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Electrospray ionization LC–MS/MS validated method to quantify griseofulvin in human plasma and its application to bioequivalence study

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Abstract

A simple, sensitive and rapid liquid chromatography/tandem mass spectrometry (LC–MS/MS) method has been developed and validated to quantify griseofulvin in human plasma using propranolol hydrochloride as internal standard (IS). Samples were prepared using solid phase extraction and analysed without drying and reconstitution. The analytes were chromatographed on Hypersil, hypurity C18 reverse phase column under isocratic conditions using 0.05% formic acid in water:acetonitrile (30:70, v/v) as the mobile phase. Total chromatographic run time was 3.0 min. Quantitation was done on a triple quadrupole mass analyzer API-3000, equipped with turbo ion spray interface and operating in multiple reaction monitoring (MRM) mode to detect parent → product ion transition for analyte and IS. The method was validated for sensitivity, matrix effect, accuracy and precision, linearity, recovery and stability studies. Linearity in plasma was observed over the concentration range 20–3000 ng/mL for griseofulvin. Lower limit of quantification (LLOQ) achieved was 20 ng/mL with precision (CV) less than 10% using 5 μL injection volume. The absolute recovery of analyte (87.36%) and IS (98.91%) from spiked plasma samples was consistent and reproducible. Inter-batch and intra-batch coefficients of variation across four validation runs (LLOQ, LQC, MQC and HQC) was less than 7.5%. The accuracy determined at these levels was within ±4.2% in terms of relative error. The method was applied to a pilot bioequivalence study of 500 mg griseofulvin tablet in six healthy human subjects under fed condition.

Keywords: Griseofulvin; LC–MS/MS; Solid phase extraction; Human plasma; Pilot bioequivalence

1. Introduction

Griseofulvin is a fungistatic agent used for the treatment of dermatophytes, since last 40 years [1]. It is a metabolic product of penicillium griseofulvin [2] and is chemically known as chloro-7-dimethoxy-4,6-oxo-3-benzo(*b*)dihydro-2-3, furane-2, spiro-4′ methoxy-3′ methyl-5′ cyclohexene-2′ one-1′. It gets deposited in keratin precursor cells, which become resistant to the invasion of dermatophytes. Other than its fungistatic effect, griseofulvin has demonstrated non-cortisone anti-inflammatory properties and some direct vasodilatory effect when used in high doses [3]. It is mainly absorbed in the duodenum and to a lesser extent in the jejunum and ileum after oral ingestion. Absorption is enhanced by administration of fatty meal. Griseofulvin is detected in the outer layer of the stratum corneum soon after it is ingested and gets diffused from extra cellular fluid and sweat. It is a highly plasma protein bound drug (80%) [4]. The drug is metabolized to 6-demethyl griseofulvin by liver's microsomal system and excreted mainly through urine and faeces. The drug is carcinogenic in animals [5] and inactive in yeast, mold and dimorphic fungi. It is contraindicated in severe hepatic diseases, pregnancy, porphyria and monilial infection [1].

Very few methods are available in literature to determine griseofulvin in biological fluids like human plasma, serum and urine. These methods are either based on TLC [6–8], HPLC [9–12] or gas chromatography [8,13–15] detection and determination. They involve cumbersome and laborious extraction procedure with large sample volumes and are either less sensitive or time consuming. Characterization/determination of griseofulvin in variety of other matrices like molds [16], marine stains [17], foods and feeds [18] and their bioavailability studies [19–21] have also been reported. Recently, a method

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for permeability assessment of griseofulvin by UPLC/MS/MS has been developed and validated [22].

Suitable methods ideally require a small specimen, with a simple sample preparation procedure. The method should be sensitive, rugged while offering high throughput to meet clinical needs. Mass spectrometry may provide an alternative to UV detection for analytes separated by HPLC. Such a combination offers the advantages of the speed of analysis associated with HPLC and the sensitivity and selectivity associated with mass spectrometry.

Thus, the aim of this study was to develop and validate a high throughput LC–MS/MS method for routine measurement of griseofulvin in human plasma in support of clinical findings. The proposed solid phase extraction (SPE) method includes a single wash (to remove the plasma matrix) and elution step, with no sample concentration and reconstitution. The plasma volume used for processing is only 0.5 mL and the final eluate volume is 1 mL. The injection volume of 5 μL minimizes matrix interference and suppression of the analyte peak, and thus helps in extending the lifetime of the column. The simple and efficient SPE procedure with a chromatographic run time of 3.0 min increases the throughput for the determination of griseofulvin in human plasma. Application of this method was tested in a pilot bioequivalence study conducted in 6 healthy human subjects after oral administration of 500 mg griseofulvin tablet formulation under fed condition.

2. Experimental

2.1. Chemicals and materials

Griseofulvin and propranolol HCl were procured from USV Limited (Mumbai, India). HPLC grade acetonitrile and methanol were purchased from Ranbaxy (New Delhi, India). GR grade formic acid (100%) was obtained from Merck Limited (Mumbai, India). Water used for the LC–MS/MS was prepared from Milli Q A10 gradient water purification system procured from Millipore (Banglore, India). Solid phase extraction cartridges (Orpheus, DVB-HL, 1 mL, 30 mg) were procured from Orochem India Pvt. Ltd. (Mumbai, India).

2.2. Liquid chromatography and mass spectrometric condition

An HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary LC-20AD prominence pump, autosampler (SIL-HTc), solvent degasser (DGU-20A_3 prominence) and Column Oven (CTO 10AVP) were used for all the analyses. The chromatographic system consisted of Hypersil, hypurity C18 (50 mm × 4.6 mm i.d., 5 μm particle size) analytical column from Thermo Electron Corporation (Cheshire, U.K.). The flow rate of the mobile phase under isocratic condition was kept at 0.4 mL/min. The auto sampler temperature was set at 10 °C and the injection volume was 5 μL. The mobile phase consisted of 0.05% formic acid in water:acetonitrile (30:70, v/v). The column oven temperature was maintained at 25 °C. The pressure of the system was 12 bars. The total LC run time was 3.0 min.

Detection of analyte and IS was performed on a triple quadrupole mass spectrometer, API-3000, (MDS SCIEX, Toronto, Canada) equipped with turbo ion spray ionization source in the positive ion mode. Analyst software Version 1.4.1 was used to control all parameters of LC and MS. Quantitation was performed using multiple reaction monitoring (MRM) mode to study parent → product ion transitions for griseofulvin (353.0 → 165.0) and IS (260.1 → 116.2), respectively. Source dependent parameters optimized were gas 1 (Nebuliser gas): 12 psi, gas 2 (heater gas): 65 psi, Ion spray voltage (ISV): 4500 V, temperature (TEM): 450 °C. Compound dependent parameters were declustering potential (DP): 32 V, entrance potential (EP): 10 V, collision energy (CE): 35 V, cell exit potential (CXP): 6 V for Griseofulvin and DP: 28 V, EP: 10 V, CE: 25 V, CXP: 12 V for IS. Focusing potential (FP) was 400 V for both analyte and IS. Nitrogen was used as collision activated dissociation (CAD) gas and was set at 6 psi. Quadrupole 1 and quadrupole 3 were maintained at unit resolution and dwell time was set at 0.2 s.

2.3. Analytical data processing

Chromatographic data were collected and integrated using Analyst software Version 1.4.1. Peak area ratio of the analyte to IS was utilized for the construction of calibration curve. A weighing of $1/x$ (least-squares linear regression analysis, where x is the griseofulvin concentration) was used for curve fitting. Concentration in unknown samples were calculated from the best-fit equation ($y = mx + c$), where y is the peak area ratio. The regression equation for the calibration curve was also used to back-calculate the measured concentration at each QC level, and the results were compared with the theoretical concentration to obtain the accuracy, expressed as percentage of the theoretical value.

2.4. Standard and quality control preparation

The stock standard solutions of griseofulvin and propranolol HCl were prepared by dissolving their accurately weighted compounds in methanol to give a final concentration of 1000 μg/mL. The griseofulvin solution was than serially diluted with methanol–water (70:30, v/v) to obtain working solutions over 20–3000 ng/mL concentration range. Standard stock solution of propranolol HCl (IS) was diluted in methanol–water (70:30, v/v) to obtain a working solution of 1.5 μg/mL. All the solutions were stored at 2–8 °C and were brought to room temperature before use. Blank human blood was collected with heparin from healthy, drug-free volunteers. After centrifugation, blank plasma was collected and stored at −20 °C until used. The calibration standards and quality control (QC) samples were prepared by spiking (5%) blank plasma with standard working solutions. Calibration samples were made at concentration of 20, 40, 100, 300, 600, 1200, 1800, 2400 and 3000 ng/mL. Quality control samples were prepared at four different levels viz. 20 (LLOQ), 60 (LQC), 1500 (MQC) and 2700 ng/mL (HQC). Aliquots of spiked plasma samples were taken in micro centrifuge tubes and stored at −20 °C.

2.5. Sample preparation

All frozen subject samples, calibration standards and quality control samples were thawed at room temperature. The samples were adequately vortexed to mix and were centrifuged at 3200 rpm at 10 °C for 5 min to precipitate solids. 0.5 mL volume of plasma was dispensed into micro centrifuge tubes with 50 μL of 1.5 μg/mL propranolol HCl (IS) and vortex to mix for 10 s. The samples were then loaded on DVB-HL cartridge previously conditioned with 1 mL of methanol followed by 1 mL of water. Plasma was drained out under vacuum, followed by washing of cartridges with 1 mL of water. After proper drying of the cartridge, elution was carried out with 1 mL mobile phase. The eluate was transferred into vials, capped and placed in an autosampler rack for injection.

2.6. Assay characteristics for method validation

A thorough and complete method validation of griseofulvin in human plasma was done following the USFDA guidelines [23].

2.6.1. Selectivity and sensitivity

These tests were performed using eight normal, one haemolysed and one lipemic plasma lot. Blank plasma and LLOQ samples were prepared for each lot and extracted. The ratio of peak height response of the analyte signal (LLOQ) over that of blank plasma (noise) was determined for each lot.

2.6.2. Linearity, accuracy, precision and recovery

The linearity of the method was determined by analysis of standard plots associated with a nine-point standard calibration curve. Eight linearity curves containing nine non-zero concentrations (20, 40, 100, 300, 600, 1200, 1800, 2400 and 3000 ng/mL) were analyzed. Back calculated concentration, slope, intercept (c) and correlation coefficient (r) values were calculated using linear regression analysis.

Intra-batch and inter-batch accuracy and precision were evaluated at LLOQ (20 ng/mL), LQC (60 ng/mL), MQC (1500 ng/mL) and HQC (2700 ng/mL) levels on four different batches. Six replicates at each level were prepared and extracted. Back calculated concentrations were calculated and compared with their nominal concentration to determine accuracy (%RE) and precision (%CV).

Recovery presents the extraction efficiency of the method. It was performed at LQC, MQC and HQC levels. The peak area response of extracted samples was compared with the response of unextracted samples, prepared by spiking extracted blank plasma externally with respective working solutions.

2.6.3. Stability, matrix effect and dilution integrity

Stability experiments were performed to evaluate the griseofulvin stability in stocks solutions and in plasma samples under different conditions, simulating the same conditions, which occurred during study sample analysis. Stock solution stability of analyte and internal standard were evaluated at room temperature and at 2–8 °C. Bench top stability, process stability, freeze and thaw stability and long term stability in plasma were evaluated at LQC and HQC levels.

To study the effect of matrix on analyte quantification with respect to consistency in signal enhancement, matrix effect was checked with six different lots of plasma. These lots of heparinised plasma comprised of: four lots of normal control plasma, one lot of lipemic control plasma and one lot of haemolysed control plasma. Three replicates were prepared from each lot of plasma at LQC and HQC levels (total 36 QC samples) and checked for the inaccuracy.

The dilution integrity experiment was performed with an aim to validate the dilution test to be carried out on higher griseofulvin concentrations (>3000 ng/mL), which may be encountered during real subject samples analysis. Dilution integrity experiment was carried out at 4500 ng/mL concentration, i.e.1.5 times the upper limit of quantification (ULOQ) concentration. Six replicates each at 1/2 (2250 ng/mL) and 1/4th (1125 ng/mL) concentration level of 1.5 ULOQ were prepared and their concentrations were calculated by applying the dilution factor of 2 and 4, respectively.

2.6.4. Bioequivalence study

The design of study comprised of "A randomized, single dose, open label, two way cross over bio equivalence study of 500 mg griseofulvin tablet in six normal healthy subjects under fed condition". All subjects were informed of the aim and risk involved in the study and informed consent were obtained. Ethics committee approved the study protocol. The study was conducted strictly in accordance with guidelines laid down by International Conference on Harmonization and USFDA [24]. Health check-up for all subjects was done by general physical examination, ECG and laboratory tests like hematology, biochemistry and urine examination. All subjects were negative for HIV, hepatitis B surface antigen (HBSAg) and hepatitis C virus (HCV) tests. Subjects were orally administered a single dose with 240 mL of water after a heavy fat breakfast. Drinking water was not allowed and supine position was restricted 2 h post dose. Standardize meals were provided as per schedule. Blood samples were collected in tubes containing heparin before and after 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.33, 3.67, 4.0, 4.33, 4.67, 5.0, 5.5, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0, 36.0, 48.0, 72.0, 96.0 and 120 h of administration of drug. Blood samples were centrifuged at 3200 rpm for 10 min and plasma was separated, stored at −20 °C until use.

3. Results and discussion

3.1. Method development

Drugs bound excessively to proteins are too large to traverse the cell membrane, and thus are unable to reach the active site to generate a pharmacological response. Since griseofulvin is a highly protein bound drug (80%), the binding to plasma protein is an important determinant of pharmacokinetic studies. To obtain a clear plasma filtrate and reproducible recovery of the analyte, different extraction procedures were tried. Liquid–liquid extraction gave clear extracts but the

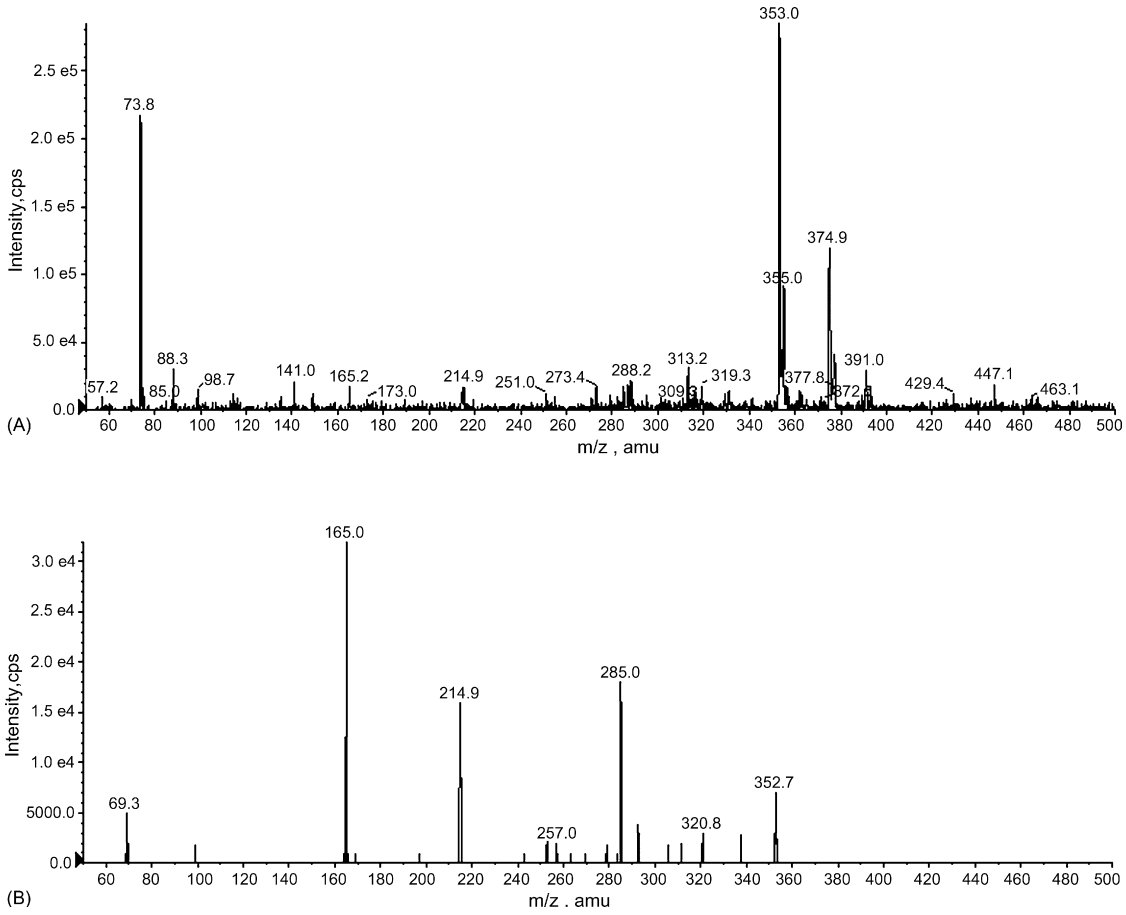

Fig. 1. Parent ion (A) and product ion (B) mass spectras of griseofulvin.

recovery was not quantitative in the solvents studied. Protein precipitation could not afford clean separation of the analyte and resulted in ion suppression. Solid phase extraction was then tried, which gave consistent and high recovery with minimum matrix interference and hence was selected for the present study. Centrifugation of spiked plasma samples at 3200 rpm and 10 °C for 5 min was sufficient to precipitate the solids. Sample preparation was simple, without addition of any additive. The samples were directly loaded on DVB-HL cartridges previously conditioned with methanol followed by water. Washing of cartridge with 1 mL of water was sufficient to remove endogenous interferences. Elution was carried out using only 1 mL of mobile phase. The sample was directly injected into LC–MS system without drying and reconstitution.

For ionization of medium polarity drugs, APCI source is generally preferable. Tuning was carried out both in positive and negative modes by direct infusion of standard solution of griseofulvin and IS. Greater intensity was achieved in positive mode and it was found more stable in TIS source than APCI for ionization. Figs. 1 and 2 show the mass spectra's of parent and product ions for analyte and IS, respectively. Most abundant parent ion at *m/z* of 353.0 for griseofulvin and *m/z* of 260.1 for IS were found after tuning in Q1 positive mode at unit resolution in 50–500 *m/z* range. Proposed fragmentation pathway for griseofulvin is shown in Fig. 3. Declustering potential (DP), focusing potential and entrance potential were set to get stable intensity. Higher DP resulted in decreased intensity as fragmentation took place in Q1 mode. Ion spray voltage and nebuliser gas further helped in increasing the intensity, but up to a certain limit. Sudden increase in intensity was observed when standard solution was infused with an acidic additive, due to generation of more number of positive species. Fragmentation was carried out in product ion scan mode by applying sufficient collision energy, keeping CAD gas at 8 psi. Lower collision energy (less than 35 V) was insufficient to fragment the parent ion of griseofulvin. Proper adjustment of cell exit potential helped in giving stable and desirable intensity.

To achieve good response with sharp peak shape, mobile phases consisting of methanol/acetonitrile with ammonium formate, ammonium acetate buffers (acidic range) in different volume ratios were tested. Considerable response was observed for mobile phases having high acetonitrile content on C18 reverse phase Hypersil, Hypurity (50 mm × 4.6 mm, 5 μm particle size) column, but the peak shape got distorted. Use of methanol in place of acetonitrile resulted in significant reduction in response with a poor peak shape. Finally, addition of formic acid (0.05%) instead of buffers in acetonitrile 30:70 (v/v) ratio gave the desired response with good peak shape. A higher back ground (noise) was observed when Q1 and Q3 were operated at unit and low resolution, respectively. Increasing gas 2 (heater gas) and decreasing CAD gas to 6 psi reduced noise level to give high S/N ratio without loss in intensity. Curtain gas did not have

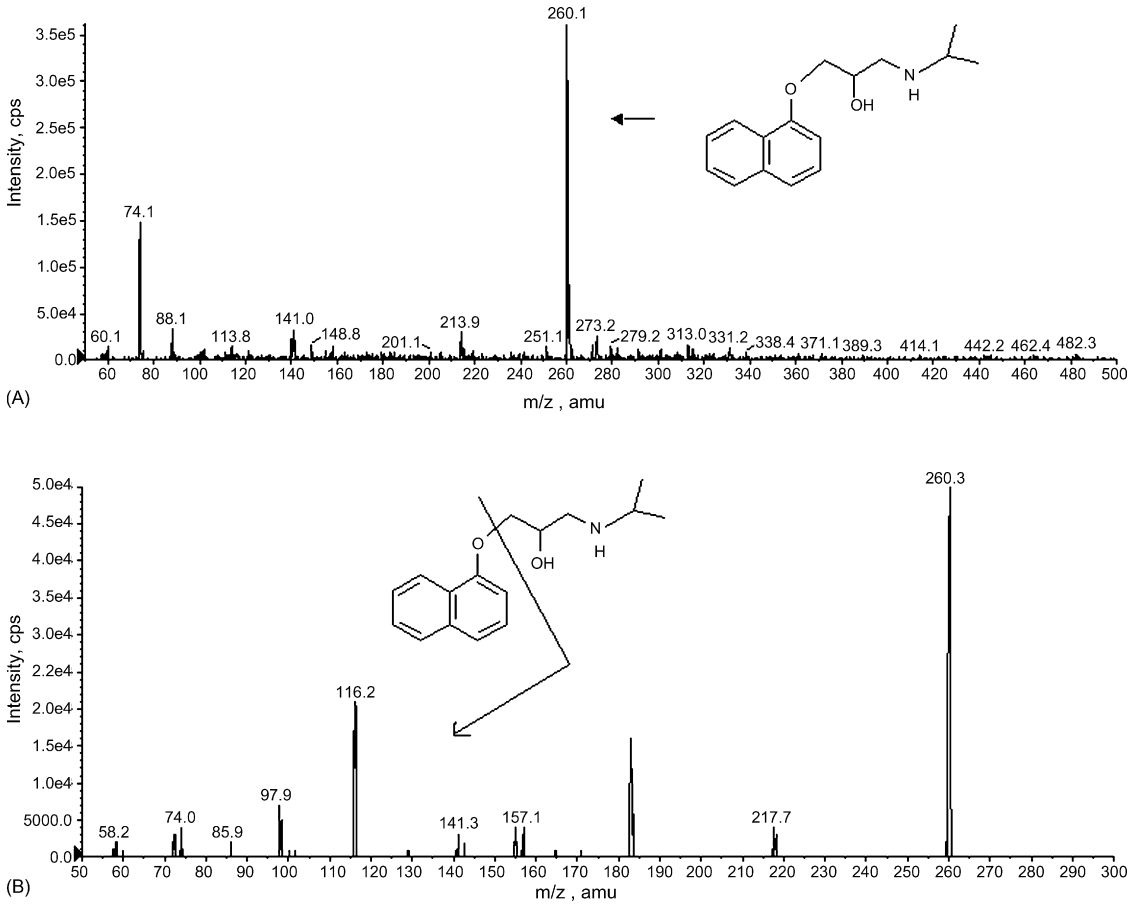

Fig. 2. Parent ion (A) and product ion (B) mass spectras of propranolol (IS).

much impact on noise level except intensity so was set at a lower value (10 psi).

The method was very fast both in analysis and sample preparation with a chromatographic run time of 3 min. Choosing an internal standard which is stable for isotope-labeled drug or structurally similar to analyte is helpful when significant matrix effect is found. It was difficult to find a compound which could ideally mirror the analyte to serve as a good IS. Several compounds were investigated to find a suitable IS, and finally propranolol hydrochloride, though belonging to a different class of compound was found to be most appropriate for the present purpose.

Table 1
Summary of calibration curve with back calculated concentrations

	Concentration (ng/mL)								
	CS-1[a] 20.0[b]	CS-2[a] 40.0[b]	CS-3[a] 100.0[b]	CS-4[a] 300.0[b]	CS-5[a] 600.0[b]	CS-6[a] 1200.0[b]	CS-7[a] 1800.0[b]	CS-8[a] 2400.0[b]	CS-9[a] 3000.0[b]
1	19.7	40.6	96.3	319.2	625.1	1319.6	1727.8	2421.4	2892.3
2	20.1	42.1	98.3	320.2	645.7	1275.7	1757.8	2511.2	2792.9
3	20.3	37.2	111.7	308.8	598.6	1232.1	2014.8	2404.5	2735.0
4	20.2	39.0	113.0	314.2	632.2	1191.7	1793.3	2407.4	2966.1
5	21.1	40.0	109.3	301.0	618.3	1245.7	1858.2	2287.6	2982.8
6	19.6	40.1	106.4	297.1	569.7	1205.0	1842.7	2339.4	3040.0
7	18.5	36.7	108.6	312.1	603.7	1228.9	1805.3	2592.3	2754.0
8	18.1	38.5	114.5	294.7	590.6	1188.5	1784.5	2756.9	2673.8
Mean	19.7	39.3	107.3	308.4	610.5	1235.9	1823.1	2465.1	2854.6
S.D.	1.0	1.8	6.7	9.8	24.6	44.6	88.2	151.0	134.0
%CV	5.1	4.6	6.2	3.2	4.0	3.6	4.8	6.1	4.7
Accuracy (%)	98.5	98.1	107.3	102.8	101.8	103.0	101.3	102.7	95.2

[a] Linearity.
[b] Added concentration.

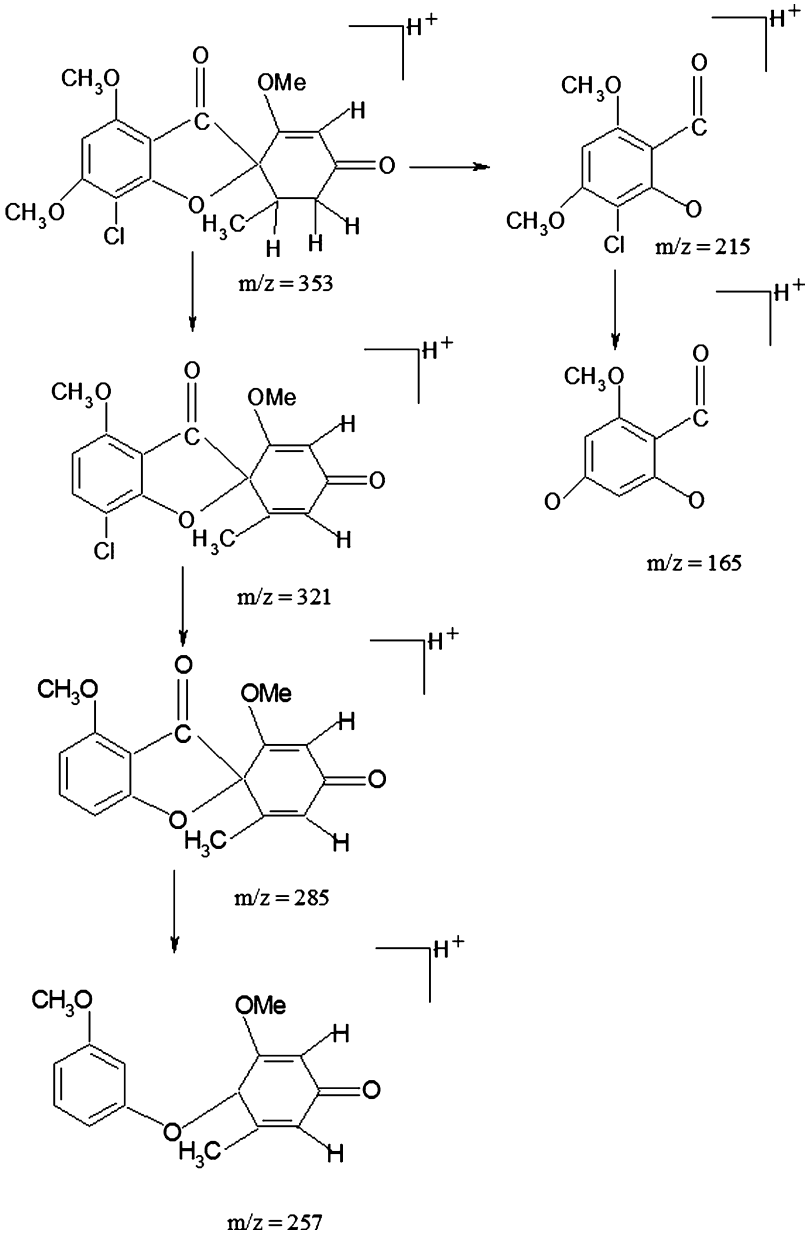

Fig. 3. Proposed fragementation pathway of griseofulvin.

3.2. Method validation

3.2.1. Selectivity and sensitivity (LLOQ)

Fig. 4 demonstrates the selectivity and sensitivity of the method as evident from the chromatograms of blank plasma and peak response of griseofulvin at LLOQ (20 ng/mL). The mean S/N ratio found was 36.6:1. The mean accuracy for back calculated concentration was 102.7% with precision (%CV) of 8.0. Thus, the proposed method can detect and quantify subject sample concentration up to LLOQ level (20 ng/mL) with desired accuracy and precision.

3.2.2. Linearity, accuracy and precision, recovery

The peak area ratio values of calibration standards were proportional to the concentration of the drug in plasma over the range tested. Calibration curves were linear from 20 to 3000 ng/mL with correlation coefficient (r) $\geq$0.9982. The observed mean back calculated concentrations at each level were within 95.2–107.3% of their nominal concentration. Precision (%CV) at each level was within 3.2–6.2 as shown in Table 1.

To determine accuracy and precision, three runs for inter-batch and a single run for intra-batch were considered. Intra-batch and inter-batch precision ranged from 2.1 to 7.4% and 5.3 to 6.8% while accuracy, expressed as relative error (RE) was within −4.2 to 3.6% and −1.5 to 3.7%, respectively, as given in Table 2.

The recovery obtained at each level was within 85–115%. Mean recovery of analyte found was 87.4% with a precision (%CV) of 7.0 across the three QC levels. The recovery of IS was 98.91%.

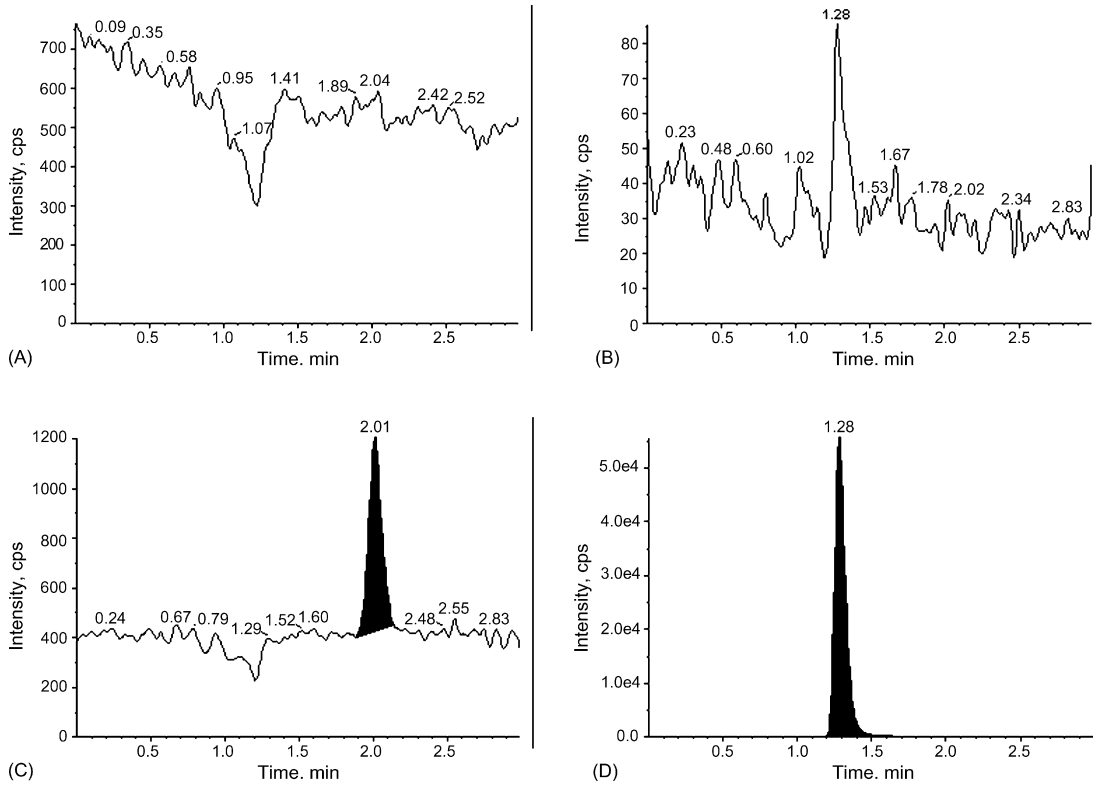

Fig. 4. Blank plasma and LLOQ chromatograms for griseofulvin (A and C) and IS (B and D), respectively.

Table 2
Intra-batch and inter-batch precision and accuracy

Level	Concentration added (ng/mL)	Intra-batch				Inter-batch			
		n	Mean concentration found (ng/mL)[a]	RE (%)	%CV	*n*	Mean concentration found (ng/mL)[b]	RE (%)	%CV
LLOQ	20	6	20.7	3.6	6.3	18	19.7	−1.5	6.8
LQC	60	6	57.6	−4.1	3.5	18	62.1	3.5	5.4
MQC	1500	6	1501.6	0.1	7.4	18	1555.6	3.7	5.3
HQC	2700	6	2586.7	−4.2	2.1	18	2775.4	2.8	5.8

RE, relative error; CV, coefficient of variance; *n*, total number of observation.
[a] Mean of six replicates observations at each concentration.
[b] Mean of 18 replicates observations over three different analytical runs.

Table 3
Stability results

Stability	Storage condition	Level	Mean comparison sample concentration found (ng/mL)	%CV	Mean stability sample concentration found (ng/mL)	%CV	%Mean change
Bench top	Room temperature (24 h)	LQC	61.3	3.8	59.3	5.7	−3.2
		HQC	2603.3	4.9	2494.0	3.1	−4.0
Process	Autosampler (10 °C, 43 h)	LQC	62.6	3.1	60.2	5.3	−3.9
		HQC	2784.0	3.7	2646.3	2.3	−5.0
Freeze and thaw	After 5th cycle at −20 °C	LQC	58.0	4.3	59.0	5.2	1.6
		HQC	2527.6	6.01	2490.7	6.7	−1.5
Long term stability	62 days at −20 °C	LQC	65.0	3.2	63.0	2.5	−3.1
		HQC	2914.0	2.9	2838.2	4.0	−2.6

Table 4
Matrix effect

	Lot-1	Lot-2	Lot-3	Lot-4	Lot-5	Lot-6
LQC (60.000 ng/mL)						
Mean calculated concentration	61.1	66.7	57.2	60.7	57.6	63.9
RE (%)	1.8	11.2	−4.7	1.2	−3.9	6.6
HQC (2700.000 ng/mL)						
Mean calculated concentration	2822.7	2933.4	2709.1	2885.8	2799.4	2819.5
RE (%)	4.6	8.6	0.3	6.9	3.7	4.4

RE, relative error.

3.2.3. Stability, matrix effect and dilution integrity

The stability results obtained were well within the acceptable limit. Stock solution of griseofulvin and IS were stable at room temperature for 24 h and at 2–8 °C for 16 days. Griseofulvin in control human plasma at room temperature was stable at least for 24 h and for minimum of five freeze and thaw cycles. Stability in the final solid phase extract at 10 °C up to 43 h in autosampler (process stability). Spiked griseofulvin plasma samples stored at −20 °C for long term stability experiment were stable for at least 62 days. Different stability experiments in plasma and the values for the precision and percent change are shown in Table 3.

For matrix effect assessment, the mean back calculated value at each level was within 85–115% of their nominal concentration. Accuracy in terms of relative error is given in Table 4 for each lot. Moreover, the minor enhancement of analyte signal due to endogenous matrix interferences does not affect the quantification of griseofulvin and IS peak.

The mean back calculated concentrations for dilution integrity experiment at 1/2 and 1/4th dilutions were within 85–115% of their nominal. The coefficient of variation for 1/2 and 1/4th dilution samples of griseofulvin were 3.23 and 1.49%, respectively.

3.2.4. Application to bioequivalence study

The proposed validated method was successfully applied to a pilot bioequivalence study in six healthy human male subject samples for reference and test formulations of 500 mg griseofulvin. All 311 samples including the calibration, QC and volunteer samples were run and analysed in only 2 days and precision and accuracy for calibration and QC samples were within acceptable limits. The 90% confidence interval of the individual ratio geometric mean for test/reference was within 80–125% for $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and C_{max} (AUC: area under curve, C_{max}: peak plasma concentration). Mean plasma concentration versus time profile for the treatment under fed condition is presented in Fig. 5.

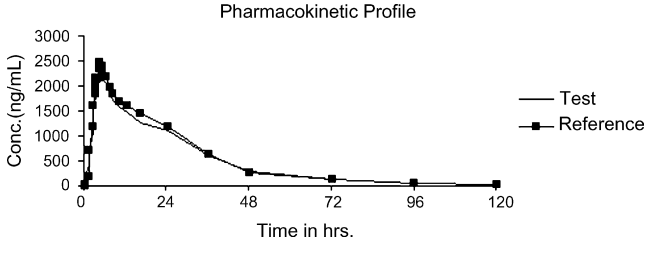

Fig. 5. Mean plasma concentration of griseofulvin after oral administration of single dose of test and reference formulation to 6 healthy male volunteers.

4. Conclusion

The developed LC–MS/MS assay for griseofulvin is sensitive, selective, rapid and rugged, suitable for routine measurement of subject samples. This method has significant advantages over other reported methods, in terms of clean and reproducible SPE extraction procedure and a shorter chromatographic run time (3 min). The extraction method gave consistent and reproducible recoveries for analyte and IS from plasma, with minimum interference and matrix enhancement. The SPE eluate (5 μL) is directly submitted for LC–MS analysis without drying and reconstitution to give an on-column loading of 100 pg per injection. The established LLOQ of 20 ng/mL is sufficiently low to conduct a pharmacokinetic study with 500 mg tablet formulation of griseofulvin.

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