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A Rapid and Sensitive HPLC Method for Determination of Roxatidine in Human Plasma

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ABSTRACT

A rapid and accurate assay for the determination of roxatidine, a selective H_2-receptor blocker, in human plasma was developed. Analysis was performed by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detector. Roxatidine acetate, a prodrug of roxatidine, is metabolized rapidly to roxatidine following oral administration. Roxatidine and the internal standard (ranitidine) were extracted from plasma by solid-phase extraction. The mobile phase of HPLC was consisted of 20 mM KH_2PO_4 (pH 7.0) and acetonitrile (5:1, v/v). The calibration curve for roxatidine was linear over the range of 5 to 1000 ng/mL. The precision and accuracy of within- and between-run were within 10% for roxatidine. The recovery of roxatidine was over 87% for both low and high concentrations (15 and 500 ng/mL) and the lower limit of quantitation (LLOQ) was 5 ng/mL. The method was successfully applied to a pilot pharmacokinetic study of roxatidine in healthy subjects.

Key words: HPLC, roxatidine, solid-phase extraction

INTRODUCTION

Upper gastrointestinal (GI) bleeding is one of the most common medical emergencies. Acute upper GI hemorrhage results in hospitalization of over 300,000 patients annually in the United States[(1)]. Among the upper GI bleeders, peptic ulcer is the source in more than 50%[(2)]. Therefore, efficient prevention of peptic ulcer will be an important issue. The description of selective H_2-receptor blockade is a landmark in the treatment of acid-peptic disease[(3)]. Roxatidine, a selective histamine H_2-antagonist, has a 3-6 times higher antisecretory potency than cimetidine, and about twice that of ranitidine[(4-7)]. It acts by blocking the H_2-receptor on gastric parietal cells, thus antagonizing the normal stimulatory effect of endogenous histamine on gastric acid secretion[(8)].

Roxatidine acetate, a prodrug of roxatidine[(9)] (Figure 1), following oral administration, is metabolized rapidly by esterases in small intestine, liver and serum to its active deacetylated metabolite, roxatidine[(10)]. Roxatidine acetate is almost completely (93-95%) absorbed after oral administration. The major disposition of roxatidine is via kidney (95% of renal excretion). Elimination half-life of roxatidine following oral administration is around 4 to 8 hr[(11)].

Despite the fact that roxatidine acetate has more potency, it is also demonstrated to have fewer side effects. For instance, it has no antiandrogenic activity, and does not have any significant effect on hypothalamic-pituitary-gonadal or hypothalamic-adrenal function. Moreover, roxatidine acetate does not interfere with the elimination of drugs that undergo hepatic metabolism so that it causes less drug-drug interaction than cimetidine does[(12)].

Since roxatidine acetate is more potent and safer according to some animal studies and clinical trials, it is important to have a rapid and sensitive analytical assay to accurately determine the concentration of roxatidine in plasma samples. Gas chromatography was the most used method to analyze roxatidine in the past[(13)], and recently, a LC/MS[(14)] method was developed. Though these two analytical methods are robust and sensitive, the instrumentation is hard to access. Therefore, an accurate, sensitive and validated high-performance liquid chromatography was set up to determine the roxatidine plasma level and applied to pharmacokinetic studies of roxatidine in human.

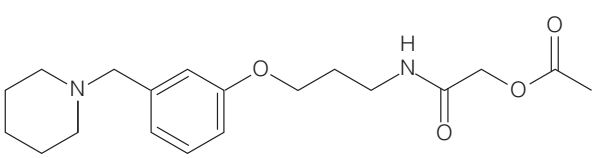

Figure 1. Structural formula of roxatidine acetate.

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MATERIALS AND METHODS

I. *Chemicals*

The roxatidine standard with a purity of 99.5% was kindly provided by Sanofi-Aventis. Ranitidine was purchased from Sigma-Aldrich and used as an internal standard. Acetonitrile (ACN) and Methanol (MeOH), both HPLC grades, were from Merck KgaA (Darmstadt, Germany). Twenty five percent ammonia solution (Pro. Analytical grade E) and potassium dihydrogen phosphate (KH_2PO_4) were also from Merck KgaA (Darmstadt, Germany). Triethylamine (TEA) of analytical grade was purchased from Riedel-deHaen®. Drug-free human plasma used in this study was obtained from Tri-Service General Hospital (Taipei, Taiwan) and stored at -80°C until use. Milli-Q water was used throughout this study.

II. *Chromatography*

The HPLC system consisted of a pump (SHIMADZU LC-10AD*VP*, Japan), an autosampler (SHIMADZU SIL-10 AD*VP*, Japan), an UV detector (SHIMADZU SPD-6AV, Japan) and a column oven (SHIMADZU CTO-10AD*VP*, Japan). The octadecyl (C18) disposable extraction column (Bakerbond spe™) was used. The chromatographic separation was performed on a Nova-Pak® C18 column (150 mm × 3.9 mm, 4 μm particle size, Waters) at 40°C in a column oven. The mobile phase consisting of 20 mM KH_2PO_4 (pH 7.0) and ACN (5:1, v/v) was used. The flow rate was set at 0.8 mL/min. The UV detector was set at 198 nm and the autosampler was maintained at room temperature. Sample volume injected was 50 μL and the run time was set at 8 min.

III. *Sample Preparation*

Plasma samples were obtained from subjects' blood by centrifugation at 4°C. Then 100 μL of 10 μg/mL ranitidine solution as internal standard was added into 1 mL of plasma sample. Solid-phase extraction was conducted using octadecyl (C18) disposable extraction cartridges conditioned with 1 mL of methanol and 1 mL of H_2O. One milliliter of plasma sample was then loaded. The loaded cartridges were washed once with 1mL of H_2O followed by twice with 1 mL of 5% MeOH - 0.1% NH_4OH (pH 10) and once with 1 mL of 40% MeOH - 0.1% NH_4OH (pH 10). Roxatidine was then eluted from the column with 1 mL of MeOH. The eluates were evaporated to dryness under N_2 stream, reconstituted with 100 μL of mobile phase and vortexed for 30 sec. Finally, the sample was transferred to the autosampler vial and 50 μL was injected into HPLC.

IV. *Calibration Graphs*

Roxatidine standard stock or working solutions and internal standard (ranitidine) stock solution were all prepared in 10% ACN and stored at 4°C. The concentrations of the standard working solutions for the calibration were from 5 to 1000 ng/mL. One hundred microliter of 10 μg/mL internal standard solution was added into 1 mL of plasma where 0.1 mL of standard working solution was included. Quality control (QC) samples at 4 levels of 5, 15, 500, and 900 ng/mL were prepared and analyzed to monitor the calibration. Sample extraction and HPLC analysis were carried out as described above.

V. *Validation*

The method validation assays were performed following the currently accepted US Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance[15]. The following parameters were determined for the validation of the analytical method developed for roxatidine in human plasma: linearity, range, precision, accuracy, lower limit of quantitation, recovery and stability.

For linearity, a line (Y = a + bX) was fitted through the standard curve ranged by a weighted linear regression (weight = $1/x^2$) of peak height ratio (Y) vs. concentration (X). The equation Y = a + bX was used to calculate the concentration of roxatidine in plasma from peak height ratio obtained by HPLC.

The within-run precision and accuracy were determined by analyzing a set of QC samples (n = 6) at each of the four levels, 5, 15, 500, 900 ng/mL. The between-run precision and accuracy (n = 6) were also carried out by analyzing QC samples at the above four concentrations. The precision of the assay for roxatidine was evaluated by the coefficient of variation (CV) and the accuracy was evaluated by the relative error {(mean calculated concentration-concentration spiked) × 100% / concentration spiked}.

The lower limit of quantitation (LLOQ) is defined in the presented study as the lowest plasma concentration in the calibration curve that can be measured with precision and accuracy.

The relative recovery for roxatidine in human plasma was determined by comparing the mean peak height ratios of plasma samples spiked with various concentrations of roxatidine (15 and 500 ng/mL) to solvent samples and extracted as described (roxatidine in solvent without extraction) (n = 3).

The stability assays in this study, including freeze and thaw, benchtop, long-term and working solution, were conducted in concentrations of 15 and 500 ng/mL. Freeze and thaw stability for roxatidine in plasma samples was studied in 3 cycles by frosting and defrosting the samples in 3 replicates. Benchtop stability for roxatidine in plasma samples was studied in triplicate at 0, 4, and 12 hr prior to processing. Long term stability for roxatidine in plasma samples was studied in triplicate at 0, 15, 30, 60, and 90 days prior to processing. Working solution stability for roxatidine was studied with 150

ng/mL and 5000 ng/mL at 0 and 6 hr at ambient temperature. QC samples were prepared at three concentrations, low (15 ng/mL), medium (500 ng/mL) and high (900 ng/mL), before assayed and analyzed routinely with clinical samples to monitor the performance of the assay. All QC sample concentrations were assayed and interpolated from the concentration standard curve to evaluate the precision and accuracy.

VI. *Pilot Pharmacokinetic Study*

The pharmacokinetic study was approved by the Institutional Review Board (IRB) of Tri-Service General Hospital. All the volunteers were signed informed consent prior to participating in this study. Three healthy volunteers aged of 25, 27 and 28 were selected for the pilot pharmacokinetic study. All three subjects underwent an overnight fasting before the treatment. A regimen of 150 mg controlled-release roxatidine acetate capsule (Roxane® 75 mg/cap) was administered orally by the subjects once daily for 7 days. Each dose was administered with 240 mL of water and no food or fluid (except water) was permitted 4 hr after administration. Blood samples were collected on days 5, 6 and 7 just prior to daily dose. On day 7, blood was drawn at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 24 hr. All the plasma samples were stored at -80°C before analysis.

RESULTS AND DISCUSSION

I. *Chromatography*

Representative HPLC chromatograms of drug-free plasma and plasma sample spiked with 5 ng/mL roxatidine and 1000 ng/mL ranitidine are shown in Figure 2A and 2B, respectively.

The retention time for rantidine and roxatidine was 3.5 and 7.7 min, respectively. No significant interfering peaks were observed at the retention time of roxatidine and the internal standard of blank plasma due to endogenous compounds or reagents. These chromatograms indicated that roxatidine and internal standard could be resolved from the rest endogenous substances.

II. *Linearity, Accuracy, and Precision*

The linear response of calibration curve was observed over the range of 5-1000 ng/mL with correlation coefficients of at least 0.995. The mean peak height ratio vs. concentration of roxatidine curve was shown in Figure 3, and the quality control samples of between- and within-run data was shown in Table 1. LLOQ was 5 ng/mL, which can be measured with precision and accuracy. The precisions were 5.9% and 7.5% and the accuracies were 101.3 and 107.7 for within- and between-run validation, respectively (Table 1). As shown in Table 1, the CVs and relative errors of within-run were less than 5.9% and ranged from -4.7 to +3.2%, respectively. For between-run, CVs and relative errors were less than 7.5% and ranged from +1.4 to +7.7%, respectively. These results indicated that the method was precise and accurate within the analytical range.

III. *Recovery*

Human plasma samples spiked with 2 different concentrations of roxatidines (15 and 500 ng/mL) in trip-

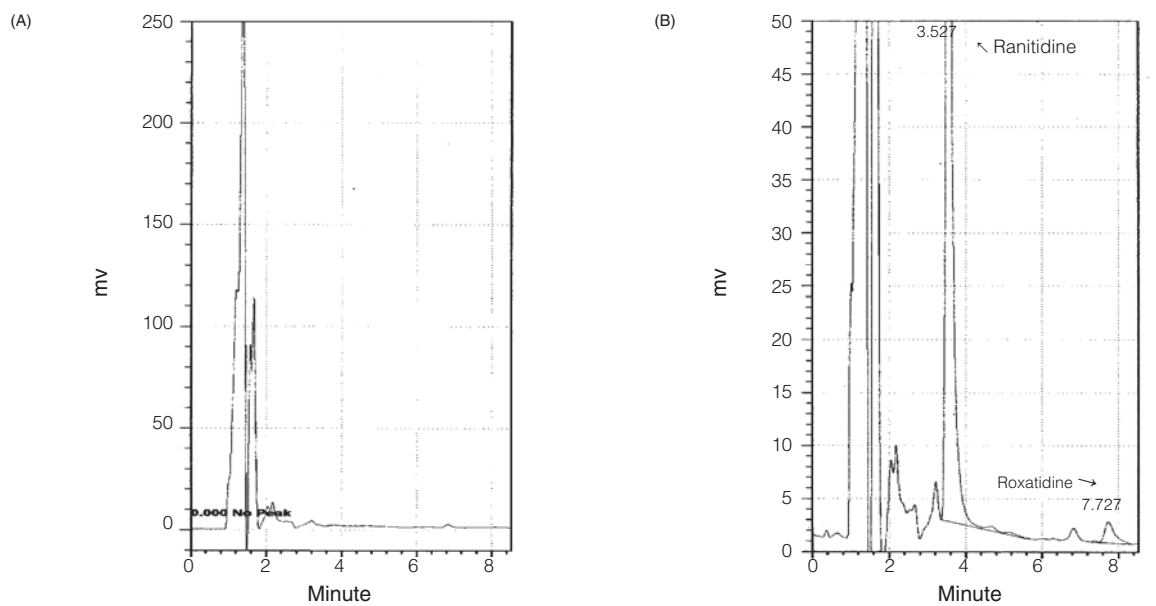

Figure 2. Representative chromatograms obtained from (A) drug-free human plasma and (B) human plasma spiked with roxatidine (5 ng/mL) and ranitidine (1000 ng/mL).

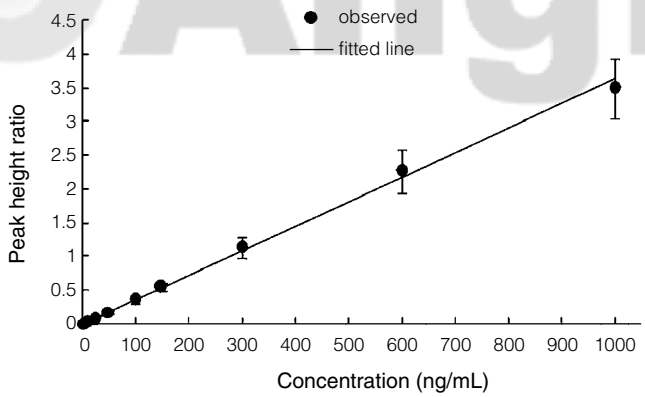

Figure 3. A peak height ratio-concentration curve of roxatidine at concentration ranging 5-1000 ng/mL (n = 6). Y= -0.000946 + 0.00363 X, r = 0.998 (weight by $1/x^2$).

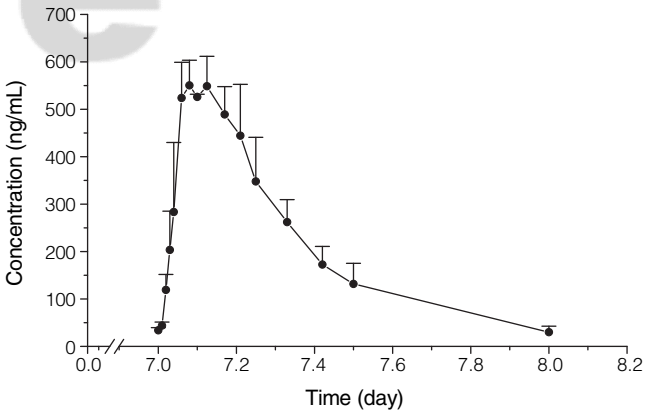

Figure 4. Concentration - time profile of 3 subjects taken 150 mg of Roxane® 75 mg/cap orally QD for 7 days.

Table 1. Quality control of within- and between- run (n = 6) for roxatidine calibration standards in human plasma determined by the HPLC

	Within-run			Between-run		
Known Conc. (ng/mL)	Conc. found (ng/mL)	CV (%)	Error (%)	Conc. found (ng/mL)	CV (%)	Error (%)
5	5.1 ± 0.3	5.9	1.3	5.4 ± 0.4	7.5	7.7
15	15.5 ± 0.5	3.2	3.2	15.2 ± 0.6	3.9	1.4
500	476.3 ± 8.4	1.8	-4.7	532.4 ± 36.6	6.9	6.5
900	897.8 ± 9.7	1.1	-0.2	962.4 ± 46.7	4.9	6.9

Results are expressed as mean ± S.D.

licate were extracted and injected into HPLC. The relative recovery was 92.3% and 87.8% for 15 and 500 ng/mL, respectively.

IV. *Stability*

The stability tests of roxatidine in plasma samples including freeze and thaw, benchtop, long-term and working solution were all determined by evaluating the relative errors. The results for the freeze and thaw stability as well as for the benchtop stability were within 10%, indicating that roxatidine was stable in multiple freeze and thaw cycles and was stable for at least 12 hr at ambient temperature. Moreover, the relative errors of working solution and long term stability at high concentration (500 ng/mL) and low concentration (15 ng/mL) were within 3%, 15% and 20%, respectively; the results showed that roxatidine in plasma samples was not only stable for at least 90 days at -80°C but also for at least 6 hr at ambient temperature.

V. *Pilot Pharmacokinetic Study*

This precise and accurate HPLC method was applied to a pilot pharmacokinetic study and yield satisfactory results for the determination of roxatidine in human plasma samples following multiple oral administration. The plasma concentration-time profile of roxatidine at steady state followed by a regimen of 150 mg Roxane® (75 mg/cap) orally QD for 7 days is shown in Figure 4. The results indicated that plasma level of roxatidine would reach steady state after 4 days regimen, which was consistent with literature reported[16]. The mean trough concentration at day 7 was 34.07 ng/mL. Roxatidine was converted from the prodrug rapidly and reached to maximum plasma concentration (C_{max}) of 583.13 ± 16.82 ng/mL at 1.83 ± 0.29 hr (T_{max}). The mean area under the curve (AUC) of roxatidine was 4968.3 ± 601.18 ng • hr/mL. The trough concentration and AUC were similar to those described previously by Lassman, H.B. *et al.* (30.9 ± 10.67 ng/mL and 4362.9 ± 757.18 ng • hr/mL, respectiverly)[16]. However, C_{max} was higher and T_{max} was shorter than their study (435.6 ± 106.5 ng/mL and 3.1 ± 0.55 hr, respectively), which implied that the absorption of roxatidine in Chinese might be faster than Caucasian.

CONCLUSIONS

The presented study describes and validates a HPLC

method for the determination of roxatidine in human plasma. The method proved to be linear in the concentration range studied (5-1000 ng/mL) as well as accurate, precise and selective. This method of analysis demonstrated that the roxatidine can be successfully used in human plasma for clinical studies, which indicated that it could apply to pharmacokinetic, bioavailability and bioequivalent studies.

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