Volume 8, Issue 3, 2019, 671 - 674 **ISSN 2284-6808**

Letters in Applied NanoBioScience

www.NanoBioLetters.com

https://doi.org/10.33263/LIANBS83.671674

Original Research Article **Open Access Journal**

Received: 28.06.2019 / Revised: 10.09.2019 / Accepted: 12.09.2019 / Published on-line: 17.09.2019

A sensitive and reliable RP-HPLC method for the detection of cimetidine - a H2 receptor antagonist in human plasma

Abul Bashar Mohammed Helal Uddin [1,*], Abul Kalam Azad [2], Abdur Rashid Mia [3], Liyana Zafirah [4]

[4]Analytical and Bioanalytical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

[2]Advanced Drug Delivery Laboratory, Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

[3]Dosage Design Laboratory, Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

*corresponding author e-mail address: ***abmhelal@iium.edu.my***

ABSTRACT

Bioanalytical methods for bioequivalence studies require high sensibility and rapidity due to the large number of samples and the low plasma concentration of drugs. The present study aimed to develop and validate a high-performance liquid chromatography method to quantify cimetidine (CMT) in human plasma and to apply it in a bioequivalence study. Spiked plasma of 500 µl (l, m and h concentration) was used for the assay. The HPLC injection volume was 20µl of the reconstitute sample where, 2 ml of ethyl acetate used for extraction purposes. Cimetidine was prepared separately for low (80 ng/ml), medium (2000 ng/ml) and high (3600 ng/ml) concentrations and internal standard (ranitidine) concentration was 3000 ng/ml. Freeze thawing and long terms stability were conducted at -25º c. The individual calibration curve for spiked standards was linear with R2= 0.99. The inaccuracy values for QC samples were within 15% of the actual value and not more than 20% for the LOQ. The limit of quantitation (LOQ) was 40 ng/ml, which was also the lowest concentration of cimetidine that was quantitated with the variability of 5.9%. The within day precision and between day precision for LOQ were 10.8 and 5.9 respectively. The retention time for the analyte was 4.1-4.5 minutes during the within a day and between day results. The mean % inaccuracy values for low, medium and high concentration were 6.8, 5.6 and 7.8 respectively for within day and 2.4, 6.1 and 7.9 respectively for between days. The within day and between day % inaccuracy for LOQ concentration was 12.4 and 5.5 respectively. The mean recoveries for low, medium and high concentration of cimetidine were 80.2, 70.9 and 74.2. The overall mean recovery for cimetidine was 75.1%. The maximum inaccuracy for freeze thaw cycle and long term stability samples for low, medium and high was found with CV less than 15% for all concentrations, indicating that cimetidine is stable. The developed method was precise and accurate and was suitable to be applied for the bioequivalence study of cimetidine.

Keywords: *HPLC, human plasma, cimetidine, ranitidine*.

1. INTRODUCTION

Cimetidine has been used as histamine H2 receptors antagonist. It is specifically inhibiting the secretion of gastric acid as well as decrease the formation of pepsin in the stomach. It has been used for the treatment of acidity, gastroesophageal reflux disease, heartburn and ulcers [1]. Alcohol and polyethylene glycol 400 is usde to dissolve this chemical generally. However, it is partially soluble in chloroform, but it is unable to dissolve with ether. According to the Biopharmaceutical Classification System (BCS), it is a Class III substance, with low permeability and high solubility [2]. The route of administration of cimetidine is oral due to its very high drug release profile and absorption rate in acid media. After administration, the maximum plasma peak concentration (Cmax) appeared within 60 to 120 min. Cimetidine plasma elimination half-life is 120 to 180 min. Previous study reported the percentage of bioavailability of cimetidine is 62 to 100 % among the healthy volunteers. About 70% of the drug is excreted through the kidney in its unchanged form **[3].** To determine the cimetidine in human plasma have been reported previously using different techniques such as HPLC-UV [1] [4] [5] and CE- UV) (Lukša and Josić, 1995). Also, cimetidine has been characterized from human plasma using different technique such as a combination of HPLC, high performance thin layer chromatography (HPTLC), and fast atom bombardment mass spectrometry (FAB- MS) Jenko et al. (1983), liquid chromatography coupled to mass spectrometry with atmospheric pressure chemical ionization (LC/MS/APCI) [6] [7]. The aim of this study is to develop and validate, sensitive and accurate high-performance liquid chromatographic method for analysis of cimetidine in human plasma with improved recovery and shorter analysis time.

2. EXPERIMENTAL SECTION

Chemicals and reagents. All reagents were analytical grade. Water used for the aqueous preparations as HPLC grade. Acetonitrile was HPLC grade from fisher, sodium dihydrogen phosphate was from merck. Triethylamine, NaOH and K2CO3 were purchased from merck.

Standards. Cimetidine and ranitidine were from sigma USA. 0.1 mg/ml stock standard solutions were prepared for each standard. Human plasma calibration standards of the analytes (40-4000 ng/l) were prepared by spiking an appropriate amount of the working standard solutions into drug free human plasma. Quality control

(QC) samples were prepared at low (80 ng/ml), medium (2000 ng/ml) and high (3600 ng/ml) concentrations.

HPLC eluent. Aqueous phase consists of 20 mm sodium dihydrogen phosphate and 1% trielthyl ammine. The aqueous and acetonitrile ratio was 95: 5. The mixture was filtered through a 0.45 µm filter prior to the use [7] [8] [9].

Chromatographic condition. Agilent 1100 HPLC system was used for the study. Data acquisition was performed with the agilent chemstation processor. The analytical column employed was a water (nova pak) c18 (150 mm x 3.9 mm, i.d, 5 µm.) With waters (nova pak) guard column (20 mm x 3.9 mm i.d., 5µm) of the same packing material. The analytes were detected using a UV-Vis detector and detection wavelength was set at 228 nm. 20µl sample was injected by the programme controlled auto injector [10] 11]. Chromatographic separation was performed at ambient temperature and flow rate was maintained at 1.0 ml/min.

Plasma sample preparation. Plasma sample (500 µl) was treated with 50 µl of 2.5 m sodium hydroxide. 150 µl of K2CO3 was added and mixed well. 2 ml of ethyl acetate was added to this mixture and vortex well for a minute [5] [6]. The organic layer was separated by centrifugation and dried under nitrogen at 50º c. The residue was reconstituted with 100 µl mobile phase before injected to the HPLC injection port.

Identification of analyte. The high-performance liquid chromatography (HPLC) method was validated to detect cimetidine (analyte) between 40 to 4000 ng/ml in human blood plasma matrix, using ranitidine as an internal standard. The identification of the analyte was achieved by comparing the peaks for both compound (analyte and IS) given at 228 nm wavelength in human plasma with the standard solution, at a similar retention time.

Quantification. The concentration of the sample was determined by using the inverse prediction method for the best fit linear regression curve using Weighted Least Square (WLS) 1/x, obtained from the standard concentrations [12] [13].

3. RESULTS SECTION

Calibration curve. The calibration line was validated from 40 to 4000 ng/ml. The individual calibration curve was linear with R2 value more than 0.99. From the seven batches of calibration curves of cimetidine (Figure. 1) the slope and intercept values with 95% confidence interval (c. i.) were as follows:

$$y = a1\ x + a0$$

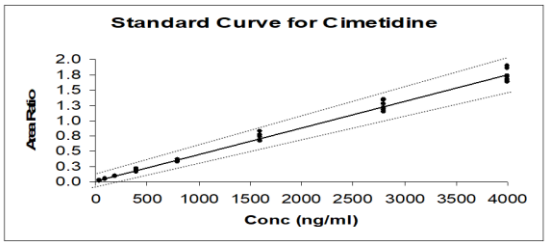

Figure 1. Mean calibration plots (± 95% ci) of peak area ratio (cimetidine to IS) vs cimetidine concentration (7 replicates). Y=0.000438[± 0.000009] x + 0.004492[± 0.004104], r2 = 0.995

From the calibration curve the QC concentrations (80, 2000 and 3600 ng/ml and LOQ 40 ng/ml for cimetidine) were calculated by inverse prediction from the equation y=a1 x + a0 as shown in the calibration curve. From the concentrations value, the percentage inaccuracy was calculated by following the formula

% inaccuracy=(Expected concentration-Predicted concentration)/(Expected concentration) X100

The inaccuracy values for QC samples were within 15% of the actual value and not more than 20% for the LOQ.

Specificity. Specificity is the ability of an analytical method to differentiate and quantitate the analyte in the presence of other constituents in the sample. It refers to the ability of a method to produce a response for a single analyte. Three samples of blank plasma along with the LOQ concentration of the analyte were prepared following the same extraction procedure and analysis was carried out under the same chromatographic conditions. The chromatograms obtained were compared for any interfering peaks. The response for the interfering peaks at a similar retention time with the analyte should be less than 15% of the response of LOQ concentration. The interfering peak at the retention time of internal standard should be less than 5% of the response of the internal standard concentration used for the study. The limit of quantitation (LOQ) was 40 ng/ml, which was also the lowest concentration of cimetidine that can be quantitated with the variability of 5.9%. The peak at this concentration is ten times higher than the noise at the retention time of cimetidine. The response of interfering peaks at the retention time of cimetidine (table -1) internal standard ranitidine (table-2) was close to 0%.

Table 1. Interference peak for cimetidine at LOQ 40 ng/ml for triplicate blank plasma.

	Retention time	Peak area of blank plasma at the same rt	Peak area of cimetidine	% interference (%)
	4.719	0	16.0	**0**
	4.712	0	14.9	**0**
	4.735	0	13.1	**0**
Mean	**4.722**			**0**

Table 2. Interference peak for ranitidine at 3000 ng/ml (internal standard) for triplicate blank plasma.

	Retention time	Peak area of blank plasma at the same rt	Peak area of ranitidine	% interference (%)
	5.210	0	733.3	**0**
	5.196	0	759.8	**0**
	5.213	0	710.9	**0**
Mean	**5.206**			**0**

Precision. Precision of an analytical method describe the closeness of the individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. Precision was measured using minimum of seven determinations per concentration. Three concentrations within the calibration range were chosen, these concentrations were the QC concentration labeled as low, medium and high (80, 2000 and 3600 ng/ml). Precision was again subdivided into within day and between day precision. For the within day precision seven replicate for each QC concentrations were prepared and injected in a series on the same day and the ratio of the analyte and internal standard was calculated for each sample. For the between day precision the samples were prepared every batch for seven batches and were analysed batch by batch each day. The coefficient of variation

(CV) was measured for every QC concentration from the mean and standard deviation of the seven sets of results. The precision around the mean value should not exceed 15% of c.v. For low, medium and high concentrations and should not exceed 20% for the LOQ. CV for within day precision were 8.6, 6.5 and 6.5 respectively (table-3), and between day precision were 2.4, 6.7 and 4.2 respectively (table-5) for low, medium and high concentrations. The LOQ concentration was 40 ng/ml, which was same as the lower point of the calibration concentrations. The within day precision and between day precision for LOQ were 10.8 and 5.9 respectively. Retention time for the analyte was 4.1-4.5 minutes during the within day and between day results. A typical within day mean and maximum variation of rt for the analyte and is, representing a QC concentration, is given in table 4.

Table 3. Within day precision for the assay of cimetidine using ranitidine as internal standard.

	High concentration 3600 ng/ml	Medium concentration 2000 ng/ml	Low concentration 80 ng/ml	LOQ concentration 40 ng/ml
Mean	3818.14	2017.94	82.29	35.43
Sd	248.77	131.50	7.05	3.84
CV (%)	6.5	6.5	8.6	10.8

Table 4. Within day retention time variation for cimetidine and ranitidine for high concentration.

	Rt for cimetidine (high concentration, 3600 ng/ml)	Rt for ranitidine
Mean	4.563	**5.350**
Sd	0.019	**0.015**
CV (%)	0.4	**0.3**
Range	4.551-4.606	**5.338-5.383**
Maximum % deviation from mean	**0.9**	**0.6**

Table 5. Between day precision for the assay of cimetidine using ranitidine as internal standard.

	High concentration 3600 ng/ml	Medium concentration 2000 ng/ml	Low concentration 80 ng/ml	LOQ concentration 40 ng/ml
Mean	3864.51	2049.46	78.74	38.39
Sd	161.03	136.97	1.91	2.27
CV (%)	4.2	6.7	2.4	5.9

Table 6. Within day accuracy for cimetidine using ranitidine as internal standard.

Concentration		% inaccuracy
High concentration (3600 ng/ml)	Mean	**7.8**
	Sd	**4.5**
Medium concentration (2000 ng/ml)	Mean	**5.6**
	Sd	**2.8**
Low concentration (80 ng/ml)	Mean	**6.8**
	Sd	**5. 8**
LOQ concentration (40 ng/ml)	Mean	**12.4**
	Sd	**8.2**

Accuracy. The accuracy of an analytical method describes the closeness of test result obtained by the method to the true value of the analyte. The values are back predicted from the equation y = a1 x + a0 which is obtained from the calibration curve. The mean value for the low, medium and high concentrations should be within 15% of the actual value and not more than 20% for the LOQ concentration. The mean % inaccuracy values for low, medium and high concentration were 6.8, 5.6 and 7.8 (table-6) respectively for within day and 2.4, 6.1 and 7.9 respectively (table-7) for between day. The within day and between day % inaccuracy for LOQ concentration was 12.4 and 5.5 respectively.

Table 7. Between day accuracy of cimetidine using ranitidine as internal standard.

Concentration		% difference (% inaccuracy)
High concentration (3600 ng/ml)	Mean	**7.9**
	Sd	**3.2**
Medium concentration (2000 ng/ml)	Mean	**6.1**
	Sd	**3.2**
Low concentration (80 ng/ml)	Mean	**2.4**
	Sd	**1.4**
LOQ concentration (40 ng/ml)	**Mean**	**5.5**
	Sd	**4.3**

Recovery. The recovery result obtained from an amount of the analyte added to and extracted out from biological matrix, compared to the result obtained from the non-extract analyte. Spiked plasma of 500 µl (l, m and h concentration) was used for assay. 20µl of the reconstitute sample was injected to the HPLC system for analysis after solvent extraction using 2 ml ethyl acetate.

Seven extracted and seven standard sample of the corresponding concentration of cimetidine was prepared separately for low (80 ng/ml), medium (2000 ng/ml) and high (3600 ng/ml) concentrations. Internal standard ranitidine concentration used was 3000 ng/ml. From the chromatogram thus obtained, the peak area of the extracted sample was divided by the peak area of the standard sample.

Recovery= (Area of extracted samples)/ (Area of standard samples) X100

The mean recoveries for low, medium and high concentration of cimetidine were 80.2, 70.9 and 74.2 (table-8). The overall mean recovery for cimetidine was 75.1.

Table 8. Percentage recovery of cimetidine based on peak area.

Concentration		% recovery
High Concentration (3600 ng/ml)	Mean	**74.2**
	Sd	**2.3**
	CV (%)	**3.1**
Medium concentration (2000 ng/ml)	Mean	**71.0**
	Sd	**2.4**
	CV (%)	**3.4**
Low Concentration (80 ng/ml)	Mean	**80.2**
	Sd	**4.9**
	CV (%)	**6.1**
LOQ Concentration (40 ng/ml)	Mean	**86.5**
	Sd	**19.0**
	CV (%)	**22.0**
	Over all mean	**75.1**

Table 9. Determination of freeze thaw cycle stability assay (concentration, ng/ml).

Thaw cycle	Higher concentration (3600ng/ml)	Medium concentration (2000 ng/ml)	Low Concentration (80 ng/ml)
Cycle 0	3532.89	1866.39	**77.39**
Cycle 1	3955.34	2075.92	**77.36**
Cycle 2	3734.26	2084.95	**74.93**
Cycle 3	3781.10	2096.01	**77.91**
Mean	3750.90	2030.82	**76.90**
Sd	173.70	109.93	**1.34**
CV (%)	4.6	5.4	**1.7**
% inaccuracy (max)	**9.9**	**6.7**	**8.0**

Stability. Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix and the container system. The types of stability test performed in this study are freeze thawing and long terms stability at -25º C. For the stability determinations, a set of standard samples were

prepared by spiking a previously selected master stock solution of cimetidine in drug free plasma. The samples were kept in the same conditions as the samples to be analyzed.

Freeze thaw cycle stability was carried out for three cycles. The maximum inaccuracy for low, medium and high was 8.0, 6.7 and 9.9 respectively with CV of less than 15% for all concentrations, indicating that cimetidine is stable at least for three freeze thaw cycle (table-9). In frozen stability all the aliquots were stored at -25º c. The sample was stable for at least one months (table-10). The stability results show good consistency of the stability of cimetidine in stated refrigerated condition This also implies that the study sample can be kept for validated periods of time prior to analysis.

Table 10. Determination of frozen stability at -25 º c for the assay (concentration, ng/ml).

	Low Concentration (80 ng/ml)	Medium concentration (2000 ng/ml)	High Concentration (3600 ng/ml)
Fresh	88.2	2123.9	**3977.1**
1st week	71.5	2057.4	**3866.0**
2nd week	73.2	1982.5	**3788.8**
3rd week	79.2	2015.1	**3685.7**
1 month	80.2	2223.8	**3946.0**
Mean	78.5	2080.5	**3852.7**
Sd	6.6	96.0	**118.7**
CV (%)	8.4	4.6	**3.1**
% inaccuracy (max)	**10.6**	**11.2**	**10.5**

4. CONCLUSIONS

The developed method demonstrates an excellent recovery of cimetidine from plasma sample using a simple single step extraction method. The method also shows a shorter run time for the analysis of cimetidine and IS. All the validation parameters for cimetidine using the developed method are in good agreement with the standard guideline reflecting its strength to be employed in routine analysis of bioequivalence study sample. Overall the developed method is simple reliable and sensitive for the determination of cimetidine is plasma sample with good precision and accuracy.

5. REFERENCES

1. Ashiru, D.A.I.; Patelb, R.; Basit, A.W. Simple and universal HPLC-UV method to determine cimetidine, ranitidine, famotidine and nizatidine in urine: Application to the analysis of ranitidine and its metabolites in human volunteers. *Journal of Chromatography B* **2007**, *860*, 235–240, https://doi.org/10.1016/j.jchromb.2007.10.029.
2. Kawabata, Y.; Wada, K.; Nakatani, M.; Yamada, S.; Onoue, S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. *International journal of pharmaceutics* **2011**, *420*, 1-10, https://doi.org/10.1016/j.ijpharm.2011.08.032.
3. Jantratid, E.; Prakongpan, S.; Dressman, J.B.; Amidon, G.L.; Junginger, H.E.; Midha, K.K.; Barends, D.M. Biowaiver monographs for immediate release solid oral dosage forms: cimetidine. *Journal of Pharmaceutical sciences* **2006**, *95*, 974–984, https://doi.org/10.1002/jps.20614.
4. Iqbal, T.; Karyekar, C.S.; Kinjo, M.; Ngan G.C.; Dowling, T.C. Validation of a simplified method for determination of cimetidine in human plasma and urine by liquid chromatography with ultraviolet detection. *Journal of Chromatography B* **2004**, *799*, 337–341, https://doi.org/10.1016/j.jchromb.2003.10.030
5. Jantratid, E.; Prakongpan, S.; Foley, J.P.P.; Dressman, J.B. Convenient and rapid determination of cimetidine in human plasma using perchloric acid-mediated plasma protein precipitation and high-performance liquid chromatography. *Biomedical Chromatography* **2007**, *21*, 949–957, https://doi.org/10.1002/bmc.838.
6. Xu, K.Y.; Arora, V.K.; Chaudhary, A.K. Quantitative analysis of cimetidine in human plasma using LC/APCI/SRM/MS. *Biomedical Chromatography* **1999**, *13*, 455–461, https://doi.org/10.1002/(SICI)1099-0801(199911)13:7%3C455::AID-BMC911%3E3.0.CO;2-K.
7. Naeeji, A.; Mozdarani, H.; Monfared, A.S.; Faeghi, F.; Ahmadi, A.A.; Gholami, M.; Behzadi, R.; Momtaz, M.R. Oral administration of vitamin C, cimetidine and famotidine on micronuclei induced by low dose radiation in mouse bone marrow cells. *Journal of biomedical physics & engineering* **2017**, *7*, 117.
8. Liu, X.; Jia, Y.; Chong, L.; Jiang, J.; Yang, Y.; Li, L.; Ma, A.; Sun, Z.; Zhou, L. Effects of oral cimetidine on the reproductive system of male rats. *Experimental and therapeutic medicine* **2018**, *15*, 4643-50, https://doi.org/10.3892/etm.2018.6065.
9. Eftekhari, A.; Ahmadian, E.; Azarmi, Y.; Parvizpur, A.; Fard, J.K.; Eghbal, M.A. The effects of cimetidine, N-acetylcysteine, and taurine on thioridazine metabolic activation and induction of oxidative stress in isolated rat hepatocytes. *Pharmaceutical Chemistry Journal* **2018**, *1*, 965-9, https://doi.org/10.1007/s11094-018-1724-6.
10. Rezaei, Z.B.; Rastegarzadeh, S.; Kiasat, A. A Sensitive and Selective Colorimetric Probe for Cimetidine Determination Based on Surface Plasmon Resonance of Chitosan-Encapsulated Gold Nanoparticles. *Plasmonics* **2019**, 1-9, https://doi.org/10.1007/s11468-019-00905-0.
11. Silva, G.M.; Köppl, A.; Unholzer, S.; Haen, E. Development and validation of an HPLC-UV method for the simultaneous determination of the antipsychotics clozapine, olanzapine and quetiapine, several beta-blockers and their metabolites. *Biomedical Chromatography* **2017**, *31*, e3968, https://doi.org/10.1002/bmc.3968.
12. Logue, B.A.; Manandhar, E. Percent residual accuracy for quantifying goodness-of-fit of linear calibration curves. *Talanta* **2018,** *1*, 527-33, https://doi.org/10.1016/j.talanta.2018.07.046.
13. Miraghaei, S.; Mohammadi, B.; Babaei, A.; Keshavarz, S.; Bahrami, G. Development and validation of a new HPLC-DAD method for quantification of sofosbuvir in human serum and its comparison with LC–MS/MS technique: Application to a bioequivalence study. *Journal of Chromatography B.* **2017**, *15*, 118-22, https://doi.org/10.1016/j.jchromb.2017.06.047.

6. ACKNOWLEDGEMENTS

This study was supported by the Research Management Centre, International Islamic University Malaysia [EDW-B130620947, RPDF-190020012 and RIGS-162920456]; and BIOPEP Solution Inc Canada for the grant SP-17-0012-0264.