

Pharmacokinetic study of granisetron in human plasma measured by UPLC-MS/MS and its use in healthy Chinese subjects

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Abstract: In this study, a rapid, simple and sensitive UPLC-MS/MS method was established for the quantification of granisetron in human plasma for the prevention of vomiting after radiotherapy and chemotherapy. The precipitated proteins were extracted and gradient eluted on a ZORBAX Eclipse Plus C18 column (2.1×50mm, 1.8µm) to achieve ideal chromatographic separation. Multiple reaction mode (MRM) was performed using a Turboion Spray API5500 mass spectrometer equipped with Electron Spray Ionization (ESI). For method validation, good linearity was observed for each analyte of interest in the validation concentration range of 0.05 to 20.0ng/mL. The CV% of inter-batch and intra-batch precision were in the range of -3.6% to 4.7% and the precision of both inter-batch and intra-batch was ≤15.0%. In addition, the method had the advantage of a low matrix effect. In human plasma, all analytes remained stable for 2 hours when kept at room temperature; samples were stable within the autosampler (5°C) for 141 h after preparation and after four freeze-thaw cycles at -20°C and -70°C for 48 days. The UPLC-MS method that had been validated was later utilized for the pharmacokinetic investigation of granisetron hydrochloride tablets in orally administered doses to healthy Chinese volunteers, both before and after meals.

Keywords: Granisetron, UPLC-MS, validation, pharmacokinetic.

INTRODUCTION

Granisetron is a specific antagonist of the 5-HT₃ receptor and has strong antiemetic effects. This medication is efficient and well-tolerated since it has fewer adverse effects and a lesser likelihood of interacting with other drugs compared to other 5-HT₃ receptor antagonists. (Krishna *et al.*, 2018; Zhu *et al.*, 2017; Cupissol *et al.*, 1993; Chatterjee *et al.*, 2020; Chua *et al.*, 2020; Rathore *et al.*, 2019). Per oxidation and binding are the primary pathways for granisetron metabolism in the liver, with 7-OH-granisetron and its sulfate and glycyrlurea conjugates constituting the major compounds. Currently, fluorometry and LC (Hamed *et al.*, 2020; Yehia *et al.*, 2019; Boppana *et al.*, 1995; Maksić *et al.*, 2016; Pinguet *et al.*, 1996; Balakumaran *et al.*, 2017; Huang *et al.*, 1998; Chen *et al.*, 2017), and tandem MS are used to analyze biological samples and determine the concentration of granisetron. (Boppana *et al.*, 1996; Ramakrishna *et al.*, 2006). The routine analysis of large batches of biological samples is hindered by several factors, including the need for huge sample volumes, intricate extraction procedures, limited sensitivity, and extended chromatographic run times. Consequently, existing fluorescence or UV detection technologies are deemed inadequate for this purpose. To detect granisetron and its 7-hydroxyl metabolites directly in plasma, Boppana *et al.* (Boppana *et al.*, 1996) used an inner surface reverse-phase protection column and an

automatic column switching device. Using atmospheric pressure chemical ionization, the tandem MS was put to use for selected reaction monitoring (SRM). The entire process (purification of samples via chromatography) takes approximately 6 minutes to complete, but it does necessitate some advanced planning. Nirogi *et al.* have devised an additional liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique to measure the concentration of granisetron in human plasma. The extraction recovery yielded a modest 62.5%, while the lower limit of quantification (LLOQ) was determined to be a mere 0.1ng/ml. In this study, a rapid and highly responsive UPLC-MS/MS method was developed to quantify granisetron hydrochloride. The internal standard used for this analysis was granisetron d3. The material was prepared for examination using the protein precipitation procedure. The detection time was only 2.8 minutes, the LLOQ was 0.05ng/ml and the extraction recovery rate was 101%. After successfully completing the validation process, the approach was used for a pharmacokinetic research with healthy individuals and tablets containing 1 mg of granisetron hydrochloride.

MATERIALS AND METHODS

Chemicals

Reference preparation Granisetron hydrochloride tablets, supplier (Waymade Plc); Rasetron: purity 99.8%, source (China Institute for Food and Drug Control); Internal standard granisetron d3, Supplier (TLC Pharmaceutical),

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the purity was 98.7%. Human heparin sodium (The First Affiliated Hospital of Xiamen University), the batch number of the heparin sodium used was SBL2021101811. The supplier of Acetonitrile (HPLC grade) and MeOH (HPLC grade) was Merck. Supplier of Ammonium Formate (AR grade) was aladdin; supplier of FA (ACS grade) was sigma.

Instruments and workstations

Liquid chromatography was 30 Series UPLC System (SHIMADZU), Shimadzu Enterprise Management (China) Co., LTD.; MS: TurboIonSpray API 5500, Applied Biosystems/Sciex; Centrifuge 5810R, Eppendorf; Balance (Sartorius) MSA6.6S-0CE, CPA225D; Ultrasonic cleaner (Good Ultrasonic) GT SONIC-D20; Data acquisition and processing software Analyst (V.1.6.3), Watson LIMS 7.5.

UPLC conditions

Mobile phase A: aqueous solution of 0.2%FA & 0.5mM NH₄FA; B: 95% CAN of 0.2% FA & 0.5mM NH₄FA; flow rate: 0.3mL/min, injection volume: 10uL; column temperature: 40°C, collection time: 2.8min, injector temperature control: 5°C. Column: Zorbax Eclipse Plus C18 column (2.1×50mm, 1.8μm).

The mass spectrometric conditions

CUR: 35.00; TEM: 500.00; IonSpray Voltage: 5500 V; GS1: 45.00; GS2:50.00; CXP: 12.00; Ihe: ON; EP: 10.00; Collision gas CAD: 8.00; Mode ESI, Positive, MRM; Declustering Potential: 55 (granisetron), 80 (granisetron d3), Collision Cell Exit Potential: 33(granisetron),33 (granisetron d3); Dwell Time (msec): 100.00/100.00; Transitions: 331.300/138.100 (granisetron), 316.100/138.300 (granisetron-d3).

Solution preparation

A solution was developed for the standard curve and quality control sample. The granisetron hydrochloride was precisely quantified and diluted in MeOH to provide a stock solution for the standard curve with a concentration of 1mg/mL. The standard curve working solution and concentration were prepared by diluting with a 50% methanol solution to achieve concentrations of 1.00, 2.00, 8.00, 20.0, 80.0, 160.0, 320.0 and 400ng/mL. The working solutions for the LLOQ QC (1.00ng/mL), LQC (3.00ng/mL), GMQC (60.0ng/mL), MQC (200ng/mL), HQC (300ng/mL) and DQC (600ng/mL) QC samples were also prepared and stored in a refrigeration unit at a temperature of -20°C.

The standard curve sample solution was prepared by diluting the standard curve working solution. Specifically, 20 uL of the relevant working solution was added to 380 uL of a blank matrix. This resulted in a sample working solution with a concentration range of 0.05, 0.10, 0.40, 1.00, 4.00, 8.00, 16.0 and 20.0ng/mL.

Working solution of internal standard(IS): The appropriate quantity of granisetron hydrochloride-d3(internal standard) in amber glass bottle was precisely weighed and completely dissolved with proper amount of MeOH to prepare stock solution of IS with final concentration of 1.00mg/mL. To obtain an 8ng/mL working solution of the internal standard, 50% methanol was added to the stock solution.

QC sample solution: Transferring the corresponding working solution (20uL) to the blank matrix (380uL) and diluting it to the concentration: LLOQ QC (0.05ng/mL), LQC (0.15ng/mL), GMQC (3.00ng/mL), MQC (10.0ng/mL), HQC (15.0ng/mL), DQC (30.0ng/mL).

Sample pretreatment

100uL of sample was added to the relevant well in the 96-well plate, followed by 50.0uL of IS working solution. For the blank sample, 50.0uL of 50% methanol was substituted. The mixture was then given another 300uL of methanol and mixed for 10 minutes. At 4°C and 4 min, the cells were centrifuged at 4000 rpm. A fresh 96-well plate was used to receive 100uL of the supernatant. After adding 300uL of 5% acetonitrile solution, sealing the plate and shaking it for 10 min.

Calibration procedure

Analyst V1.6.3 was used to collect and analyse the samples chromatograms and the internal standard and analytes were automatically integrated into the sample to produce the peak area. The standard curve data were subjected to linear regression using Watson LIMS (7.5), with a weight coefficient of 1/X².

Suitability of the system

For the system suitability sample, the s/n of analyte and IS should be ≥5; The ratio of the peak area of the analyte to the IS and the %CV of the retention time for six injection samples should ≤15.0%.

Selectivity

Peak area detected by the analyte in blank plasma matrix should be ≤20.0% of peak area of the effective lower limit of quantification standard curve samples (LLOQ), which should be done during 6 consecutive injections. A maximum of 5.0% of the average peak area of the IS of the LLOQ sample should not be identified in the internal standard channel on average.

The interference of the IS with the analyte was evaluated by adding the IS (without the analyte) to blank matrix to working concentration of the IS compared with the lower limit of quantification standard curve sample (LLOQ). Peak area in the analyte channel must not be ≥20.0% of peak area of the LLOQ sample in this assay batch for six consecutive injection needles.

A sample with internal standard and no analyte was prepared from a pooled batch of human heparin sodium plasma for three consecutive injections. The peak area in the analyte channel must be no more than 20.0% of the peak area of the LLOQ standard curve sample. The analyte interference with the IS was evaluated by adding granisetron (without IS) to the blank matrix to the ULOQ level. The average peak area detected in the internal standard channel should be no more than 5.0% of the peak area of the effective upper limit of quantification standard curve (ULOQ) sample after 3 consecutive injections.

Standard curve

The standard curve samples were freshly prepared granisetron standard curve samples (two sets) containing eight concentration levels, one set of standard curve samples was injected at the beginning of an analysis batch and the other set was injected at the end. The concentration of granisetron was 0.0500, 0.100, 0.400, 1.00, 4.00, 8.00, 16.0 and 20.0ng/mL, respectively. For each concentration level, at least 50% of the standard curve samples must be within $\pm 15.0\%$ of the theoretical value, and the standard curve correlation coefficient R^2 must be ≥ 0.99 .

Lower limit of quantification

No specific prerequisites existed for the preparation of LLOQ substrates and replicates; both single and mixed substrates were acceptable. Sensitivity testing, accuracy and precision assay batches were evaluated together (by QC samples at the LLOQ level in accuracy and precision assay batches). The SNR of the response signal of the LLOQ samples was evaluated by the system applicability experiment of assay batch in which the samples were tested. The deviation must be within $\pm 20.0\%$ the precision must be less than 20.0%. The SNR of the response signal of the LLOQ sample must be no less than 5.

Accuracy and precision of within-run batch

The assessment of Granisetron's precision and accuracy within a single run was conducted by repeatedly evaluating QC samples. The study included a total sample size of $n=6$. Various working solutions were incorporated into human heparin sodium plasma in order to generate the subsequent concentrations: LLOQ QC (0.0500 ng/mL), LQC (0.150ng/mL), GMQC (3.00ng/mL), MQC (10.0ng/mL), and HQC (15.0ng/mL). Calculating the %CV of the detected concentration allowed us to assess the precision. Accuracy was assessed by calculating the divergence between the theoretical and measured concentrations, expressed as the mean percentage difference (%Diff). The acceptable deviation was $\pm 15.0\%$ for all samples, and $\pm 20.0\%$ for the LLOQ QC samples. Precision requirements were set at $\leq 15.0\%$ for all samples, and $\leq 20.0\%$ for the LLOQ QC samples. For each concentration of quality control sample, there must be more than or equal to 50% of the sample number

to meet the deviation requirements. Overall, at least two-thirds of all QC samples must comply with the deviation criteria.

Accuracy and precision of between-run batch

Calculating QC samples from 3 distinct batches over the course of 2 days allowed researchers to assess the granisetron's inter-batch precision and accuracy. Each batch of QC samples should be freshly prepared ($n=6$) at the same concentration as the QC samples for intra-batch testing (LLOQ QC, LQC, GMQC, MQC, HQC). Deviation (%) must be $\pm 15.0\%$ and deviation of LLOQ QC must be $\pm 20.0\%$. Precision (%) must be $\leq 15.0\%$ and deviation of LLOQ QC must be $\leq 20.0\%$.

Extraction recovery rate

The determination of analyte and IS recovery was based on concentration levels employed throughout the analysis. The LQC group prepared six samples, the MQC group prepared six samples, and the HQC group prepared six samples. Additionally, a concurrent extraction of 18 blank samples, devoid of both analyte and internal standard (IS), was performed. Following the extraction of the blank matrix sample, the analyte and IS concentrations were included into the extract to guarantee consistency with the samples that had low, medium and high extraction. The assessment of extraction recovery involved the comparison of the peak area of a single QC sample, which underwent extraction with both the analyte and IS, with peak area of a blank extract sample that had the analyte and IS added. The precision of the extraction recovery for both the analyte and the IS should not exceed 15.0% at each concentration level and across different concentration levels. In cases where the established conditions for extraction recovery are not satisfied, the assessment of IS normalized recovery becomes possible by employing the isotopic IS. The calculation of the IS normalized recovery involves determining the ratio between the peak areas of the analyte and the IS. The IS normalized recovery must have a precision of no more than 15.0% at each concentration level, as well as across all concentration levels.

Matrix effect

A total of six separate batches of individual human plasma were collected for the purpose of examining matrix effects, as documented in previous studies (Shi *et al.*, 2022; Trivedi *et al.*, 2020; Raposo *et al.*, 2021). Following the extraction process, the analytes and IS were introduced into plasma samples devoid of any substances to attain concentrations that align with the injection concentrations of LQC, MQC and HQC. This was done in triplicate for each concentration level and blank matrix. A reference solution was created, which consisted of the analyte and IS at equimolar concentrations. The determination of the matrix effect was performed by evaluating the ratio between the peak area of the analyte and the IS, as described in previous studies (Gabrail *et al.*,

2015; Castaman *et al.*, 2020; CHEN *et al.*, 2020). The matrix effect precision should be within 15.0%.

To assess the matrix effect of hemolyzed plasma, we added six HQC and LQC samples to it and compared the results with the standard curve and QC samples from conventional plasma. To evaluate the matrix effect of hyperlipemia, we utilized a batch of hyperlipemia samples (with six replicates each) in both the LQC and HQC samples. Heparin sodium hyperlipidemia plasma with final lipid content ≥ 4 mg/mL. The precision of the matrix effect should be within 15.0%, and the mean deviation from the theoretical value must be within $\pm 15.0\%$.

Stability

There was no requirement to conduct an investigation on the stability of the isotopic internal standard. The plasma samples of granisetron underwent freeze-thaw cycles at temperatures of -20°C and -70°C . This study aimed to investigate the cyclic stability and long-term conditions of granisetron human plasma samples stored at ambient temperature, as well as the stability of granisetron in human whole plasma at room temperature. Throughout the entirety of the validation process, it is imperative that any IS solution successfully undergoes the test of specificity. This entails ensuring that, in the analyte channel, there are no interference peaks exceeding 20.0% of the average effective lower limit of quantification (LLOQ) peak area. It is crucial to ascertain that the results of the specificity test align with the requirements of the entire validation process.

Sampling process

Recommendations for subjects enrolled according to the "Technical Guidelines for Human Bioequivalence Studies of Generic Chemical Drugs with pharmacokinetic parameters as endpoint Evaluation Indicators" issued by NMPA: According to the guidelines of FDA for bioequivalence study, it was recommended that the test be carried out under fasting/postprandial conditions in healthy volunteers. A total of 51 subjects (36 males, 15 females) with an average age of 27.7 ± 6.9 years were enrolled in the postprandial test. The average weight was 63.8 ± 11.3 kg; Body mass index was 22.6 ± 2.0 kg/m²; The average height was 167.4 ± 10.8 . A total of 28 subjects (18 males and 10 females) were enrolled in the fasting test, with an average age of 27.3 ± 5.2 years. The average body weight was 63.2 ± 10.5 kg. The body mass index was 22.6 ± 2.5 kg/m²; The average height was 166.7 ± 10.0 cm. The drug was given as a single dose of 1 tablet (standard 1mg) with 240 mL water.

Fasting subjects were required to fast overnight for at least 10 hours before administration. After taking a blank blood sample the next day, the subjects were given a reference preparation orally (Specification: 0.1mg), given in 240mL of water. At time points of 0 hours (within 60 minutes prior to administration), 15 min, 0.5 h, 0.75 h, 1 h,

1.33 h, 1.67 h, 2 h, 2.33 h, 2.67h, 3 h, 3.5h, 4 h, 5 h, 6 h, 8h, 12h, 24h, 36 h, 48h, 72 h following administration. After meal, the subjects fasted for more than 10 hours before eating, ate a high-fat meal 30 minutes before administration and took one reference preparation orally (Specification: 0.1mg), taken in 240mL of water. At 0h (within 60 min before administration) and 15 min, 0.5h, 0.75h, 1h, 1.33h, 1.67h, 2h, 2.33h, 2.67h, 3h, 3.3h, 3.7h, 4 h, 4.5h, 5 h, 5.5h, 6h, 8 h, 12h, 24 h, 36 h, 48 h and 72 h after administration. Venous blood samples were collected at 21 time points in each group. Each collection involved the extraction of 4mL of blood from the blood vessels, which were pre-treated with heparin sodium anticoagulant. Following the collection process, the blood samples were subjected to centrifugation within a time frame of 1.5h. Subsequently, the blood samples underwent pretreatment within a period of 2h and were subsequently stored at -60°C .

Pharmacokinetic study

(Spartinou *et al.*, 2017; Cupissol *et al.*, 1993; Wada *et al.*, 2001; Chen *et al.*, 2023; Gabrail *et al.*, 2020) The study's ethical approval process adhered to the Drug Administration Law of the People's Republic of China, Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, and pertinent domestic laws and regulations. The study adhered to ethical approval procedures that aligned with the Drug Administration Law of the People's Republic of China, the Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki and pertinent domestic laws and regulations. The Ethical code: GSSEY2021-YW012-02. The present investigation employed a single-center approach, utilizing a randomized and open-label design. The trial consisted of two cycles and two sequences, employing a crossover methodology. The study focused on the evaluation of pharmacokinetic parameters in healthy adult volunteers, utilizing established methodologies. The assessment was conducted in both fasting and postprandial stages. A graphical representation was created to illustrate the correlation between the average plasma concentration of granisetron and the passage of time. Subsequently, the participants' pharmacokinetic parameters were determined using a non-compartmental model (Castaman *et al.*, 2020; CHEN *et al.*, 2020), taking into account the precise timing of sample collection. The parameter $\text{AUC}_{0-\infty}$ represents the integral of the concentration-time curve throughout the interval from 0 to ∞ . The AUC_{0-t} represents the integral of the concentration-time curve from the initial time to the final time. C_{max} denotes the highest concentration observed, while T_{max} indicates the time taken to attain this maximum concentration. The $t_{1/2}$ refers to the time required for the concentration of a substance to decrease by half during the elimination phase. Lastly, λ_z represents the apparent terminal elimination rate constant. The study involved plotting the average plasma concentration of granisetron against time. The

patients' pharmacokinetic parameters were then calculated using the non-compartment model, taking into account the actual sampling time.

RESULTS

System suitability result

Table 1 displayed the CV% for the retention time of the tested substances, the RT of the IS and the peak area ratio. The CV% for the RT of the tested substances was found to be 0.1%, while the CV% for the RT of the IS was 0.2%. Additionally, the CV% for the peak area ratio was determined to be 3.9%. Notably, all the values were within the acceptable range specified by the standard, i.e., $\leq 15.0\%$. Consequently, the verification results can be deemed satisfactory.

Specificity

Interference of the blank plasma matrix on the analytes was seen to range from 0.0% to 1.3%, as indicated in table 2 and table 3. Additionally, the interference on the IS was found to be 0.0%. The observed interference resulting from the presence of an IS ranged from 0.0% to 2.0%. The tested chemical exhibited an interference of 0.2% with the IS and its result was confirmed to comply with the acceptance criteria.

Standard curve and LOQ

The production of samples for the granisetron standard curve followed the experimental technique, after which they were introduced into the UPLC-MS/MS for the purpose of calibrating the curve. Fig. 6 depicted the linear relationship between the peak area signal of the analyte and the concentration. The minimum detectable concentration was 0.05ng/mL. To further substantiate the accuracy of the calibration curves, we conducted the generation of six calibration curves which demonstrated R^2 values surpassing 0.99. Furthermore, it was determined that the discrepancies observed for every concentration level and standard concentration fell within a range of $\pm 15.0\%$ of the corresponding theoretical value.

Precision, accuracy of within-run batch and between-run batch

According to the data presented in table 4, the highest level of precision within the assay, excluding the LLOQ QC samples, was 4.4%. Additionally, the range of accuracy deviation within the assay was observed to be between -3.6% and 4.7%. The highest level of precision observed within the assay for the LLOQ QC samples was 3.7%. The range of accuracy deviation within the assay was found to be between -2.2% and 0.4%. The highest level of precision observed between assays, excluding the LLOQ QC samples, was 3.1%. The range of accuracy within assay varied from -2.1% to 2.7%. The LLOQ QC samples had a maximum inter-assay precision of 3.1%, whereas the inter-assay accuracy deviated by -1.2%. The

forementioned findings demonstrated a high level of precision and accuracy in the employed methodology.

Extraction recovery results

The results in tables 5 and table 6 showed that the maximum precision of the recovery of granisetron and the IS was 1.2% and 2.4%, respectively, which was much less than the standard value, indicating a good recovery.

Matrix effect

Table 7 presented the matrix effect of normal plasma. It demonstrated that the precision of the matrix effect for the analyte at each concentration level (across all tested lots) and the IS working solution concentration level was found to be $\pm 15.0\%$. Additionally, the precision of the mean of the normalized matrix effect within the three concentration levels of the analyte was also within 15.0%. The level of accuracy in measuring the matrix effect in hemolyzed plasma and hyperlipidemic plasma was found to be within a range of 15.0%. The presence of a positive matrix effect indicated the absence of interference between the matrix and the material under investigation. Consequently, this improved the precision and accuracy of the detection outcomes.

Solution stability results

The stock solution, utilizing methanol as the solvent, exhibited stability for a duration of 26 hours at ambient temperature and 48 days when stored at a temperature of -20°C . The stock solution, which utilized a solvent composed of 50% MeOH, exhibited stability while stored at room temperature for a duration of 25 hours and at a temperature of -20°C for a period of 48 days. The entire blood matrix remained stable when stored at room temperature for a duration of 2 hours. The stability of the plasma matrix was seen at ambient temperature for a duration of 24 hours, as well as at temperatures of -20°C and -70°C for a period of 48 days. Additionally, the samples exhibited stability even after undergoing four cycles of freezing and thawing. The samples that were prepared underwent stabilization at a temperature of 5°C for a duration of 141 hours within the auto sampler. The isotope internal standard of the investigated drug was utilized in this experimental study. The stability test for the solution containing the internal standard was not conducted, since it was determined that the properties of the internal standard were comparable to those of the substance being evaluated, and it was established that the internal standard did not interact with the compound in each analysis batch.

Pharmacokinetic statistics analysis

Fig. 8 and table 8 illustrated the diverse pharmacokinetic parameters seen in healthy individuals following both fasting and postprandial treatment. The elimination half-lives ($T_{1/2}$) during fasting and after a meal were found to be 9.36 ± 5.58 h and 8.95 ± 5.71 h, respectively.

Table 1: System suitability

Sample ID	RT of the analyte	RT of the analyte CV%	Internal standard RT	CV% of Internal standard RT	Area ratio	Area ratio CV%
1	1.142		1.136		4.522	
2	1.143		1.140		4.591	
3	1.143	0.1	1.135	0.2	4.417	3.9
4	1.146		1.140		4.302	
5	1.145		1.139		4.665	
6	1.145		1.140		4.792	

Table 2: The phenomenon of interference caused by a blank matrix on both the chemical and internal target

Granisetron					Internal standard				
ID	Blank matrix peak area	LLOQ peak area-1	LLOQ peak area-2	Interference %	ID	Blank matrix peak area	LLOQ peak area-1	LLOQ peak area-2	Interference %
1	167			1.3	1	172			0.0
2	0			0.0	2	131			0.0
3	38	12175	13174	0.3	3	271	735033	7826663	0.0
4	0			0.0	4	159			0.0
5	123			1.0	5	177			0.0
6	76			0.6	6	169			0.0

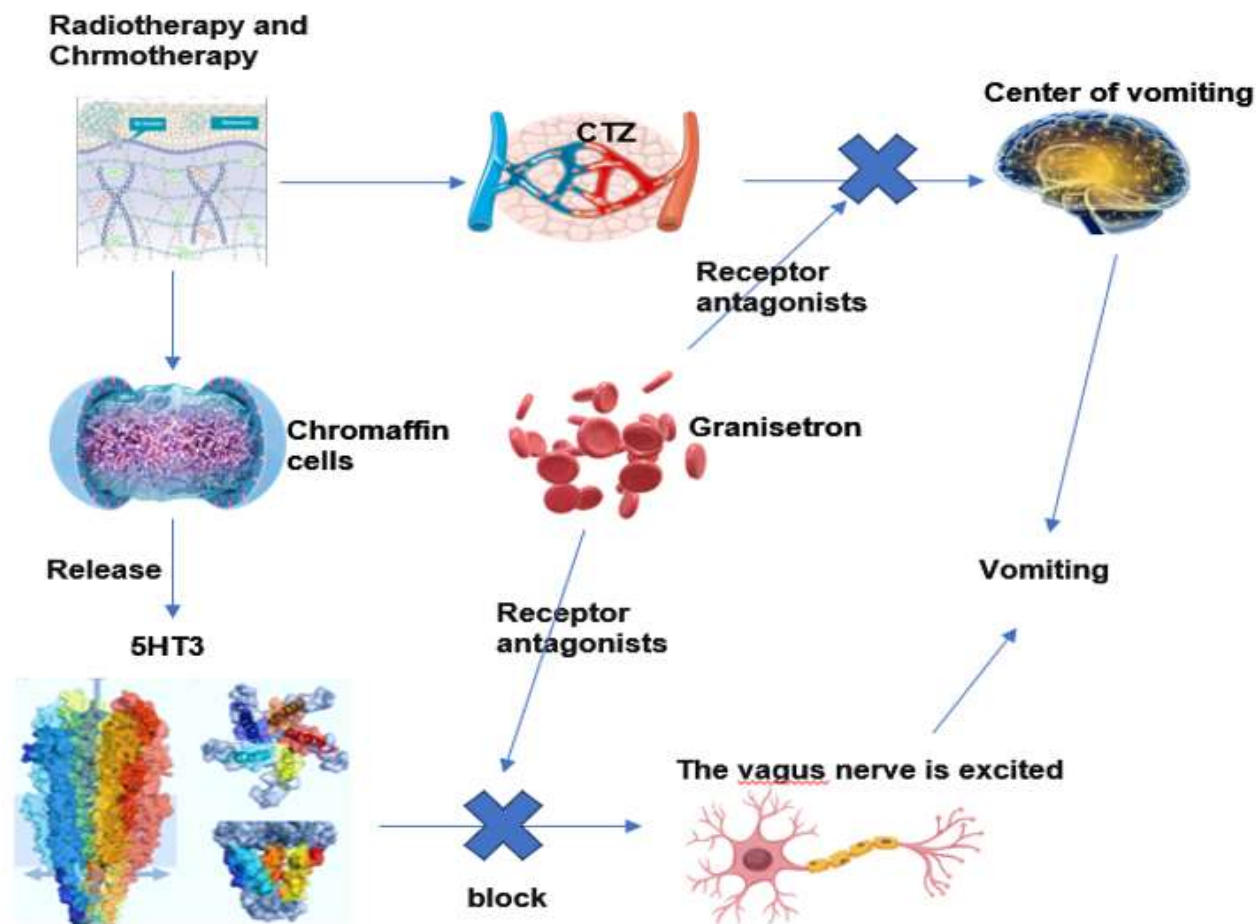


Fig. 1: Mechanism of action of granisetron

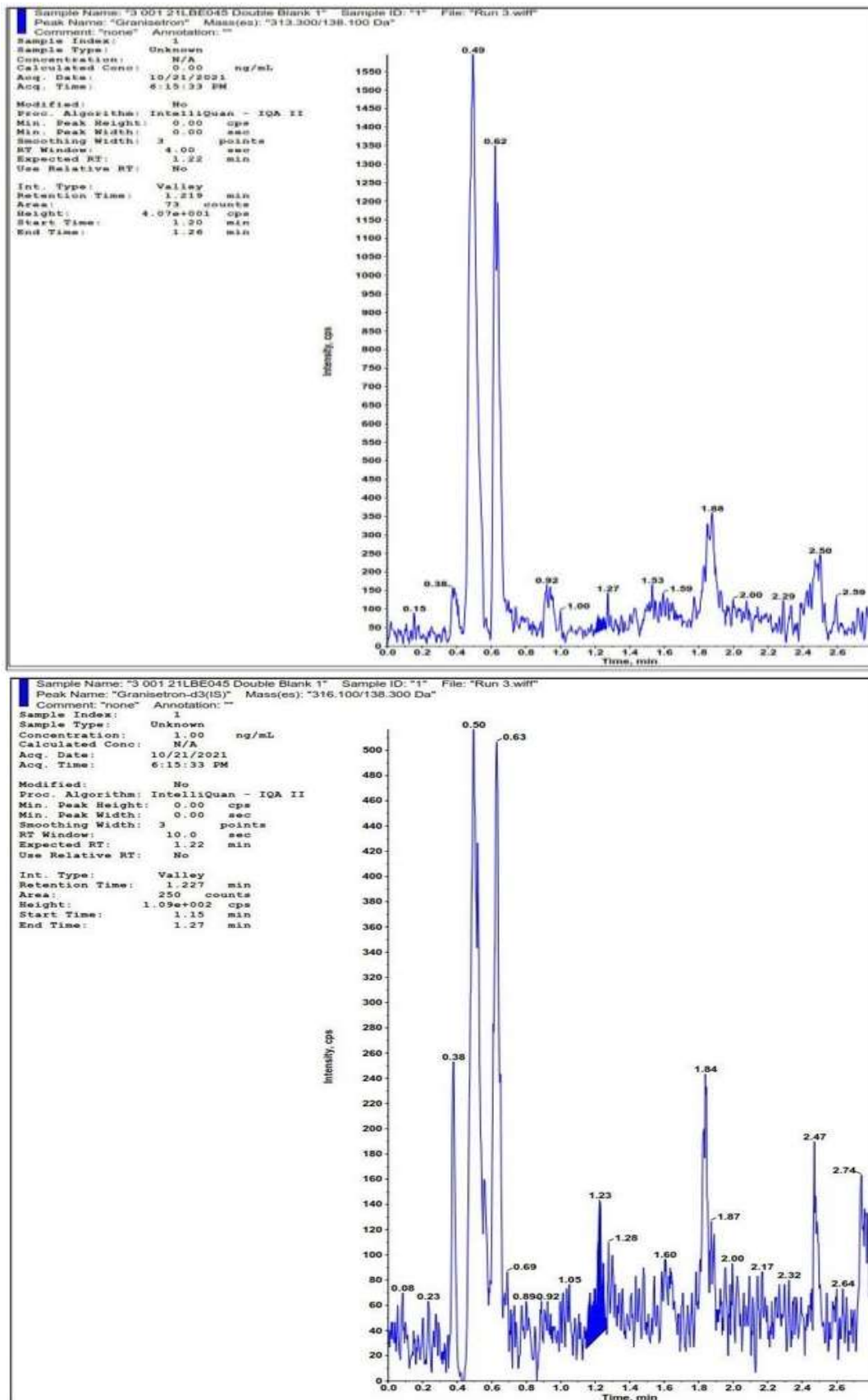


Fig. 2: Double blank matrix

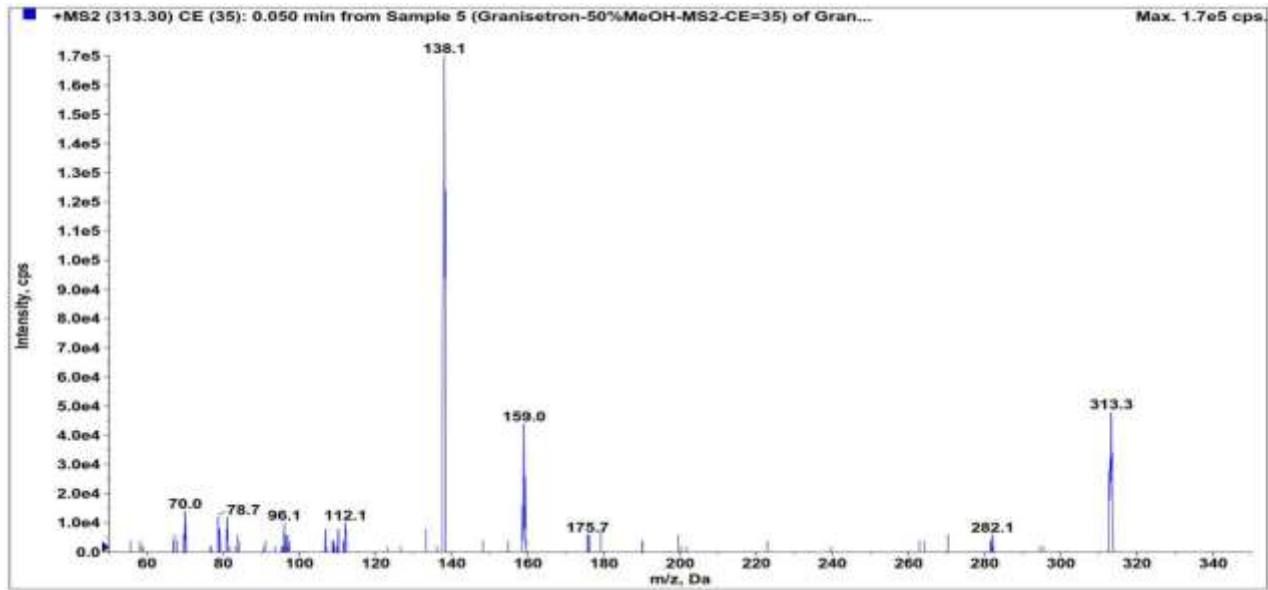


Fig. 3: Granisetron mass spectrogram

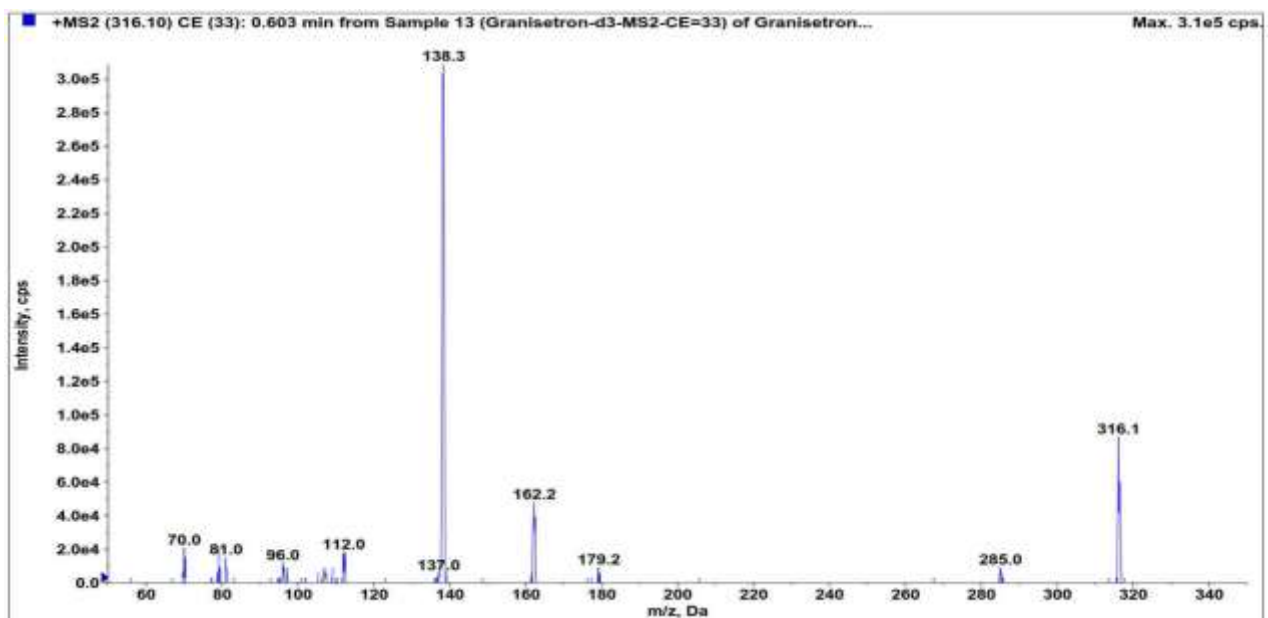


Fig. 4: Granisetron-d3 mass spectrogram

Table 3: Mutual interference of internal standards and analytes

Granisetron					Internal standard				
ID	QC0	LLOQ peak area-1	LLOQ peak area-2	Interference %	ID	ULOQ without IS	ULOQ peak area-1	ULOQ peak area-2	Interference %
1	0			0.0	1	1865			
2	0			0.0	2	1812			
3	89			0.7	3	1829			0.2
4	0	12175	13174	0.0			784538	771641	0.2
5	158			1.2					0.2
6	257			2.0					

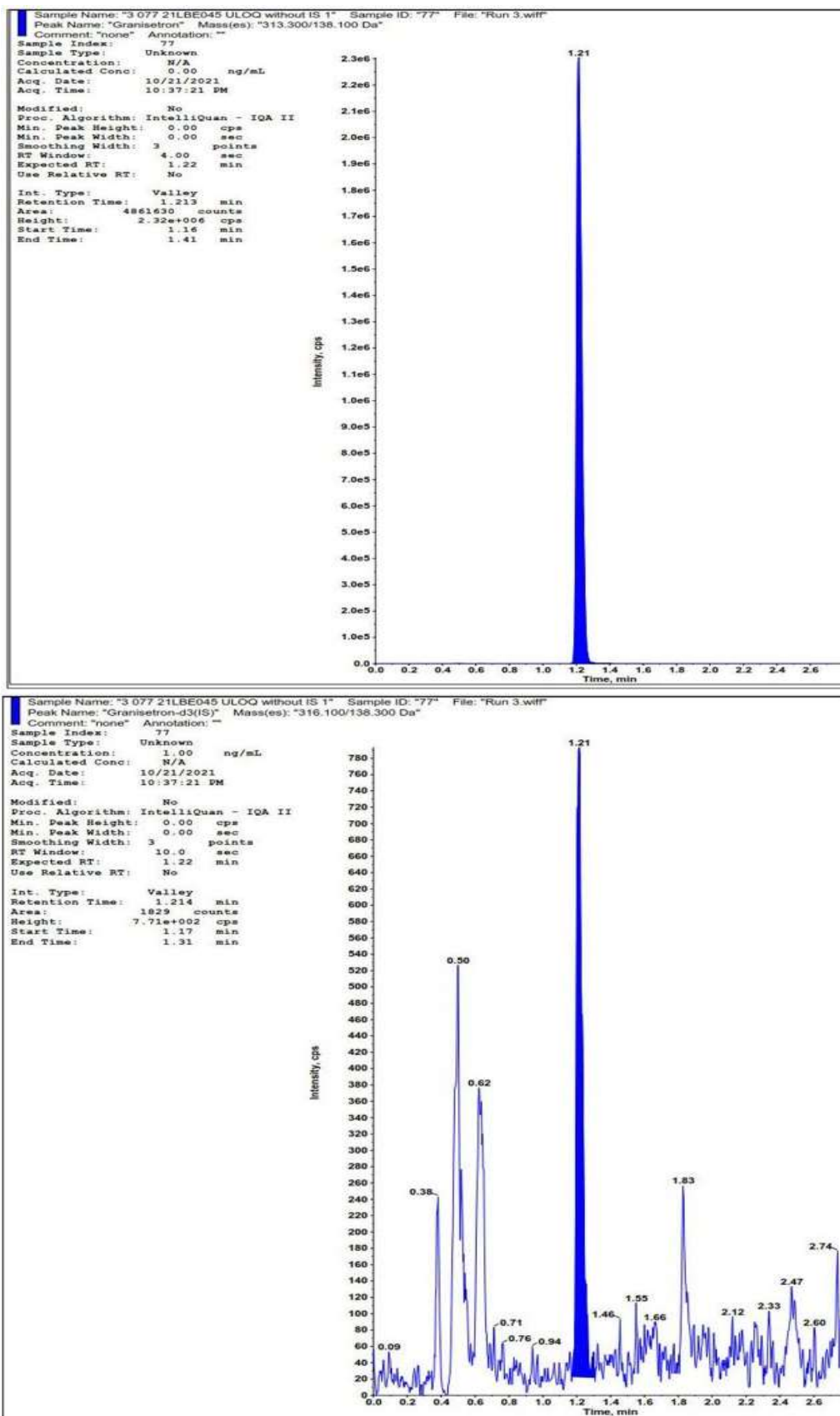


Fig. 5: ULOG without IS

Table 4: Precision and accuracy of within-run batch and between-run batch

Experiment number	LLOQ QC (ng/mL)	Accuracy deviation %	LQC (ng/mL)	Accuracy deviation %	GMQC (ng/mL)	Accuracy deviation %	MQC (ng/mL)	Accuracy deviation %	HQC (ng/mL)	Accuracy deviation %
1#	0.048	-4.2	0.148	-1.3	2.97	-1.0	9.75	-2.5	15.6	4.0
	0.049	-1.2	0.152	1.3	2.99	-0.3	9.65	-3.5	15.0	0.0
	0.049	-2.2	0.151	0.7	2.97	-1.0	9.90	-1.0	15.4	2.7
	0.050	-0.8	0.155	3.3	3.05	1.7	10.0	0.0	14.9	-0.7
	0.049	-3.0	0.156	4.0	3.12	4.0	9.65	-3.5	15.3	2.0
	0.050	-0.2	0.151	0.7	3.03	1.0	10.3	3.0	15.0	0.0
	Average	0.049	NA	0.152	NA	3.02	NA	9.88	NA	15.2
Within-run SD	0.0007	NA	0.0029	NA	0.0581	NA	0.2500	NA	0.2760	NA
Within-run %CV	1.5	NA	1.9	NA	1.9	NA	2.5	NA	1.8	NA
Within-run Accuracy deviation %	-2.0	NA	1.3	NA	0.7	NA	-1.2	NA	1.3	NA
2#	0.049	-1.8	0.147	-2.0	3.07	2.3	10.3	3.0	14.0	-6.7
	0.046	-8.0	0.159	6.0	2.92	-2.7	9.71	-2.9	14.0	-6.7
	0.050	0.8	0.159	6.0	3.08	2.7	9.96	-0.4	14.5	-3.3
	0.048	-4.6	0.147	-2.0	2.99	-0.3	9.76	-2.4	15.2	1.3
	0.050	-0.8	0.161	7.3	3.03	1.0	9.41	-5.9	14.3	-4.7
	0.051	1.6	0.148	-1.3	3.03	1.0	10.1	1.0	14.9	-0.7
	Average	0.049	NA	0.154	NA	3.02	NA	9.87	NA	14.5
Within-run SD	0.0018	NA	0.0068	NA	0.0587	NA	0.315	NA	0.488	NA
Within-run %CV	3.7	NA	4.4	NA	1.9	NA	3.2	NA	3.4	NA
Within-run Accuracy deviation %	-2.2	NA	2.7	NA	0.7	NA	-1.3	NA	-3.3	NA
3#	0.052	4.6	0.159	6.0	3.05	1.7	9.88	-1.2	15.2	1.3
	0.050	-0.6	0.159	6.0	3.02	0.7	9.71	-2.9	14.3	-4.7
	0.052	4.0	0.156	4.0	3.04	1.3	9.30	-7.0	15.2	1.3
	0.049	-1.4	0.155	3.3	3.11	3.7	9.83	-1.7	14.9	-0.7
	0.050	0.6	0.154	2.7	2.90	-3.3	9.57	-4.3	14.8	-1.3
	0.048	-5.0	0.156	4.0	3.02	0.7	9.52	-4.8	15.0	0.0
	Average	0.050	NA	0.157	NA	3.02	NA	9.64	NA	14.9
Within-run SD	0.0018	NA	0.0021	NA	0.0689	NA	0.2160	NA	0.335	NA
Within-run % CV	3.6	NA	1.3	NA	2.3	NA	2.2	NA	2.2	NA
Within-run Accuracy deviation %	0.4	NA	4.7	NA	0.7	NA	-3.6	NA	-0.7	NA
Between-run SD	0.0016	NA	0.0046	NA	0.0583	NA	0.2730	NA	0.4650	NA
Between-run %CV	3.1	NA	3.0	NA	1.9	NA	2.8	NA	3.1	NA
Between-run Accuracy deviation %	-1.2	NA	2.7	NA	0.7	NA	-2.1	NA	-0.7	NA

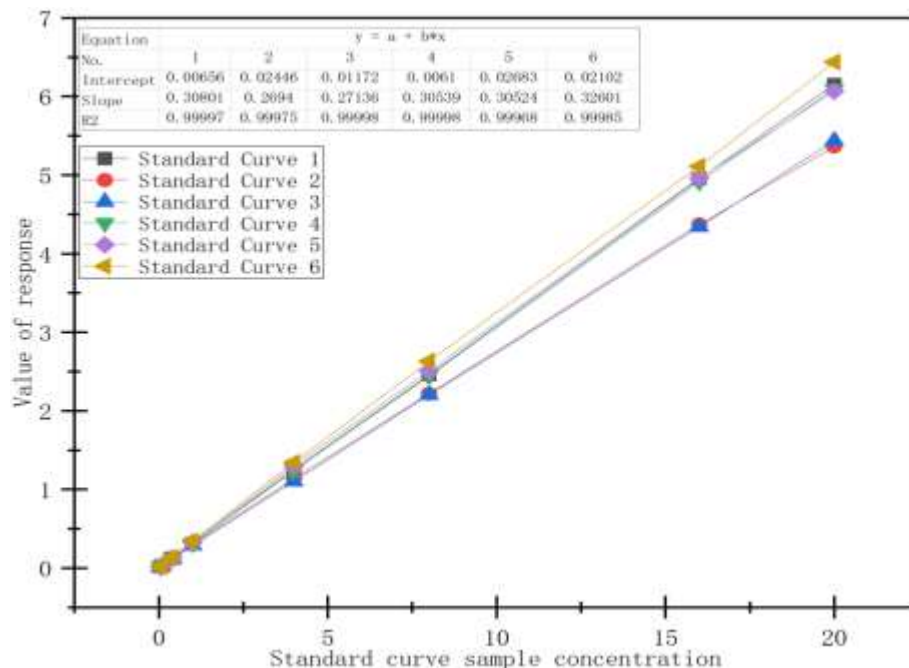


Fig. 6: Granisetron standard curve

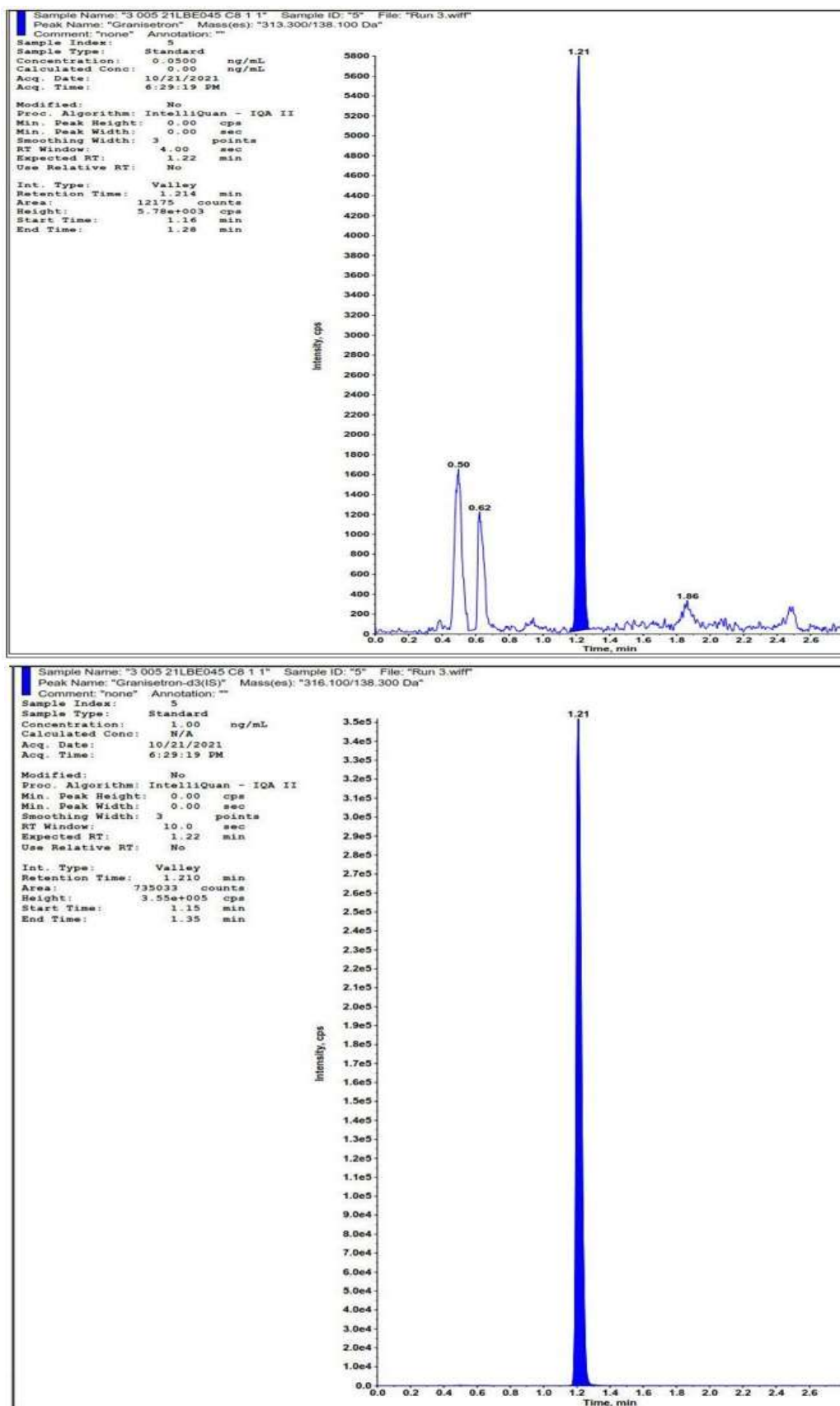


Fig. 7: LLOQ

Table 5: Extraction recovery rate of granisetron

Experiment No.	HQC peak			MQC peak			LQC peak		
	Area after extraction	Pre-extraction	Recovery %	Area after extraction	Pre-extraction	Recovery %	Area after extraction	Pre-extraction	Recovery %
1	2012795	1928681	105.0	1388473	1248690	106.6	21011	19667	106.1
2	1957537	1896456	102.1	1303051	1327612	100.1	21672	19882	109.4
3	1984571	1963668	103.6	1399376	1300610	107.5	21946	20382	110.8
4	2049766	1853393	107.0	1323963	1312684	101.7	19962	20304	100.8
5	1988940	1885711	103.8	1287808	1294368	98.9	21147	19222	106.8
6	2021948	1970396	105.5	1386547	1327710	106.5	20332	19384	102.7
Average	2002593	1916384	104.5	1348203	1301946	103.6	21012	19807	106.1
SD	12600	10800	1.71	49000	29400	3.76	760	474	3.82
% CV	2.4	1.8	1.6	3.6	2.3	3.6	3.6	2.4	3.6
Overall recovery%						104.7			
Overall % CV						1.2			

Table 6: Recovery rate of IS

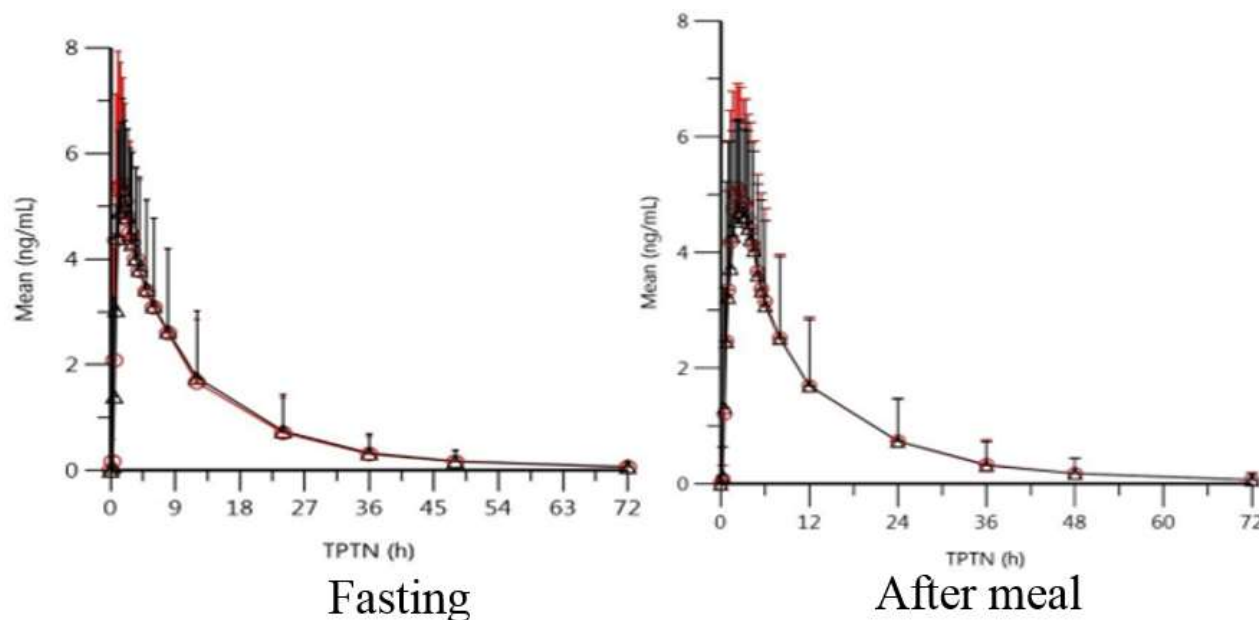
Experiment No.	IS peak area	Concentration: 300 ng/mL		Recovery%
		Pre-extraction	Internal standard peak area	
1	495865	481181		104.1
2	475022	471502		99.7
3	479182	463194		100.6
4	471654	472952		99.0
5	457163	469084		95.9
6	477112	451226		100.1
7	472870	469776		99.2
8	472756	483957		99.2
9	495158	487204		103.9
10	477870	493228		100.3
11	482234	479790		101.2
12	483611	477103		101.5
13	506543	493244		106.3
14	494286	466492		103.7
15	480822	474243		100.9
16	475909	477214		99.9
17	489205	480961		102.7
18	477204	485717		100.1
Average	481359	476559		NA
SD	11400	10600		2.40
%CV	2.4	2.2		2.4
Average Recovery %			101.0	

Table 7: Matrix Effect results

Concentration	Matrix effect					
	Granisetron matrix effect	Precision of Granisetron matrix effect	IS Matrix effect	Precision of IS Matrix effect	IS working solution concentration level (all batch substrates tested) matrix effect precision	The precision of IS normalized matrix effect mean
LQC	0.960	2.0	0.965	2.4	1.1	0.7
MQC	0.969	2.0	0.962	1.9		
HQC	0.957	0.6	0.958	0.4		
Hemolytic plasma matrix effect			Hyperlipidemic matrix effect			
	Accuracy deviation %	Precision deviation%	Accuracy deviation %	Precision deviation%		
LQC	-2.7	3.7	-1.3	5.2		
HQC	-5.3	3.1	-6.0	2.9		

Table 8: Pharmacokinetic parameters of Granisetron

Parameters	Fasting (Mean±SD)	After meal (Mean±SD)
T _{max} (h)	2.00(0.75,4.50)	1.33(0.75,2.67)
C _{max} (ng/mL)	6.20±1.95(31.43)	6.41±2.20(34.4)
AUC _{0-t} (h*ng/mL)	63.66±41.86(65.77)	63.08±39.86(63.18)
AUC _{0-∞} (h*ng/mL)	65.96±44.46(67.40)	64.76±41.21(63.64)
%AUC %Extrap	3.01±2.02(67.12)	2.50±1.26(50.24)
λ _z (h ⁻¹)	0.11±0.08(74.88)	0.14±0.12(86.05)
T _{1/2} (h)	9.36±5.58(59.64)	8.95±5.71(63.74)

**Fig. 8:** Subjects mean (SD) blood concentration-time curve (fasting and after meal)

The average value plus or minus the standard deviation (SD) of AUC_{0-t} was 63.66±41.86ng·h/mL and 63.08±39.86ng·h/mL, while the average value plus or minus the standard deviation (SD) of AUC_{0-∞} was 65.96±44.46ng·h/mL and 64.76±41.21ng·h/mL, respectively. The median and range of T_{max} were 2.00 (0.75-4.50) hours and 1.33 (0.75-2.67) hours, respectively. The arithmetic mean plus or minus the standard deviation of the maximum concentration (C_{max}) were 6.20±1.95ng/mL and 6.41±2.20ng/mL, respectively.

DISCUSSION

Through methodological validation of the detection method, it has been demonstrated that the method has good durability, simplicity and accuracy in detecting the content of granisetron hydrochloride. At the same time, a rapid detection time of 2.8 minutes can greatly improve the detection efficiency. After administration, we detected the content of granisetron hydrochloride in human plasma and found that granisetron hydrochloride can be quickly absorbed in the body and widely distributed throughout the body. After oral administration of granisetron

hydrochloride tablets to healthy subjects on an empty stomach and after meals, the drug concentration in the plasma rapidly increases, reaching a peak concentration of about 6.2ng/ml and 6.4ng/ml, respectively, with peak times of about 2.0 and 1.3h (Spartinou *et al.*, 2017).

The results of this study indicate that although postprandial oral administration of granisetron can reach maximum plasma concentration faster, the overall pharmacokinetic data before and after meals are similar, indicating that diet has no significant effect on the oral bioavailability of granisetron tablets. Through this study, we can obtain data on the concentration changes of the drug *in vivo*, provide guidance for clinical doctors in terms of dosage, duration, etc., and ensure that the drug can function safely and effectively. At present, we have not seen any literature that simultaneously studies the pharmacokinetics of granisetron before and after meals, so our study fills this gap.

The half-life of granisetron hydrochloride in the body is influenced by various factors, including age and disease status. The results of this study show that the half-life of

granisetron is about 9 hours in healthy subjects, while it may be significantly prolonged in cancer patients. In addition, the half-life of elderly and young people is slightly different and this study did not conduct relevant studies. This is also the limitation of our research. In the future, we will try to supplement these studies. Based on the research results, attempts are made to improve the absorption and distribution of drugs in vivo by adjusting their structure, thereby enhancing their bioavailability and therapeutic efficacy.

CONCLUSION

In this paper, the UPLC-MS/MS method was established and validated to successfully study the content of granisetron in 2.8min of human heparin sodium plasma. Compared with the traditional liquid chromatography method, the detection speed and sensitivity of this method are faster. At the same time, the selectivity, extraction recovery, precision and accuracy of the method meet the detection requirements. The method was also successfully applied to the granisetron PK study, which evaluated the main PK parameters of granisetron in plasma of healthy Chinese subjects, with package results comprising both premeal and postprandial. In the future, the method will also be applied to the bioequivalence study of granisetron.

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