the base (0.1N NaOH), acid (0.1N HCl) and hydrogen peroxide (10 % v/v). Griseofulvin was also studied for its thermal degradation at 80 °C for 2 d and photolytic degradation for 10 d, exposed to white fluorescent light (1.2 million lux) near UV fluorescent light (200 w/m^2).

Powdered tablet equivalent to 10 mg drug was accurately weighed and transferred to 50 ml clean volumetric flask and volume were made up with 0.1N NaOH solution, 0.1N HCl and hydrogen peroxide 10 % v/v kept at room temperature to accelerate the degradation. 5 mL of sample was taken out at various time intervals, and it was neutralized with 0.1N HCl solution and 0.1N NaOH diluted with solvent to get the final concentration of 100µg/ml of griseofulvin.

Similarly, placebo solution was prepared. Sample and placebo solutions were analyzed as per methodology, calculated the percentage degradation. The results of stability studies are presented in table 4.

Table 4: Forced degradation study of griseofulvin

Test preparation	Area of griseofulvin in the test preparation	% drug remained	% drug degraded
Control sample	22676.2	100	0
Base Stress-4 h	20037.1	88.36	11.64
Base Stress-8 h	13596.4	59.95	40.05
Base Stress-24 h	9160.0	40.39	59.61
Acid Stress-4 h	20787.0	91.66	8.34
Acid stress-8 h	8273.6	36.48	63.52
6% Peroxide treated-4 h	17698.4	78.04	21.96
6% Peroxide treated-8 h	8930.9	39.38	60.62
Heat-24 h	22435.3	98.93	1.06
Heat-48 h	21349.3	94.14	5.86
Light-5days	21308.9	93.97	6.03
Light-10days	12945.5	57.08	42.92

CONCLUSION

An economical, accurate, sensitive and precise HPLC method with ultraviolet detection was developed and fully validated for quality control analysis of griseofulvin in tablets. The proposed method is very rapid, where the total analytical run time is 5.16 minute. Griseofulvin was exposed to acidic, alkaline, oxidative, thermal and photolytic stress conditions and the sample was taken at different time intervals. The stressed samples were analyzed by the proposed method. The proposed method can be used for routine analysis stability testing and assay of griseofulvin in quality control laboratories.

CONFLICT OF INTERESTS

Declared none

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