Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 Issue 2, 2014

Research Article

DEVELOPMENT AND VALIDATION OF RANITIDINE HYDROCHLORIDE IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY AND ITS APPLICATION

SENTHIL KUMAR K. R.*, J.S.K.NAGARAJAN, S.N. MEYYANATHAN

Department of Pharmaceutical Analysis, JSS College of Pharmacy, Off Campus JSS University, Mysore, Udhagamandalam-643001, Tamil Nadu, India. Email: senalysis005@gmail.com

Received: 31 Jan 2014, Revised and Accepted: 11 Mar 2014

ABSTRACT

Objective: A rapid and sensitive bioanalytical method based on liquid chromatography coupled with mass spectrometry detection with positive ion electrospray ionization was developed for the determination of ranitidine hydrochloride in human plasma using clarithromycin as the internal standard.

Methods: A simple protein precipitation method was performed using perchloric acid 5%. The analyte and internal standard were subjected to chromatographic analysis on phenomenex C_{18} column (100×4.6 mm i.d., 5µ) using acetonitrile and water (90:10v/v) as a mobile phase at a flow rate of 0.5 ml/min. A shimadzu 2010A Liquid Chromatographic coupled with mass spectrometry was operated in the selected ion monitoring (SIM) mode.

Results: The described method was linear over the range of (0.025 µg/ml to 2.50 µg/ml) (r = 0.997) and the total analysis time for each chromatograph was 3 min. Mean extraction recovery was 87.91% to 92.69%. Sample was stable at room temperature for 6.0hr and at -70^{0}C over 4 weeks respectively.

Conclusion: The method provides good sensitivity and excellent precision and reproducibility relatively short term analysis and will be useful tool in the pharmacokinetic study of ranitidine hydrochloride in humans.

Keywords: Ranitidine hydrochloride, Validation, Human plasma, Liquid chromatography coupled with mass spectrometry.

INTRODUCTION

Ranitidine hydrochloride (HCl), a histamine H_2-receptor antagonist. Chemically it is N [2-[(5-[(dimethylamino) methyl] -2furanyl] methyl] thio] ethyl] -N'-methyl-2-nitro-1, 1-ethenediamine, HCl (Fig. 4). It is histamine H_2 receptor antagonist that inhibits stomach acid production [1, 2].

Ranitidine has been found to be effective as maintenance therapy for patients following healing of acute duodenal ulcers. As with other H_2-antagonists, the factors responsible for the significant reduction in the prevalence of duodenal ulcers include prevention of recurrence of ulcers, more rapid healing of ulcers that may occur during maintenance therapy [3].

It is a selective and competitive histamine H2-receptor antagonist with an extensive clinical history in the treatment of gastric and duodenal ulcers, gastroesophageal reflux disease (GERD) and Zollinger-Ellison syndrome [4]. Although proton pump inhibitors(PPIs) are considered as first line treatment for many conditions associated with gastric hyperacidity, Ranitidine continues to be of great value in combination with PPIs for treating night-time heartburn[3], with antacids for treating mild GERD symptoms, and as Ranitidine bismuth citrate for treating Helicobacter pylori infection [5,6].

The pharmacokinetics of RAN after oral administration indicates that 50% of a dose is rapidly absorbed *via* the small intestine with the peak serum level occurring at approximately three hours [7, 8]. Ranitidine is mainly cleared by metabolism of it to N- and S-oxides by flavin monooxygenases [9]. Keeping this fact in mind we have developed and validated a bioanalytical method for ranitidine hydrochloride.

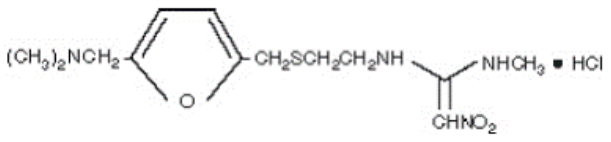

Fig. 4: Structure of Ranitidine hydrochloride

MATERIALS AND METHODS

Chemicals and reagents

Ranitidine hydrochloride and clarithromycin (internal standard) were obtained from Glaxo pharma Mumbai and Alembic Ltd Baroda (Gujarat, India). Acetonitrile, methanol of HPLC grade and obtained from Merck (Mumbai, India) and Formic acid is from analytical grade by Qualigens Fine Chemicals and S.D. Fine chemicals, Water HPLC grade from Milli-Q RO system were used through analysis.

Instruments

A shimadzu 2010 A LC-MS (including LC-10 AD-vp pumps an online vacuum deaerator, a constant temperature automatic sampler, a quadrouple mass spectrometer equipped with an electrospray ionization interface (ESI) source and LC-MS solution (Version 2.04) was used for data processing. Liquid chromatographic separation were achieved by using phenomenex column C_{18} (100×4.6 mm i. d., 5µ) the column and autosampler tray temperature were kept constant at 40^o and 4^{o}C respectively. The mobile phase consist of a mixture water (A) and acetonitrile (B) (10:90v/v) and was delivered at a flow rate of 0.5ml/min, the sample injection volume was 10µl respectively. Samples were ionized by positive ion electrospray ionization mode under the following source conditions: Gas flow 1.5L/min, curved desolvation line (CDL) voltage was fixed as tuning, CDL temperature 250^{o}C, and block temperature 200^{o}C. Mass spectra were obtained at a dwell time of 0.2 and 1.5 for SIM and SCAN mode accordingly. Analysis was carried out by using selected ion monitoring (SIM) for specific m/z 315.05 for ranitidine hydrochloride and m/z 748.15 for clarithromycin. Peak areas for all components were automatically integrated using LC-MS lab solution Version 2.04, 2010 Shimadzu Corp.

Preparation of standard solution and quality control samples

Stock solution of ranitidine hydrochloride and clarithromycin were prepared in water at a concentration 1mg/ml solution respectively. It was further diluted to spike the calibration curve standards and quality control samples. Calibration curves for ranitidine hydrochloride were prepared by spiking blank plasma at concentrations of 0.50, 1.00, 2.50, 5.00, 10.00, 20.00, 30.00,

50.00 µg/ml respectively. Quality control samples were prepared by spiking blank plasma at concentration of 1.50, 20.00, and 40.00 µg/ml, representing low, medium, high concentration respectively.

Sample preparation

A volume 0.5ml of sample was pipetted into 2.0ml centrifuge tube with this 50µl of internal standard (clarithromycin) 5.0µg/ml and 1.0 ml of precipitating agent (perchloric acid 5%) was added. The resulting solution was vortexed for 5 minutes and centrifuged at 3500 rpm for 10 minutes, supernatants from the above solution were separated and used for the analysis.

Method validation

The method was validated according with to currently accepted principles of the US Food and Drug Administration (FDA) industry guidance for validation of bioanalytical methods [10]. The validation parameters addressed were linearity, precision, accuracy, specificity, sensitivity and stability.

Sensitivity and specificity

The lower limit of quantification was determined as the minimum concentration that could be accurately and precisely quantified (lowest data of the standard curve). The specifity of the assay for the analytes verses endogenous substances in the matrix was assessed by comparing the lowest concentration in the calibration curve with the reconstitutions prepared with drug free plasma from six different humans.

Accuracy and precision

The accuracy and precision (presented as relative standard deviation R.S.D.) of the assay were determined by using quality control (QC) samples at 0.075, 1.000, 2.000 µg/ml. Accuracy (%) was determined by the percentage ratio of the measured over spiked quality control samples concentration (mean of measured/spiked %). Intra-day precision was determined by analysing replicate aliquots of quality control samples (n=3 per each concentration) on the same day. Inter-day precision was similarly evaluated over two-week period.

Recovery

To investigate the recovery of ranitidine hydrochloride by the protein precipitation method, plasma samples were spiked with ranitidine hydrochloride at concentration (0.075, 1.000, 2.000 µg/ml). The resulting peak-area ratios (analyte: internal standard) were compared with that of the standards prepared in mobile phase to provide the recovery values.

Stability

The stability studies of plasma samples spiked with ranitidine hydrochloride were subjected to three Freeze- thaw cycles, Short term stability at room temperature, three replicates of quality control samples at each of the low, middle and high concentrations were subjected respectively.

The plasma samples of the long term stability were stored in the freezer at -70^{o}C until the time of analysis. The mean concentrations of the stability samples were compared with theoretical concentration.

RESULTS AND DISCUSSION

Specificity

The full scan mass spectra of ranitidine hydrochloride and clarithromycin (IS) after direct injection in mobile phase are presented in Fig 1. The protonated molecules found for ranitidine hydrochloride were m/z 315.05. The mass spectrometric parameters were optimized to obtain the higher signal from the selected ion m/z 315.05, which also shows less internal interferences, observed retention time for ranitidine hydrochloride and clarithromycin were 2.47 and 4.34 min respectively Fig 2.

(Standard solution)

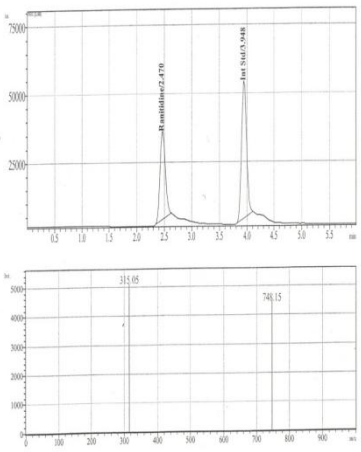

Fig. 1: chromatogram of ranitidine hydrochloride and internal standard

(Sample solution)

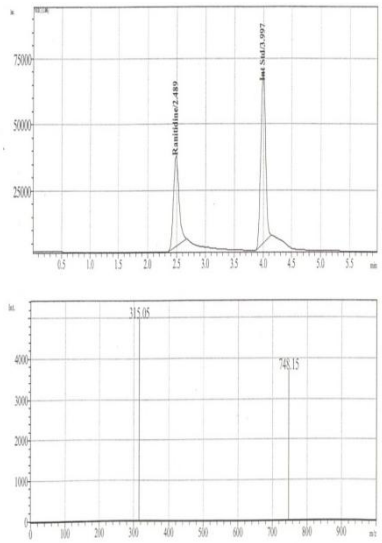

Fig. 2: chromatogram of ranitidine hydrochloride and internal standard

Linearity

The linear regression analysis of ranitidine hydrochloride constructed by plotting the peak area of response factors verses concentration. The average regression equation of these curves and their correlation coefficients (r) were calculated, it showed good linear relationship between the peak area and concentration (Fig 3).

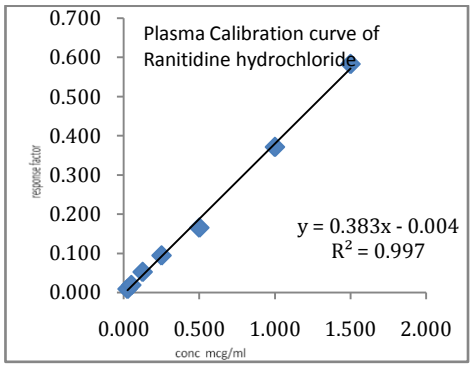

Fig. 3: Calibration curve of Ranitidine hydrochloride

Lower limit of quantification: The lower limit of quantitation was 10ng/ml for the determination of ranitidine hydrochloride in human plasma. **Precision :** The intraday precision presented as relative standard deviation. The accuracy defined as (measured concentration/spiked concentration) ×100%, the interday precision was similarly evaluated over two-week period (Table 1).

Table 1: Results of Precision studies from quality control samples

S. No.	Concentration of drug	Obtained concentration	Mean concentration
1	0.075	0.067	0.067
2	0.075	0.064	
3	0.075	0.069	
4	1.000	0.920	0.912
5	1.000	0.905	
6	1.000	0.912	
7	2.000	1.835	1.828
8	2.000	1.817	
9	2.000	1.832	

Recovery

The absolute recoveries of ranitidine hydrochloride at concentrations 0.075, 1.000, 2.000 µg/ml were 87.91%, 92.48%, and 92.69% respectively (Table 2).

Table 2: Results of Extraction recovery of Analyte

Level	Concentration of drug added µg/ml	Amount of drug recovered (µg/ml) in plasma sample	Recovery %	Amount of drug recovered (%)in mobile phase	Relative Recovery %
Level-I	0.075	0.065 ± 0.008	Mean : 86.66 CV: 12.30 N : 6	Mean : 98.57 CV: 8.14 N : 6	87.91
Level-II	1.000	0.913 ± 0.094	Mean : 91.30 CV: 10.29 N : 6	Mean : 98.72 CV: 5.28 N : 6	92.48
Level-III	2.000	1.821 ± 0.113	Mean : 91.05 CV : 6.20 N : 6	Mean : 98.23 CV: 3.51 N : 6	92.69

Stability

Stability of ranitidine hydrochloride for short term stability at room temperature for 6 hrs and long term stability at -70ºC over four weeks (Table 3).

Table 3: Stability of Analyte at different conditions

Storage Condition	Conc (µg/ml) 0.075	Conc (µg/ml) 1.000	Conc (µg/ml) 2.000
Freeze and Thaw			
Cycle 1	0.068	0.921	1.827
Cycle 2	0.063	0.907	1.803
Cycle 3	0.062	0.902	1.813
Short term plasma sample at room temperature After 1hr	0.065	0.917	1.820
After 2hr	0.067	0.904	1.793
After 3hr	0.062	0.892	1.791
Long term plasma sample at-70ºC			
After 1week	0.067	0.913	1.825
After 2week	0.063	0.902	1.807
After 3week	0.064	0.884	1.794
Standard Solutions			
After 6hr at room temperature	0.073	0.987	1.963
After 2weeks in freeze	0.070	0.968	1.925
After 4weeks in freeze	0.068	0.952	1.893

Ionization

It was shown that protein precipitation method improves the sample cleanup to remove the internal substances of plasma and there by decrease the amount of matrix injected onto the column, thus the ion suppression was minimized. The results indicated that there was no significant difference between the signals of analytes extracted from human plasma and the mobile phase, which proves that there were no matrix effects.

CONCLUSIONS

Protein precipitation of ranitidine hydrochloride from plasma was found to be more precise than the other extraction methods.

The current method shows high precision, accuracy, recovery, and relatively short term analysis and will be useful tool in the pharmacokinetic study of ranitidine hydrochloride in humans.

ACKNOWLEDGMENT

The authors are grateful to His Holiness Jagadguru Sri Sri Shivarathri Deshikendra Mahaswamigalavaru of Sri Suttur Mutt, Mysore for his blessings and the facilities provided to complete the research work successfully.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCE

1. Rouge, N., Buri, P., & Doelker, E. Drug absorption sites in the gastrointestinal tract and dosage forms for site-specific delivery, Int. J. Pharm 1996; 136: 117-139.
2. Brogden RN,Carmin AA, Heel RC.Ranitidine,A review of its pharmacology and therapeutic use in peptic ulcer disease and other allied diseases, Drugs 1982; 24:267-303.
3. Jeffery, G.H. Basselt, J. Vogel's Text Book of Quantitative Chemical Analysis, 5th edition, 1991, 217-235.
4. Higuchi K., Watanabe T., Tominaga K., Shiba M., Nakagawa K., Uno H., Kitada K., Satoh H., Chono S., Uchida T., Fujiwara Y., Arakawa T., *Int. J. Clin. Pharmacol. Res.*, 2005, *25*, 187
5. Améndola R., de los Santos M. C., Massa J. M., Vazquez H., Smecuol E., *Acta* Gastroenterol. Latinoam., 2007, *37*, 231
6. Mesquita M. A., Lorena S. L., Almeida J. R., Montes C. G., Guerrazzi F., Campos L. T., Zeitune J. M., *World J.* Gastroenterol., 2005, *11*, 3566
7. Gisbert J. P., Gonzalez L., Calvet X., *Helicobacter*, 2005, *10*, 157
8. Gschwend M. H., Guserle R., Erenmemişoglu A., Martin W., Tamur U., Kanzik I., Hincal A. A., Arzneimittelforschun, 2007, *57*, 315
9. Zhou Q., Ruan Z. R., Yuan H., Jiang B., Xu. D. H., *World J.*Gastroenterol., 2006, *12*, 2742
10. U.S. Food and Drug Administration, Guidance for industry, Bioavailability and Bioequivalence studies for orally administered Drug Products-General considerations. Rockville, MD (2000) Centre for Drug Evaluation and Research (CDER)