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Rapid determination of granisetron in human plasma by liquid chromatography coupled to tandem mass spectrometry and its application to bioequivalence study

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Abstract

A simple, sensitive and rapid method for analysis of granisetron in human plasma, utilizing liquid chromatography tandem mass spectrometry (LC-MS/MS), has been developed and validated to satisfy FDA guidelines for bioanalytical methods. The analyte and internal standard (IS) were isolated from 100 μl plasma samples by liquid–liquid extraction (LLE). A Varian 1200 l tandem mass spectrometer equipped with an electrospray ionization source was operated in selected reaction monitoring (SRM) mode with the precursor-to-product ion transitions *m/z* 313.4/138 for granisetron and *m/z* 270/201 for the IS used for quantitation. The assay exhibited a linear dynamic range of 0.02–20 ng/ml for granisetron in human plasma. The lower limit of quantification (LLOQ) was 0.02 ng/ml with a relative standard deviation of less than 15%. The mean extraction recovery from spiked plasma samples was 97.9%. The intra-day accuracy of the assay was within 10% of nominal and intra-day precision was better than 15% C.V. A run time of 2.0 min for each sample made it possible for high-throughput bioanalysis. The method was employed in a bioequivalence study of two formulations of granisetron hydrochloride 1 mg rapidly disintegrating tablets/1 mg capsules.

Keywords: Granisetron; LC-MS/MS; Plasma; Pharmacokinetics; Bioequivalence

1. Introduction

Granisetron, endo-1-methyl-*N*-[9-methyl-9-azabicyclo (3.3.1) non-3-yl]-1H- indazole-3-carboxamide, is a selective 5-HT_3 receptor antagonist which may have beneficial therapeutic effects in the treatment of vomiting and nausea resulting from cancer therapy [1–3]. It has an improved side effect and tolerability profile, a lower risk of drug interactions and a longer duration of action than other 5-HT_3 receptor antagonists. Granisetron is an effective and well-tolerated agent in the management of chemotherapy-induced, radiotherapy-induced and post-operative nausea and vomiting in adults and children [4].

The analytical methods used to determine granisetron concentrations in biological samples include liquid chromatography (LC) with fluorescence [5–12], UV [13] and tandem mass spectrometric [14,15] detector. All the reported fluorescence or UV detection methods have inconveniences for routine analysis of large batches of biological samples for the reasons of large sample volumes, complex extraction procedures, inadequate sensitivity or long chromatographic run time. Boppana et al. [14] reported a direct plasma LC-MS/MS method for granisetron and its 7-hydroxy metabolite utilizing internal surface reversed-phase guard columns and automated column-switching devices. The tandem mass spectrometer was

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operated in selected reaction monitoring (SRM) using atmospheric pressure chemical ionization (APCI). The total run time, including both sample enrichment and chromatography, was about 6 min, but this kind of method requires special arrangements. Nirogi et al. [15] developed another LC-MS/MS method for quantification of granisetron in human plasma with a sample volume of 0.5 ml to achieve a lower limit of quantification (LLOQ) of 0.1 ng/ml and an extraction recovery of 62.5%.

The purpose of this study was to develop a more rapid, sensitive and highly selective LC-MS/MS method for determination of granisetron using rizatriptan as the IS. It was essential to establish an assay capable of quantifying granisetron at concentrations down to 0.02 ng/ml. LLE was used to extract the analyte from 0.1 ml plasma with recovery of above 95% for both granisetron and the IS. After full validation, the method was applied to a bioequivalence study of 1 mg granisetron hydrochloride rapidly disintegrating tablets versus 1 mg granisetron hydrochloride capsules in 20 healthy volunteers.

2. Experimental

2.1. Materials

Granisetron hydrochloride and rizatriptan benzoate (IS) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). The purities of granisetron hydrochloride and rizatriptan benzoate were 99.5%. Granisetron hydrochloride rapidly disintegrating tablets were from CINMED Pharmaceutical (Fujian, PR China) and granisetron hydrochloride capsules were from Xincat Pharmacy (Shandong, PR China). Ammonium acetate, acetic acid, sodium hydroxide and ethyl acetate (analytical reagent grade) were purchased from Shanghai Chemical Reagent Company (Shanghai, PR China). Acetonitrile (chromatographic grade) was purchased from Merck (Darmstadt, Germany). Human control plasma (sodium heparin as an anticoagulant) was obtained from Shanghai Blood Center (Shanghai, PR China). Deionized (18 MΩ/cm) water was generated in-house using a Millipore (Bedford, MA, USA) Milli-Q Plus system.

2.2. Instrumentation

A Varian LC-MS/MS system (Palo Alto, CA, USA) consisted of a ProStar 410 autosampler, two ProStar 210 pumps, and a 1200 l triple quadrupole mass spectrometer equipped with an electrospray ionization source. Varian MS workstation version 6.3 software was used for data acquisition and processing.

2.3. Chromatographic conditions

The chromatographic separation was performed on a Lichrospher C18 column (4.6 mm × 50 mm, 5 μm), which was purchased from Hanbon Science & Technology Co. Ltd. (Jiangsu, PR China). The column was thermostated at 30 °C. The mobile phase consisted of acetonitrile-water (containing 10 mM ammonium acetate and 0.5% acetic acid) (40:60, v/v) at a flow rate of 1.0 ml/min. Before use, the mobile phase was filtered through a 0.45 μm nylon membrane filter. The injection volume was 20 μl and the analysis time was 2.0 min per sample.

2.4. Mass spectrometer conditions

The HPLC eluent was split 1:5 to flow 200 μl into the mass spectrometer. The electrospray ionization (ESI) mass spectrometer was operated in the positive ion mode. The electrospray capillary Voltage was set to 30 V. Nitrogen was used as a drying gas for solvent evaporation. The API housing and drying gas temperatures were kept at 50 and 380 °C, respectively. Protonated analyte molecules were subjected to collision-induced dissociation using argon as the collision gas to yield product ions for each analyte. The collision energy was 16 eV for granisetron and 10 eV for the IS. The scan time was 1 s and the detector multiplier voltage was set to 1500 V. Selected reaction monitoring of the precursor-product ion transitions m/z 313.4 → 138 for analyte and 270 → 201 for IS was used for quantitation. Product ion mass spectra for analyte and IS are shown in Fig. 1.

2.5. Preparation of standard solutions

A stock solution of granisetron was prepared by dissolving accurately weighed granisetron hydrochloride in methanol to yield a final concentration of 1.0 mg/ml (calculated as free base). The solution was sonicated for 5 min to ensure complete dissolution. Following sonication, the solution was allowed to equilibrate to room temperature after which it was diluted to volume with methanol. Working standards of granisetron were prepared from the 1.0 mg/ml stock solution at 0.4–800 ng/ml using H_2O: acetonitrile (70:30, v/v) as the diluent. The stock standard solution of IS was prepared by dissolving appropriate amount of rizatriptan benzoate in methanol to give a final base concentration of 1.0 mg/ml. A 100 ng/ml IS working solution was obtained by diluting the stock solution of rizatriptan with H_2O: acetonitrile (70:30, v/v). All solutions were stored at 4 °C and were brought to room temperature before use.

Plasma standards were prepared by spiking 5 μl of each working standard into 100 μl of human control plasma. These standards were used to construct calibration curves for the quantitation of granisetron at plasma concentrations ranging from 0.02 to 20 ng/ml. Samples found to contain granisetron at concentrations above 20 ng/ml were diluted appropriately with control plasma and re-assayed.

2.6. Sample preparation

Plasma samples were removed from −20 °C storage and immersed in a heated (37 °C) water bath to thaw. After vortexing and centrifugation (9000 rpm for 5 min) of the sample tubes, a 100 μl aliquot of plasma was transferred to a 1.5 ml eppendorf tube. A 5 μl aliquot of H_2O: acetonitrile (70:30, v/v) was added to the blanks, quality controls (QC), and subject samples to compensate for the volume of diluent added during spiking of the calibration standards. Next, 5 μl of the working internal standard solution was added followed by 50 μl of 0.1 M NaOH. To the mixed samples, 1.0 ml of ethyl acetate was added and

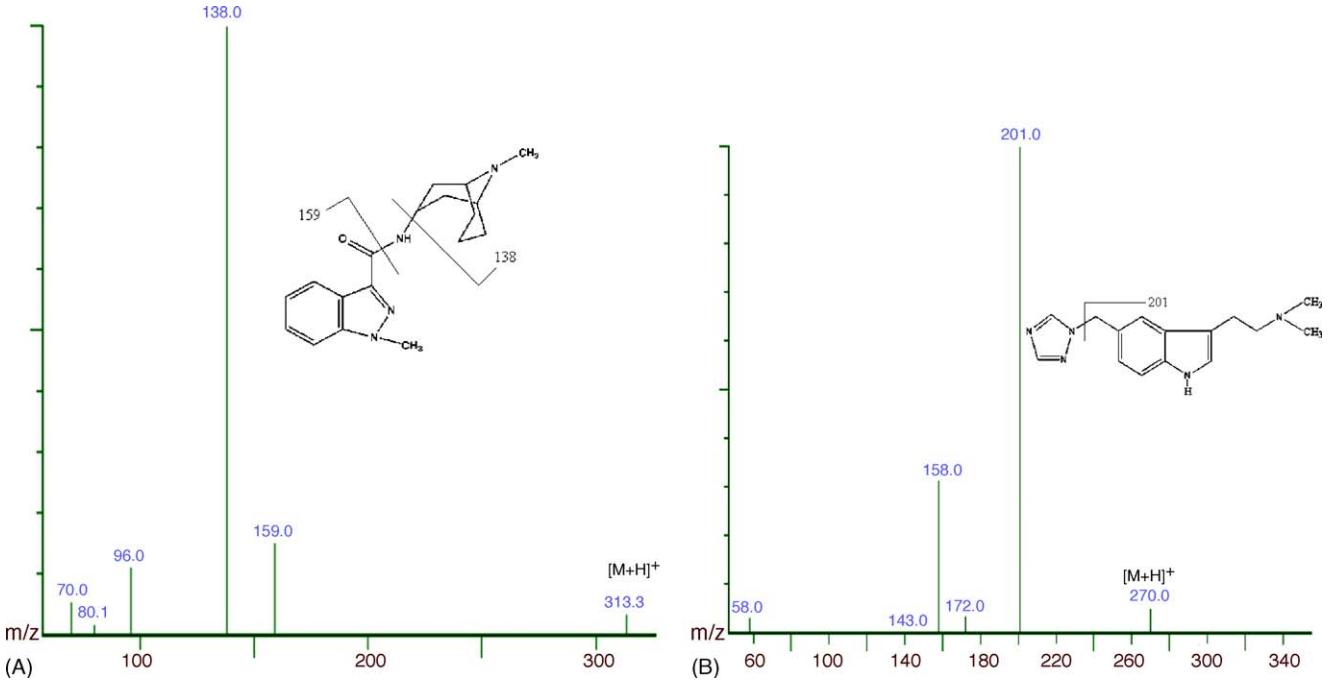

Fig. 1. Chemical structures and product ion spectra of $[M+H]^+$ of: (A) granisetron and (B) rizatriptan.

vortexed for about 2.0 min. After centrifugation at 3500 rpm for 5 min, 0.8 ml of the organic layer was transferred to the evaporation tube. The eluent was then evaporated to dryness at 40 °C under nitrogen stream. The extraction residue was reconstituted in 50 μl of mobile phase, vortexed for 30 s and centrifuged at 9000 rpm for 5 min and 20 μl of supernatant was injected onto the analytical column.

2.7. Study design

The bioequivalence of two tablets formulations of granisetron hydrochloride 1 mg rapidly disintegrating tablet (test formulation) of CINMED Pharmaceutical (Fujian, PR China) versus 1 mg granisetron hydrochloride capsules (standard reference formulation) of Xincat Pharmacy (Shandong, PR China) was conducted using an experimental design of two way crossover, single blind, open label, balanced, two period, two sequence, randomized study with a 1-week washout period [16] in Chinese healthy male subjects after they had been informed on the purpose, protocol and risk involved in the study. All subjects gave written consent and local ethics committee approved the protocol. Twenty volunteers, 22–28 years of age were enrolled in the study. The study was conducted in accordance with the current good clinical practices (GCP), International Conference on Harmonization (ICH) and FDA [16] guidelines. Subjects with history of drug allergies or idiosyncrasies, renal or hepatic impairment, history of any illness of cardiovascular system, or alcohol and drug abuse were excluded. Twenty subjects were selected after passing a clinical screening procedure including a physical examination and laboratory tests. All subjects avoided using other drugs or alcohol for at least 1-month prior to the study and until after its completion.

Subjects were admitted into hospital at 9:00 p.m. the day before the study and fasted 10 h before each drug administration. A single dose (2 mg) consisting of two granisetron hydrochloride rapidly disintegrating tablets (Fujian, PR China) or granisetron hydrochloride capsules (Shandong, PR China) according to the randomization plan was given to each subject in a fasting state for each treatment period. The drug was administered with 200 ml of water. Subjects were provided with standard meals at 4 h (lunch) and 10 h (supper) after drug administration in each treatment.

Heparinized venous blood samples, 0.3 ml, were collected by means of an indwelling venous cannula of the cubital vein on profile days according to the time schedule, which included a blank sample just prior to dosing and then at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 24.0 h after drug administration. Any deviations from the sampling times were recorded. Plasma was immediately separated by centrifugation at 3000–4000 rpm for 10 min, then was transferred to properly labeled tubes and stored at −20 °C until the LC-MS/MS analysis.

3. Results and discussion

3.1. Method development

The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for the analyte and the IS, as well as a short run time. Modifiers, such as ammonium acetate and acetic acid alone or in combination in different concentrations were added. It was found that a mixture of acetonitrile-water (containing 10 mM ammonium acetate and 0.5% acetic acid) (40:60, v/v) could achieve this purpose and was

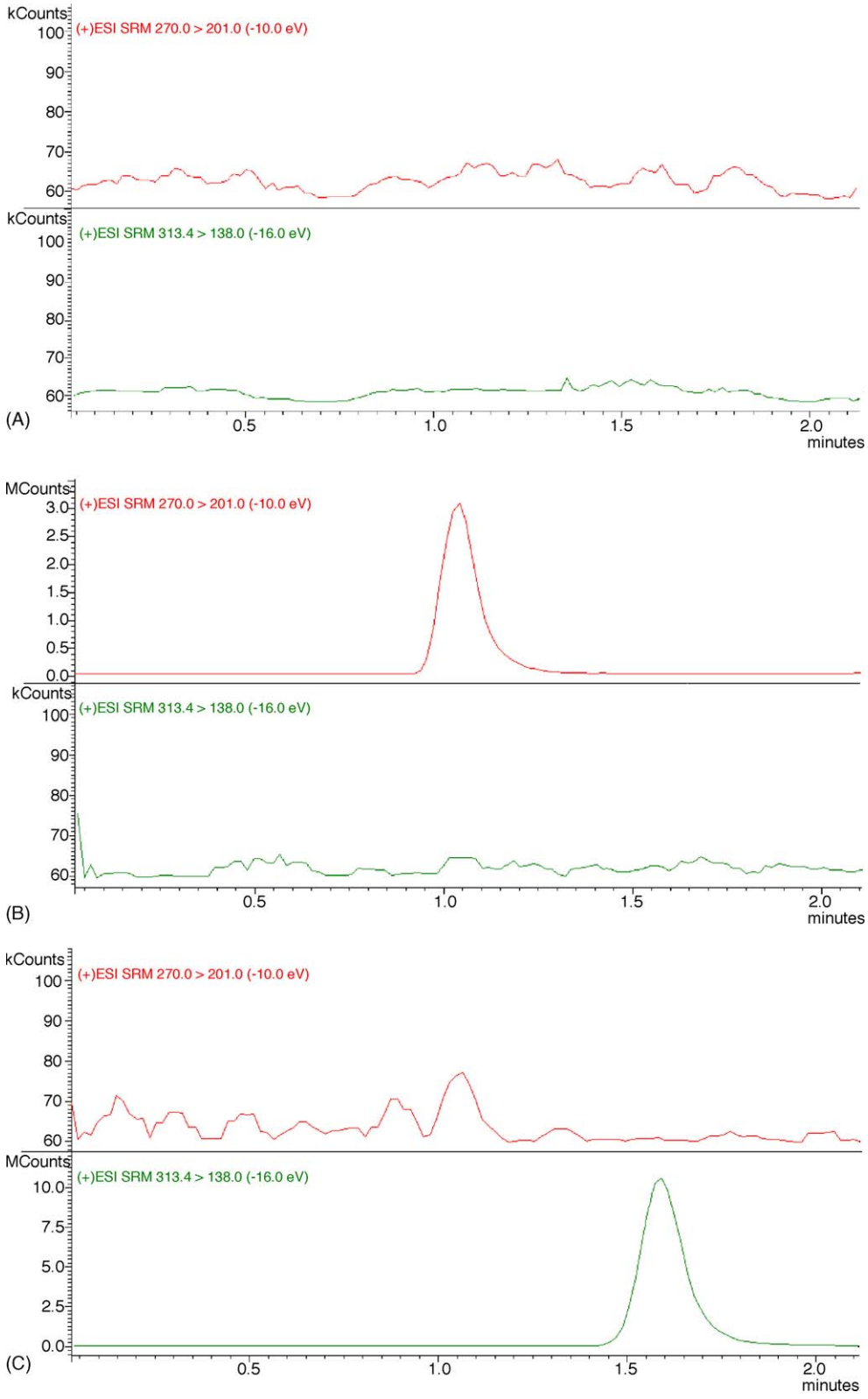

Fig. 2. Representative chromatograms: (A) control plasma double blank; (B) blank plasma spiked with 5 ng/ml of IS; (C) blank plasma spiked with 20 ng/ml of granisetron; (D) 0.02 ng/ml plasma standard; (E) plasma sample collected from a subject 3 h after receiving a 2 mg oral dose of granisetron. The assayed concentration of granisetron in this sample was 3.89 ng/ml.

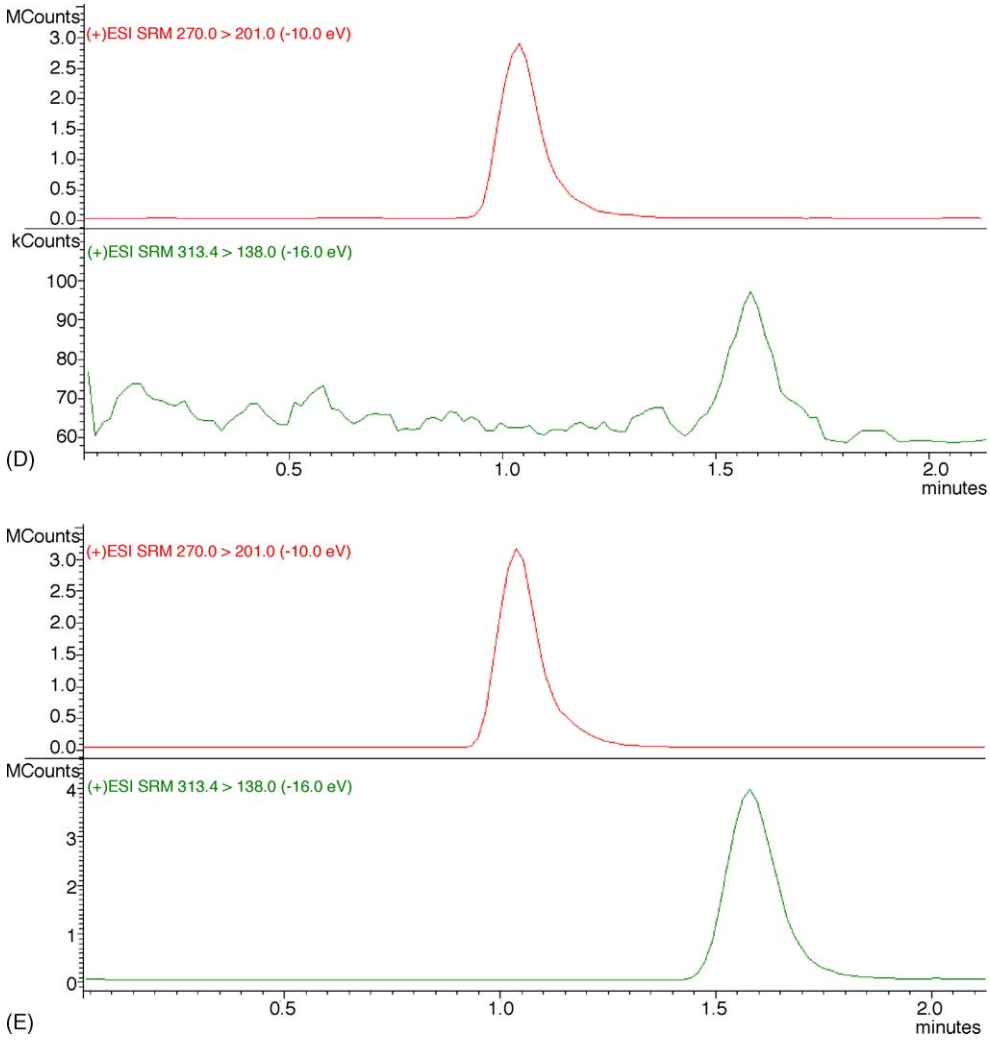

Fig. 2. (*Continued*).

finally adopted as the mobile phase. The percentage of acetic acid was optimized to maintain this peak shape while being consistent with good ionization and fragmentation in the mass spectrometer. After careful comparison of several columns, a Lichrospher C18 column (4.6 mm × 50 mm, 5 μm) was finally used with a flow rate of 1.0 ml/min to produce good peak shapes and permit a run time of 2.0 min.

In order to produce a spectroscopically clean sample and avoid the introduction of non-volatile materials onto the column and MS system, LLE was used for the sample preparation in this work. Clean samples are essential for minimizing ion suppression and matrix effect in LC-MS/MS analyses. Nirogi et al. [15] used a mixture of diethyl ether and dichloromethane (7:3, v/v) to extract granisetron from plasma with a recovery of 62.5%. Since granisetron and rizatriptan are weak bases, plasma samples were basified with 0.1 M NaOH prior to extraction in order to ensure liberation of the analyte molecules in an effort to more effectively retain the compound on the sorbent. Experience has shown that the addition of 50–100 μl of 0.1 M NaOH per 100 μl of plasma is often effective at improving the extraction recovery of analytes. In this work, a simple organic solvent ethyl acetate was used as an extraction solvent, which can produce a clean chromatogram for a blank plasma sample and yield the highest recovery for the analyte from plasma.

3.2. Method validation

The method was validated according to FDA guidelines [17]. The validation experiments and results obtained are described below.

3.2.1. Selectivity

Assay selectivity was evaluated by analyzing six separate lots of drug-free human control plasma. All plasma lots were found to be free of interferences with the compounds of interest.

In addition, the LC–MS/MS system was evaluated for the presence of "cross-talk" between the channels used for monitoring granisetron and IS. Plasma samples spiked with the working concentration of IS (5 ng/ml) in the absence of granisetron (i.e. control plasma single blanks) were prepared and analyzed. No peaks were detected in the channel used to monitor granisetron (Fig. 2B). Additionally, a plasma sample spiked with granisetron at the assay upper limit of quantitation in the absence of IS was analyzed. No "cross-talk" was observed (Fig. 2C).

Representative chromatograms of a control plasma double blank and 0.02 ng/ml plasma standard are shown in Fig. 2A and D.

3.2.2. Sensitivity and linearity

The LLOQ of the assay, defined as the lowest concentration on the standard curve that can be quantitated with accuracy within 15% of nominal and precision not exceeding 15% C.V., was 0.02 ng/ml.

Calibration curves were constructed by plotting the peak area ratios (granisetron/ IS) of plasma standards versus nominal concentration. Weighted ($1/x^2$, where x = nominal standard concentration) linear least squares regression was employed. Linear calibration curves were obtained over the range 0.02–20 ng/ml granisetron in plasma. Unknown sample concentrations exceeding 20 ng/ml were diluted appropriately with control plasma and re-assayed.

3.2.3. Accuracy and precision

The within-day accuracy and precision of the assay were determined by analyzing replicate ($n = 5$) standard curves. To more fully characterize the ruggedness of the assay, the standard curves were prepared in five different lots of plasma (i.e. lots originating from five unique donors). The results of this analysis are provided in Table 1. The ruggedness of the assay and the absence of relative matrix effects are further evidenced by the precision of the slopes of the individual standard curves, which is shown in Table 2.

Quality control samples were prepared at low (0.1 ng/ml), medium (1 ng/ml) and high (10 ng/ml) concentrations and stored under the same conditions as the clinical samples (i.e. −20°C) for the purpose of evaluating sample stability and inter-day accuracy and precision. Duplicate QC samples at each concentration were analyzed daily along with standards and clinical samples. Inter-day accuracy and precision data for QC samples analyzed over a 5-day period are provided in Table 3.

The results in Tables 1–3 show that this method is as accurate and precise as others reported in literature [12–14], while others have used higher volumes of plasma sample [10,15], or more laborious plasma extraction procedures [8], with similar results.

Table 1
Intra-day accuracy and precision for the determination of granisetron in five unique lots of human plasma

Nominal concentration (pg/ml)	Mean determined concentration (pg/ml, $n = 5$)	Accuracy[a] (%)	Precision[b] (%)
20	21.8	108.9	13.0
50	51.6	103.2	9.4
100	101.7	101.7	7.7
200	203.9	102.0	8.4
500	499.2	99.8	7.3
1000	1008.3	100.8	5.1
2000	1942.8	97.1	4.6
5000	5218.0	104.4	3.0
10000	10259.3	102.6	2.2
20000	19402.4	97.0	1.5

[a] Accuracy is expressed as [(mean observed concentration)/(nominal concentration)] × 100.
[b] Precision is expressed as the coefficient of variation of peak area ratios.

Table 2
Intra-day slope precision for standard curves prepared in five unique lots of human plasma

Control plasma lot	Slope
1	0.2754
2	0.2717
3	0.2773
4	0.2867
5	0.2851
Mean	0.2792
C.V. (%)	2.3

3.2.4. Extraction recovery and matrix effect

To investigate extraction recovery, a set of samples ($n = 5$ at each concentration in unique lots of plasma) was prepared by spiking granisetron into plasma at 0.1, 1 and 10 ng/ml. Each of the samples was also spiked with IS at the working concentration of 5 ng/ml. The samples were subsequently processed using the procedure described previously. A second set of plasma samples was processed and spiked post-extraction with the same concentrations of granisetron and IS that actually existed in the pre-extraction spiked samples. Extraction recovery for each analyte was determined by calculating the ratios of the raw peak areas of the pre-extraction spiked samples to the raw peak areas of the samples spiked after extraction. The extent of matrix suppression or enhancement of ionization was assessed by comparing the mean raw peak areas of the post-extraction spiked plasma samples to the mean raw peak areas of granisetron and IS in neat solution at three different concentrations in five different lots of human plasma. The results are indicated in Table 4.

Mean extraction recoveries of granisetron at concentrations 0.1, 1 and 10 ng/ml were 97.9, 98.4 and 97.5%, respectively, and the extraction recovery of the IS was 98.1%. Although some matrix enhancement of ionization was observed for granisetron and IS ($\text{area}_{\text{post-extraction}}/\text{area}_{\text{neat}} = 1.27$ for granisetron and 1.26 for IS), assay precision was not compromised by a relative matrix

Table 3
Inter-day QC accuracy and precision

Day	Assayed concentration (pg/ml)[a]		
	Low QC	Mid QC	High QC
1	111.2	992.5	9735.6
2	108.3	1096.4	10131.5
3	91.6	927.4	10177.2
4	105.3	1085.3	10264.8
5	94.8	1024.7	9104.3
Mean	102.2	1025.3	9882.7
Accuracy[b] (%)	102.2	102.5	98.8
C.V. (%)	8.4	6.8	4.9

[a] Data presented are the mean of duplicate QC samples at each concentration.
[b] Accuracy is expressed as [(mean observed concentration)/(nominal concentration)] × 100.

Table 4
Extraction recovery and matrix effect[a]

Nominal concentration (pg/ml)	Peak area[b] (e^6) (A)	Peak area[c] (e^6) (B)	Peak area[d] (e^6) (C)	Extraction recovery[e] (%) (A/B)	Matrix effect[f] (B/C)
100	0.95	0.97	0.78	97.9	1.24
1000	10.76	10.94	8.11	98.4	1.35
10000	112.51	115.38	94.57	97.5	1.22
5000 (IS)	31.62	32.23	25.58	98.1	1.26

[a] $n = 5$.
[b] Standards spiked before extraction.
[c] Standards spiked after extraction.
[d] Neat standards.
[e] Extraction recovery (%) expressed as the ratio of the mean peak area of the analytes spiked into plasma pre-extraction (A) to the mean peak area of the analytes spiked into plasma post-extraction (B).
[f] Matrix effect expressed as the ratio of the mean peak area of the analytes spiked into plasma post-extraction (B) to the mean peak area of the neat standards (C).

effect, as illustrated in Tables 1 and 2. The recovery of the method is comparable to other methods that use larger plasma samples [10] or SPE pretreatment [6,8].

3.2.5. Stability

Table 5 lists data for bench top, autosampler, freeze/thaw and storage stability.

Bench top stability was investigated to ensure that granisetron was not degraded in plasma samples at room temperature for a time period to cover the sample preparation. Three sets of plasma samples at concentrations of 0.1, 1 and 10 ng/ml were left at room temperature for 15 h. The samples were then processed and analyzed. The results indicated that granisetron was stable during the exposure period.

Due to the need for occasional delayed injection or reinjection of extracted samples, stability of granisetron in the final reconstituted extraction fluid was evaluated in the autosampler at 10 °C. A group of QC samples at concentrations of 0.1, 1 and 10 ng/ml were extracted, loaded onto the autosampler and kept in the autosampler for 48 h before injection. The quantitative results indicated that granisetron was stable in the autosampler up to at least 48 h.

Freeze-thaw stability was evaluated for granisetron using QC samples. The QCs were exposed to three freeze-thaw cycles, each cycle consisted of removing the QCs from the freezer, thawing them unassisted to room temperature, kept at room temperature for 4 h and re-freezing at −20 °C. The samples were processed along with a standard curve and concentrations were determined. This result indicated that granisetron had an acceptable stability after three freeze-thaw cycles in human plasma.

The storage stability at −20 °C was also tested using QC samples. The stability was closely monitored during validation and sample analysis periods, and no degradation of the compound was observed. The 6-week stability data are listed in Table 5. The result indicated that granisetron was stable in plasma for at least 6 weeks.

3.2.6. Sample dilution

To demonstrate the ability to dilute and analyze samples containing granisetron at concentrations above the assay upper limit

Table 5
Granisetron stability data

Nominal concentration (pg/ml) ($n = 3$)	Found concentration (pg/ml)	C.V. (%)	Accuracy (%)
Bench top stability[a]			
100	103.9	7.3	103.9
1000	1024.1	5.0	102.4
10000	9994.8	2.7	99.9
Autosampler stability[b]			
100	102.5	7.5	102.5
1000	1013.3	6.2	101.3
10000	9960.6	2.8	99.6
Freeze-thaw stability[c]			
100	101.8	6.9	101.8
1000	1023.6	5.2	102.4
10000	10004.7	1.4	100.0
6-week storage stability[d]			
100	102.4	7.1	102.4
1000	971.6	6.3	97.2
10000	10128.3	1.9	101.3

[a] Exposed at room temperature (25 °C) for 15 h.
[b] Kept at 10 °C for 48 h.
[c] After three freeze-thaw cycles.
[d] Stored at −20 °C.

Table 6
Mean pharmacokinetic parameters and 90.0% confidence interval for granisetron, after the administration of an oral dose of 2 mg of test and reference formulations to healthy volunteers

Pharmacokinetic parameters[a]	Reference formulation (mean ± S.D.)	Test formulation (mean ± S.D.)	Confidence limit 90.0%
T_{max} (h)	1.40 ± 0.30	1.30 ± 0.40	–
C_{max} (ng/ml)	7.32 ± 2.35	7.42 ± 2.19	93.50–111.19
AUC_{0-t} (ng h/ml)	38.41 ± 9.88	43.18 ± 13.04	105.16–116.25
$AUC_{0-\infty}$ (ng h/ml)	41.54 ± 10.84	47.27 ± 14.73	105.33–117.62
$t_{1/2}$ (h)	5.74 ± 1.96	5.62 ± 1.96	–
MRT (h)	8.14 ± 2.11	8.35 ± 2.41	–

[a] All the parameters are defined and explained in the article.

of quantitation, a set of plasma samples was prepared containing granisetron at a concentration of 50 ng/ml and placed in a −20 °C freezer overnight prior to analysis. After thawing by immersion in a 37 °C water bath, a 20 μl aliquot was withdrawn for analysis ($n = 5$), diluted with 80 μl of control human plasma, and processed as described in Section 2.6. The accuracy of the test was 94.4% with a good precision (C.V. = 1.5%).

3.3. Bioequivalence of granisetron formulations

This method was applied to a bioequivalence study of two granisetron hydrochloride formulations. A representative chromatogram from a post-dose sample is provided in Fig. 2E. The mean plasma concentrations-time profiles of granisetron after a single oral dose of 2 mg of either formulation are shown in Fig. 3. Fig. 3A shows a direct variation of plasma concentrations while Fig. 3B shows a log-transformed plasma concentrations versus time graph which indicates the kinetic characteristic of elimination of the drug. To determine the pharmacokinetic parameters of the two formulations, the concentration-time data were analyzed by non-compartmental methods using the Bioavailability Program Package (BAPP, Version 2.0, Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University). The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the concentration-time data. Area under the plasma concentration-time curve from time zero to the last sampling time (AUC_{0-t}) was calculated by the trapezoidal rule. The terminal phase was determined by visual inspection of the log-transformed concentration-time data. The elimination rate constant, k_{el}, was obtained from linear regression analysis of the terminal log-linear phase of the concentration versus time curve. Plasma $AUC_{0-\infty}$ values were estimated by the combination of AUC_{0-t} and $AUC_{t-\infty}$, where $AUC_{t-\infty}$ represents the residual area of drug from time t to infinity and were calculated by dividing the last plasma concentration value measured by the elimination rate constant. The elimination half-life ($t_{1/2}$) was calculated as 0.693 divided by k_{el}. Mean residence time (MRT) was estimated from AUMC/AUC, where AUMC is area under the first moment curve. The pharmacokinetic parameters of the two granisetron formulations are shown in Table 6, and the relative bioavailability of the test formulation was 111.41 ± 13.55%. The T_{max} and C_{max} in this paper were similar to the data reported [15]. All the statistical evaluations were performed by the software Drug And Statistics (Version 1.0, Wannan Medical College, China). Wilcoxon's signed rank test was utilized to compare T_{max}. ANOVA was performed to access period, treatment and crossover effects. Main pharmacokinetic parameters, such as C_{max} and AUC_{0-t}, $AUC_{0-\infty}$ were evaluated using the two one-sided t-test ($p > 0.05$) procedure for logarithmic transformed data. The means and standard deviations of these parameters for the two formulations were similar, indicating that the pharmacokinetics of granisetron in the two formulations are similar. The 90% confidence intervals for the ratios of test drug to reference drug in terms of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were within the range 80.0–125.0%, which is the range accepted by FDA [18].

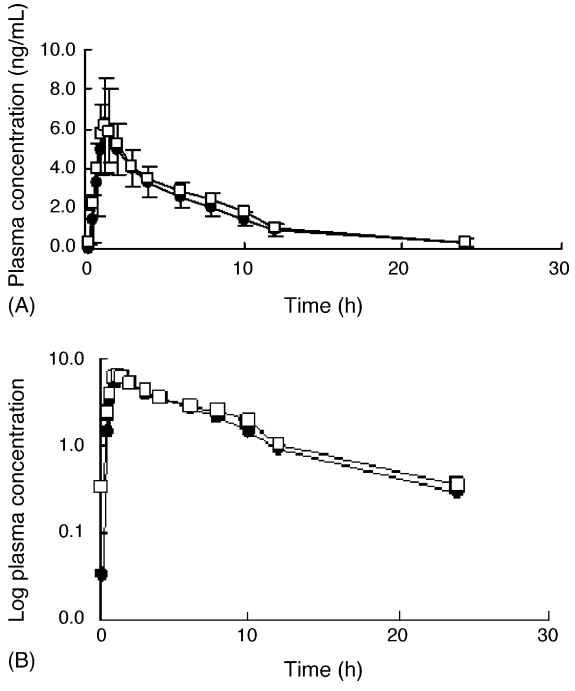

Fig. 3. Mean plasma concentration vs. time graph of granisetron after administration of test (□) and reference (●) formulations to healthy, adult, male human subjects under fasting condition. (A) linear and (B) log-transform scale.

4. Conclusion

In this paper a sensitive, selective and accurate LC-MS/MS method is described for the determination of granisetron in human plasma and offers rapid and simple sample pretreatment requiring only 0.1 ml of plasma compared to previously reported method where 0.5 ml samples were used [15]. The

Table 7
Comparison of analytical methods reported for granisetron in biological matrix for routine analysis

No.	Biological matrix and processing volume (ml)	Extraction procedure	Extraction recovery (%)	I.S.	Analytical run time (min)	Quantification limit (ng/ml)	Detection technique	Reference
1	Rat blood and brain(–)	Microdialysis	12.1–37.4 for blood and 8.1–19.9 for brain	–	8	0.1	LC- fluorescence	[5]
2	Human plasma (1)	SPE	107.9–113.0	BRL 43704	25	0.1	LC- fluorescence	[6]
3	Rat blood and brain(–)	Microdialysis	29.7 for blood and 6.1 for brain	–	8	0.1	LC- fluorescence	[7]
4	Human serum, urine and pleural effusion (0.5)	SPE	96.2–103.6	BRL 43693A	15	0.25	LC- fluorescence	[8]
5	Human plasma (1)	LLE	75.3	Granisetron	–	–	LC- fluorescence	[9]
6	Human plasma (0.5)	LLE	95.8	N-(1-Naphthyl) ethylenediamine dihydrochloride	8	0.3	LC- fluorescence	[10]
7	Human plasma and urine (–)	LLE	–	BRL 43704	–	0.1	LC- fluorescence	[11]
8	Human plasma (–)	LLE	–	An analog of granisetron	–	0.2	LC- fluorescence	[12]
9	Guinea pig plasma (0.15)	Microfiltered (It costs about 100 min)	58-59	–	3.4–3.5	19	LC-UV	[13]
10	Dog plasma (0.1)	Centrifuged (using column-switchi-ng device)	>100	BRL 43704	6	0.05	LC-APCI-MS/MS	[14]
11	Human plasma (0.5)	LLE	62.5	Tamsulosin	2	0.1	LC-ESI-MS/MS	[15]
12	Human plasma (0.1)	LLE	97.9	Rizatriptan	2	0.02	LC-ESI-MS/MS	Present method

method was capable of estimating accurately granisetron down to 0.02 ng/ml in human plasma with high degree of reproducibility. Table 7 summarizes the salient features of some methods reported in literature for the routine analysis of granisetron in biological matrix. This method was rugged and was successfully applied to bioequivalence study. The analysis of pharmacokinetic parameters confirmed that the test formulation granisetron hydrochloride 1 mg rapidly disintegrating tablets (CINMED Pharmaceutical, Fujian, PR China) when compared with the reference formulation 1 mg granisetron hydrochloride capsules (Xincat Pharmacy, Shandong, PR China), met the bioequivalence criteria in terms of rate and extent of absorption and no adverse event was reported during the study.

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