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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for the Determination of Griseofulvin in bulk and Its Solid Oral Dosage form using RP-HPLC

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A B S T R A C T

A simple, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) coupled with a SPD detector method was developed for the quantitative determination of Griseofulvin (GSF) in bulk and its pharmaceutical dosage form. The method is applicable to the quantification of drug product. Chromatographic separation was achieved on a Phenomenex Luna reversed phase C_{18} (250 mm x 4.6 mm, 5 µm) column. The optimized isocratic mobile phase consists of a mixture of methanol: water (0.1% Orthophosphoric acid) in the ratio of 55:45 % v/v. The eluted compounds were monitored at 295 nm for GSF assay, the flow rate was 1 mL/min, and the column oven temperature was maintained at ambient temperature. The developed method separated GSF from its excipients within 5.0 min. The developed method was validated as per ICH guidelines. The method was validated for specificity, linearity, precision, accuracy, robustness and system suitability. The method was linear in the drug concentration range of 10-60 µg/mL. The precision (% RSD) of six samples was found within 2. The mean recoveries were between 99.75%. The proposed method can be used successfully for routine analysis of the drug in bulk and combined pharmaceutical dosage forms.

Keywords: Methanol, Phenomenex Luna, Griseofulvin and Chromatographic separation

A R T I C L E I N F O

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C O N T E N T S

1. Introduction 27
2. Materials and Methods. 27
3. Results and Discussion. 27
4. Conclusion. 28
5. References. 28

1. Introduction

Griseofulvin (GSF) [1-3] is an antifungal drug [4]. It binds and interferes with the function of spindle and cytoplasmic microtubules by binding to alpha and beta tubulin. It also binds to fungal microtubes thus altering the fungal process of mitosis therefore a fungistatic. The chemical structure of GSF was shown in Figure 1.

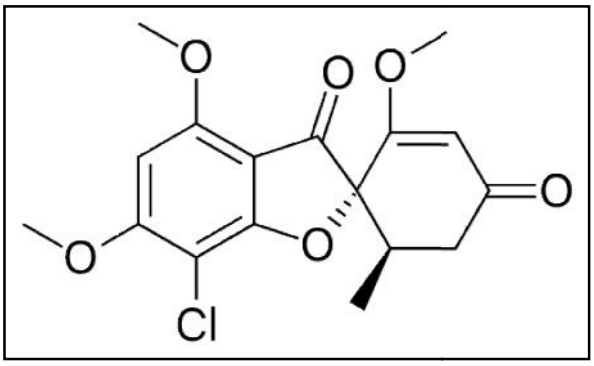

Figure 1: Chemical structure of Griseofulvin

Many of the analytical methods have been described for the detection of griseofulvin in biological samples. Methods include gas chromatography [5–7], thin-layer chromatography, high performance liquid chromatography (HPLC) [8–12], and liquid chromatography/tandem mass spectrometry (LC-MS/MS) [12-13]. The present report describes a simple, specific, precise and rapid HPLC method with SPD detection to determine griseofulvin in bulk and its formulation. The developed method to be validated in accordance to ICH guidelines [14,15].

2. Materials and Methods

Chemicals and reagents:

Pharmaceutical grade Griseofulvin (GSF) was gifted by MSN Laboratories, Hyderabad, India. Methanol (HPLC grade) was purchased from Merck Chemical Company, India. Double deionized water was generated by a Milli-Q academic ultra-pure water purification system (Millipore, Bedford, MA, U.S.A). All other reagents used in the study were of AR grade.

Instrument:

HPLC from Shimadzu (SPD 20A) Liquid Chromatography comprising Lab solutions (LC) with SPD detector and Phenomenex Luna C_{18}, (250x4.6) mm; 5µm. The HPLC system was equipped with "LC Solution" data acquisition software. The mobile phase consists of a mixture of methanol: water (0.1% Orthophosphoric acid) in the ratio of 55:45 % v/v. The mobile phase was set at a flow rate of 1.0 ml/min and an injection volume of 20µl.

Preparation of Mobile Phase:

550 ml of HPLC grade Methanol was placed in a 1000 ml volumetric flask. And 450 ml HPLC grade water was added to it. The solution was mixed, filtered through the membrane filter and sonicated for 5 min. Mobile phase was freshly prepared daily. And the same was used as diluents.

Preparation of stock solution:

10 mg of pharmaceutical grade GSF was weighed and dissolved in mobile phase in a 10 ml volumetric flask. The final volume was made up to the mark with the same to get 1 µg/ml primary stock solution. This solution was sonicated for 5 min. The required working standards for linearity were prepared from the primary stock solution.

Sample preparation

Take 25 mg equivalent tablet powder of GSF and dilute up to 25 ml with Methanol: Water (0.1% OPA) (55:45). Pipette out 0.4 ml of this solution in the 10 ml volumetric flask and make the volume with mobile phase, Sonicate it for 10 minutes. Filter the solution through Whatmann filter paper no. 41. This solution was used as sample solution.

Method Validation:

The method was optimized for system suitability parameters. The method developed was validated for various parameters as according to ICH guidelines Q2(R1).

3. Results and Discussion

Chromatographic separations were performed using isocratic elution at ambient temperature. Several trials were taken for the optimization of RP HPLC method by altering various proportions of organic phase and aqueous phase in the mobile phase using HPLC grade solvents. The optimized chromatogram was shown in Figure 2.

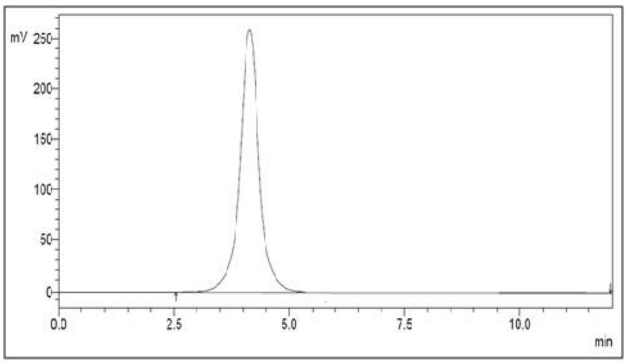

Figure 2: Chromatogram of Griseofulvin

Linearity:

Calibration curve was constructed for GSF and was linear over the concentration range of 10-60 µg/ml with correlation coefficient 0.999. Calibration curve were prepared using ratio of analyte peak area to standard peak versus concentration of analyte. The calibration curve was shown in Figure 3.

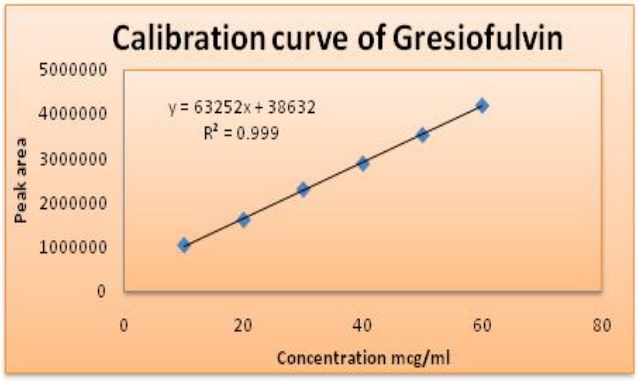

Figure 3: Calibration curve of Gresiofulvin

Accuracy: The accuracy was assessed by recovery studies of GFS in its dosage form at three different concentration levels. A fixed amount of pre analyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated for three times. The results are shown in Table 2.

Precision: The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. A minimum of six replicate sample determinations were carried together with a simple statistical assessment of the results, including the percent relative standard deviation. For intra-day and inter-day precision, %RSD for GFS was found to be 1.13 and 1.50 respectively. (Limit %RSD: < 2.0%). The results are shown in Table 3.

Limit of Detection and Quantification:

The Limit of detection (LOD) and Limit of quantitation were found to be 1.69 and 5.13 µg/ml respectively.

Robustness: The method is found to be robust as the results were not significantly affected by slight variation in flow rate (± 0.1 ml min-1) and temperature composition (± 5^{o}C). The percent RSD was calculated and was found within the acceptable limit.

5. Conclusion

The developed RP-HPLC method for the quantification of GFS has various advantages like good peak symmetry and phenomenal linearity, simple, precise and accurate. The mobile phase can be easily prepared and diluent is economical and readily available and it does not need sample preparation with sophisticated techniques or instruments. This attributes the high quality of the method. The proposed method can be used for the routine analysis of GFS in solid oral preparations and for routine application in quality control laboratories without interference of excipients.

Table 1: Results of Accuracy

Spiked Conc. (µg/ml)	Peak area	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery	% Mean Recovery
10	1480105	20.012	20.3825	101.848	100.91
	1468972	20.012	20.22919	101.0819	
	1450566	20.012	19.97572	99.81539	
20	2904681	40.025	40.00032	99.9375	99.34
	2865401	40.025	39.45939	98.58604	
	2891876	40.025	39.82398	99.49693	
30	4316545	60.038	59.44307	99.00908	99.00
	4325498	60.038	59.56637	99.21444	
	4306545	60.038	59.30536	98.77971	

Table 2: Results of Precision

Sample Injection	Peak Area	
	Intra-day	Inter-day
1	2810632	2879654
2	2831462	2908789
3	2904681	2831354
4	2865401	2790216
5	2891876	2860675
6	2854980	2913458
Mean	2859839	2864024
SD	32450.74	43239.81
% RSD	1.134705	1.509757

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