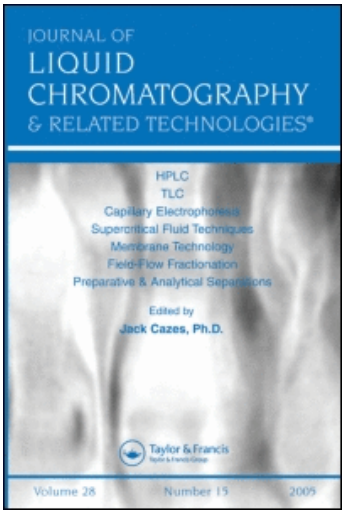

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Rapid and Sensitive Hplc Determination of Ranitidine in Plasma. Application to Pharmacokinetics Study

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RAPID AND SENSITIVE HPLC DETERMINATION OF RANITIDINE IN PLASMA. APPLICATION TO PHARMACOKINETICS STUDY

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ABSTRACT

A rapid and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) method for the separation and quantification of the H_2-receptor antagonist drug ranitidine in human plasma is described.

The extraction of ranitidine from plasma by an organic solvent was eliminated in this method. Instead, the pre-chromatography isolation of the drug was done by adding approximately 50 mg of zinc sulfate and 200 µL of acetonitrile in 1.0 mL of plasma. A short column packed with pH-stable (1-13) reversed phase PLRP-S™ particles was used with an isocratic elution of 5.0mM dibasic potassium phosphate plus 0.50mM tetraethylammonium hydroxide/ acetonitrile, 80:20 (v/v). The ranitidine was monitored at 315 nm and 0.20 to 0.002 absorption units full scale (AUFS). The completion time of the assay was less than 15 minutes and had a limit of detection of 1.0 ng/mL for a 100-µL injection volume.

After an oral dose of 150 mg of ranitidine, plasma samples were collected at several time points and were analyzed by using this method to determine various pharmacokinetic parameters.

INTRODUCTION

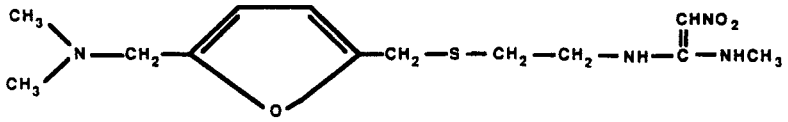
Ranitidine is a histamine H_2-receptor antagonist agent and has been found highly effective in inhibiting gastric acid secretion stimulated by

food, pentagastrin, insulin-induced hypoglycemia, histamine, natural alkaloids, and many other similar chemicals (1). It has also been proven to be effective in the treatment of prophylaxis of stress ulceration and Zollinger-Ellison syndrome (1-3). In a number of patients, ranitidine treatment failed. This failure may be related to dosage. Measuring the concentrations of ranitidine in plasma may help to obtain maximum therapy with minimum toxicity. Figure 1 shows the structural formulae of ranitidine, ranitidine S-oxide, ranitidine N-oxide, and desmethylranitidine.

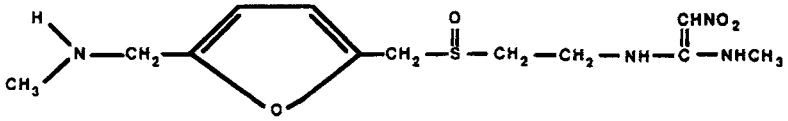
Ranitidine is a weakly-basic compound. The pKa values of the positively charged ranitidine molecule are 2.7 and 8.2 (4). Ranitidine was found to be 6 to 8 times more effective than cimetidine in inhibiting the acid secretion caused by the induction of histamine, and at least 5 times more potent in inhibiting the gastric acid secretion induced by food and similar entities (1). These properties of ranitidine make it possible to obtain an optimum therapy with significantly lower doses than cimetidine. This may also reduce the adverse effects of the drug, which include headache, dizziness, nausea, abdominal pain, and skin rash (2).

Determination of ranitidine in the plasma and serum samples of patients who have ingested the drug is based on gas chromatography (GC), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GS-MS) (4-12). The sample preparation in most of these methods involves multiple-step extraction, evaporation, and derivatization, which make these methods unsuitable for routine analyses in a clinical laboratory.

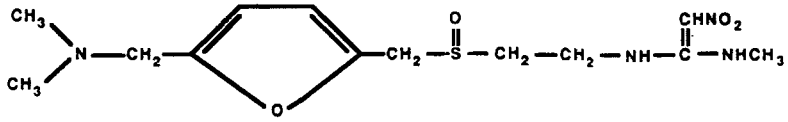
This paper describes a rapid, sensitive, and simple reversed-phase HPLC (RP-HPLC) method to estimate ranitidine levels in the plasma of patients who have ingested the drug. The method uses a slurry-packed short column with an isocratic elution of the mobile phase. Using this method, the pharmacokinetics of ranitidine was studied in a patient who had orally ingested 150 mg of the drug.



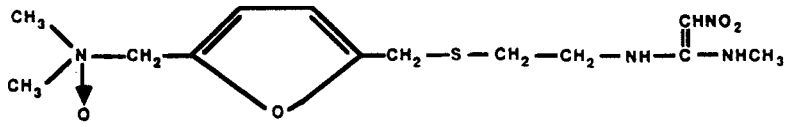
(1) RANITIDINE



(2) DESMETHYLRANITIDINE



(3) RANITIDINE S-OXIDE



(4) RANITIDINE N-OXIDE

FIGURE 1: Structural formulae of ranitidine, ranitidine S-oxide, ranitidine N-oxide, and desmethylranitidine.

MATERIALS AND METHODS

Equipment

The HPLC system consisted of a Perkin-Elmer Series 410 solvent delivery pump (Perkin-Elmer Corporation, Norwalk, Connecticut), equipped with a Rheodyne 7275 sample injector (Rheodyne, Cotati, California), equipped with a

200-µL loop. A reversed-phase, 5-cm x 4.1-mm column packed with 5-µm PLRP-S particles was used. A slurry packer from Micromeritics (Norcross, Georgia) was used to pack the analytical column. A Kratos Spectroflow 783 ultraviolet (UV)-visible variable wavelength absorbance detector with a flowcell path length of 8.0 mm (ABI Analytical, Kratos Division, Ramsey, New Jersey) was used. UV-visible chromatograms were recorded on a Houston Instrument D5000 strip chart recorder (Houston Instrument Company, Austin, Texas). A Model 2200 Branson sonicator was used to degas the mobile phase (Branson Cleaning Equipment Company, Shelton, Connecticut). A Gilson P-1000 digital pipette was used for all quantitative sampling (Gilson International, Middleton, Wisconsin). Deionized water was collected from a Milli-Q® system (Millipore Corporation, Milford, Massachusetts). An analytical balance from Mettler Instrument Corporation (Highstown, New Jersey), Model AE100, was used for weighing reagents. Samples were centrifuged by an IEC centrifuge, Model HN (Damon, IEC Division, Needham Heights, Massachusetts). A vortex mixer from Scientific Industries, Inc., (Bohemia, New York), Model Vortexer-2®, was used. A C-130B Upchurch guard column (Upchurch Scientific, Oak Harbor, Washington) packed with PLRP-S particles was used throughout. A 5.0-mL gas-tight syringe was used to filter the samples (Hamilton Company, Reno, Nevada). A 0.45-µm filter tip for the syringe was purchased from Rainin Instrument Company, Inc. (Woburn, Massachusetts).

Materials

HPLC-grade acetonitrile (ACN) (EM Science, Cherry Hill, New Jersey) was used. Ranitidine in the form of ranitidine hydrochloride (brand name Zantac®) was obtained in 2.0-mL, single-dose vials from Glaxo, Inc. (Research Triangle Park, North Carolina), and was used as received. The three metabolites of ranitidine, ranitidine S-oxide, ranitidine N-oxide, and desmethylranitidine were obtained from the Biomedical Pharmacology Division of Glaxo Group Research Limited (Ware, Hertfordshire, United Kingdom). Dibasic

potassium phosphate (K_2HPO_4) and zinc sulfate ($ZnSO_4$), both reagent-grade, were purchased from Fisher Scientific Company (Fairlawn, New Jersey). The tetraethylammonium hydroxide base and Gold Label acetone were purchased from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin). The aged, pooled plasma was obtained from the Blood Center of Southeastern Wisconsin (Milwaukee, Wisconsin). The borosilicate glass culture tubes and the disposable glass pipettes were purchased from Curtin Matheson Scientific, Inc. (Elk Grove, Illinois). A PLRP-S column (15 cm x 4.6 mm) was purchased from Alltech Associates, Inc. (Deerfield, Illinois). This column was unpacked and the stationary phase was used to pack a 5-cm x 4.1-mm column. The 5-cm x 4.1-mm column was used throughout the study.

Chromatographic Conditions

The mobile phase consisted of 20% ACN in 5.0mM K_2HPO_4 and 0.50mM tetraethylammonium hydroxide dissolved in ultrapure deionized water. The nominal pH of the mobile phase mixture was approximately 11. The mobile phase was delivered through the HPLC system at a rate of 1.0 mL/minute. Ranitidine and its metabolites were monitored with a UV-visible absorbance detector at 315 nm with sensitivity from 0.20 to 0.002 AUFS. Quantification of the ranitidine peak in plasma samples was done by comparing the peak height of ranitidine with a standard calibration curve.

EXPERIMENTAL PROCEDURES

Preparation of Stock Solution

A stock solution of ranitidine was prepared by dissolving 50.0 mg of ranitidine hydrochloride in 50.0 mL of ACN. Ten milliliters of this stock solution was diluted to 250 mL in ACN. This solution was then used to prepare plasma and water standards to construct the calibration curves. Stock solutions of ranitidine S-oxide, ranitidine N-oxide, and desmethylranitidine

were prepared separately in three vials in ACN at a concentration of 20 µg/mL. Each of the three metabolites was injected individually into the HPLC system to determine the retention times under the experimental chromatographic conditions. A solution was then prepared with all three metabolites and ranitidine. This mixture was injected into the HPLC system to determine the resolution of the metabolites with ranitidine.

Purification of $ZnSO_4$

Purification of $ZnSO_4$ crystals was done by adding 200 mL of HPLC-grade ACN to 100 g of $ZnSO_4$. This mixture was stirred on a magnetic stirrer for at least 30 minutes. The slurry was allowed to settle and the supernatant was decanted gently. The above procedure was then repeated twice with fresh ACN solution. The $ZnSO_4$ was then dried under a hood at room temperature.

Cleaning of Borosilicate Culture Tubes

Borosilicate culture tubes were placed in a container and Gold Label acetone was then added. The container (with the borosilicate tubes and acetone) was sonicated on an ultrasonic bath for at least 30 minutes. The acetone was decanted and replaced by HPLC-grade ACN. This was again sonicated for approximately 15 minutes and the ACN was decanted. The sonification with ACN was repeated once more with fresh ACN. The borosilicate tubes were then dried in an oven at a temperature of 100°C.

Slurry Packing of the Analytical Column

The 5-cm x 4.1-mm analytical column was packed by using a slurry packing method. Approximately 0.7 g of 5-µm PLRP-S microparticulate stationary phase was weighed out and transferred into the slurry packer. A 50:50 mixture of isopropyl alcohol:methanol was prepared and degassed by sonification. Approximately 30 mL of this mixture was transferred into the slurry packer. This mixture was stirred for 20 minutes on a magnetic stirring plate. The

slurry packer was then assembled and the column to be packed was connected with the outlet of the packer. The inlet of the packer was connected with a Waters M-6000 solvent delivery HPLC pump. A flow rate of 1.0 mL/minute of methanol:isopropyl alcohol (50:50) was used until the solvent started to flow through the outlet of the packer and the column. At this point the flow rate was increased to attain a back pressure of 5,500 psi. The flow rate was decreased continuously with time in order to maintain a constant back-pressure of approximately 5,500 psi; the back-pressure increased with time as the column filled up with the stationary phase. The back-pressure increased sharply when the column was completely filled with the stationary phase and the flow was halted by automatic shut-down of the solvent delivery pump at 6,000 psi. The column was then conditioned by delivering pure HPLC-grade methanol through the column for at least 20 hours at a flow rate of 0.2 mL/minute. The efficiency of the column was determined by calculating the theoretical plate number (N) for toulene and phenol when a mobile phase of methanol:0.01M aqueous monobasic potassium phosphate (65:35) at a flow rate of 1.0 mL/minute was used.

Pre-Chromatography Isolation of Ranitidine from Plasma

Isolation of ranitidine from plasma constituents before chromatography was conducted by adding 200 µL of ACN and approximately 50 mg of $ZnSO_4$ crystals to 1.0 mL of plasma. The aged plasma was stored at -10°C. This plasma was thawed at room temperature and 1.0 mL of thawed plasma was pipetted into a disposable borosilicate test tube. An aliquot of the standard stock solution of ranitidine was added to the 1.0 mL of plasma and vortexed for 30 seconds. A 200-µL aliquot of ACN and approximately 50 mg of $ZnSO_4$ was added to the plasma-ranitidine solution, mixed on a vortex mixer for approximately 2 minutes, and centrifuged at 2,200 g for 3 minutes. The supernatant was decanted into another fresh borosilicate culture test tube. This solution was filtered through a 0.45-µm filter tip using a 5.0-mL

gas-tight syringe. An aliquot of this filtered solution was injected directly onto the chromatographic system.

Construction of the Calibration Curve

The stock solution of ranitidine described in the Preparation of Stock Solution section was stored at -10°C and was found to be stable for at least 1 year. At least six solutions of ranitidine were prepared by adding enough stock solution to give concentrations of about 0.010, 0.250, 3.00, 10.0, 20.0, and 40.0 μg/mL. These solutions were prepared in each of three matrices: ACN and water (20:80); plasma; and 20:80 ACN:water and 50 mg of $ZnSO_4$/mL of the solution. The plasma solutions were treated identically as described in the Pre-Chromatography Isolation of Ranitidine section. If plasma samples of the patient had ranitidine concentrations higher than 40.0 μg/mL, then additional higher-concentration solutions were used to construct the calibration curve (the calibration curve was linear to at least 100 μg/mL). A linear calibration curve of peak height versus concentration of the drug was constructed and used for subsequent plasma quantifications.

RESULTS AND DISCUSSION

Figure 2 shows the chromatogram of ranitidine and its three major metabolites: ranitidine S-oxide, ranitidine N-oxide, and desmethylranitidine in ACN solution. Typical chromatograms for the control plasma and plasma from patients that ingested ranitidine are in Figures 3 and 4.

From the chromatogram (Figure 2) it is clear that the chromatographic conditions used in this experiment are capable of resolving ranitidine from its three major metabolites. Inspection of Figures 3 and 4 shows that the ranitidine peak is well-resolved and did not interfere with plasma creatinine and endogenous substances. A linear calibration curve of ranitidine peak height versus concentration was used instead of an internal standard method. The linear calibration curve method also eliminated the possibility of any

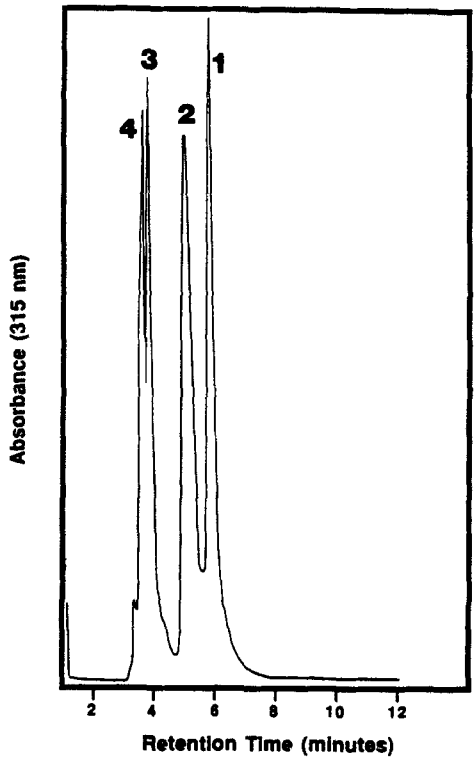


FIGURE 2: Chromatogram of ranitidine and its three metabolites in ACN solution. The peak designations 1, 2, 3, and 4 are the same as described in Figure 1.

peak interference between the internal standard and the ranitidine peak. Calibration curves for plasma and water treated with 20% ACN and approximately 50 mg of $ZnSO_4$ for 1.0 mL of plasma were constructed. A third calibration curve was constructed for water treated only with 20% ACN. The objective of constructing these calibration curves was to see whether the ACN, $ZnSO_4$, or a combination ACN and $ZnSO_4$ when used for water and plasma had any effect on the slope and other parameters of the calibration curves. Table 1 shows the correlation coefficient, slope, and intercept values of the three calibration curves.

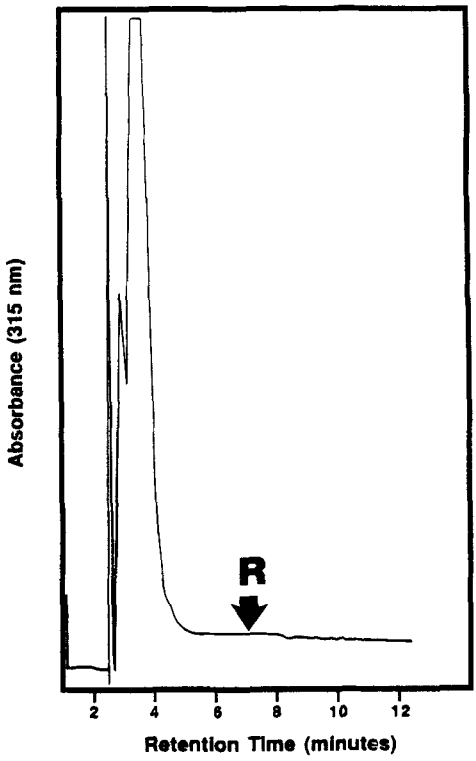


FIGURE 3: Chromatogram of plasma with no ranitidine. Detector was at 315 nm and 0.005 AUFS. Chromatographic conditions are as described in the text. R indicates the retention time of ranitidine.

TABLE 1

Parameters of Calibration Curves of Ranitidine in Different Matrices

Matrix	Slope	Correlation Coefficient	Y-Intercept
20:80, ACN:water	135	0.9997	40.3
20:80, ACN:water treated with $ZnSO_4$ (50 mg/mL)	133	0.9950	63.8
20% ACN and $ZnSO_4$ (50 mg/mL) in plasma	116	0.9996	-12.1

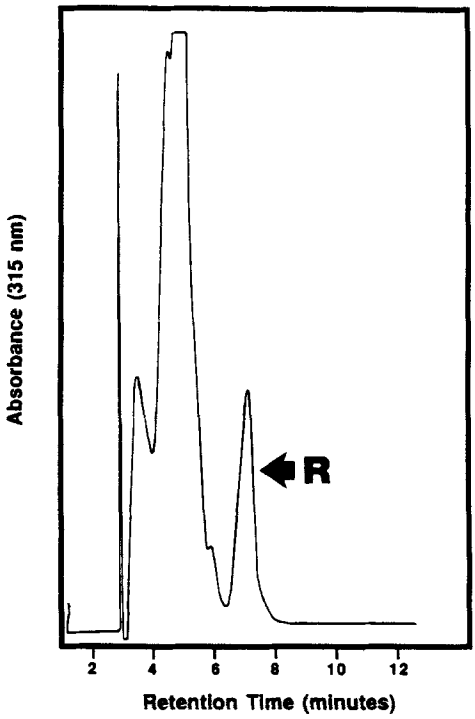


FIGURE 4: A typical chromatogram of an extract of plasma from a patient who ingested ranitidine. Concentration found was 260 ng/mL. R indicates the ranitidine peak. The detector was at 315 nm and 0.01 AUFS. The chromatographic conditions are as described in the text.

Statistical calculation at a 95% confidence interval of the slopes of the calibration curves in ACN:water (20:80) and in ACN:water (20:80) treated with $ZnSO_4$ were identical. The slope of the calibration curve in plasma is approximately 13% less than the slope in water treated identically before injection. This indicates that some of the ranitidine may have co-precipitated with the endogenous substance of the plasma. The 20% ACN in the pre-chromatography isolation of ranitidine from plasma precipitates significant amounts of proteins that are probably of high molecular weight.

The addition of $ZnSO_4$ caused an additional precipitation of the endogenous substances that were not effected by ACN. If the $ZnSO_4$ cleaning step of the plasma samples is omitted, then large plasma peaks elute with similar retention times as that of ranitidine, and therefore interfere with the quantification of the ranitidine peak.

The cleaning of the borosilicate culture test tubes and $ZnSO_4$ is a critical step of this method in order to achieve reproducible chromatographic results with no interference peaks. If the cleaning steps are not followed before sample preparation and pre-chromatographic isolation of ranitidine from plasma, then large interfering peaks overlap the ranitidine peak.

A guard column was used on the top of the analytical column and was also packed with PLRP-S particles. The analytical column showed no significant deterioration of chromatographic properties after 600 injections. The packing of the guard column needed to be replaced by fresh packing after approximately 60 injections. It was also observed that if the plasma samples were not filtered through the 0.45-μm filter tips by the aid of a gas-tight syringe, then the back-pressure of the chromatographic systems exceeds 5,000 psi after 5 to 10 injections. Therefore, filtering the plasma samples before injection into the HPLC system is extremely necessary in order to achieve longer column life and to avoid high back-pressure of the chromatographic system.

The effectiveness of a 20% (v/v) organic solvent to precipitate proteins from plasma was investigated using methanol, n-propanol, isopropyl alcohol, and ACN. It was found that ACN was most effective in plasma protein precipitation, followed by methanol, isopropyl alcohol, and n-propanol. Therefore, ACN was used in this experiment. The use of approximately 50 mg of $ZnSO_4$ was found to be optimal for 1.0 mL of plasma. Use of less than 40 mg of $ZnSO_4$ resulted in large chromatographic plasma peaks that overlapped the ranitidine peak. When too much $ZnSO_4$ (greater than 50 mg) was used, ranitidine also co-precipitated with many endogenous substances.

The reproducibility and accuracy of the assay was determined by repetitive analysis of plasma "spiked" with aliquots of standard ranitidine solution. The data for same-day and day-to-day analyses are in Tables 2 and 3.

The percent of relative standard deviation (%RSD) ranged from 2.4 to 6.0 for same-day analyses and 3.2 to 5.2 for day-to-day analyses. Inspection of Tables 2 and 3 shows that the reproducibility of the assays (%RSD) is better at higher plasma-ranitidine concentrations. Nevertheless, the %RSD was less than 10 at a ranitidine concentration of 5 ng/mL in plasma solutions.

Because ranitidine is a weakly-basic compound, an alkaline mobile phase was used in order to elute ranitidine in its molecular form. The elution of

TABLE 2

Assay Reproducibility for Same-Day Analysis

Concentration (μg/mL) (Mean ±SD)		Percent
Actual	Determined	RSD
0.025	0.021 ±0.012	6.0
0.10	0.12 ±0.005	4.2
1.5	1.7 ±0.08	4.7
5.0	4.8 ±0.14	2.9
20.0	21.6 ±0.52	2.4

TABLE 3

Assay Reproducibility for Day-to-Day Analysis

Concentration (μg/mL) (Mean ±SD)		Percent
Actual	Determined[a]	RSD
0.050	0.058 ±0.003	5.2
0.20	0.24 ±0.013	5.4
2.0	2.4 ±0.09	3.8
10.0	9.6 ±0.38	3.9
25.0	25.7 ±0.82	3.2

SD Standard deviation.
RSD Relative standard deviation.

a Samples were analyzed everyday for 5 days.

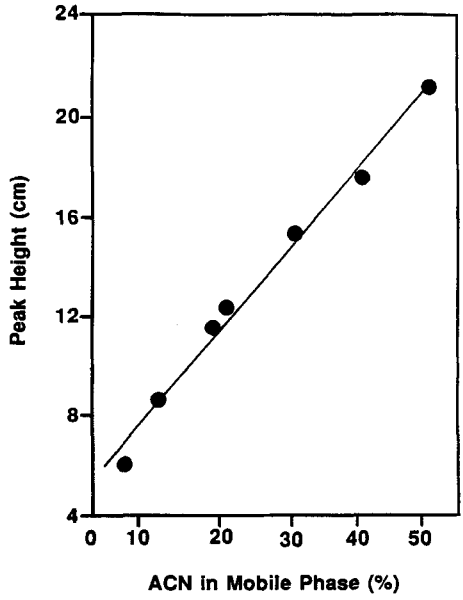


FIGURE 5: Effect of mobile phase ACN on peak height of ranitidine. Amount of ranitidine injected in each interval was 20 ng.

ranitidine in its molecular form also improved the resolution and sensitivity of the peak. The mobile phase ratios used in this experiment were optimal with respect to sensitivity and resolution of the ranitidine peak from the plasma peaks.

An experiment was conducted by injecting a constant amount of ranitidine into the HPLC system and varying the percent of ACN in the mobile phase. Figure 5 shows the linear regression plot of peak height versus percent of ACN in the mobile phase. From the linear regression data ($r = 0.994$), it appears that the peak height of ranitidine has a linear relationship at least up to 50% of ACN in the mobile phase when all other experimental conditions are kept constant. This relationship might not be linear with a higher percentage of ACN in the mobile phase because ranitidine will elute at void volume under

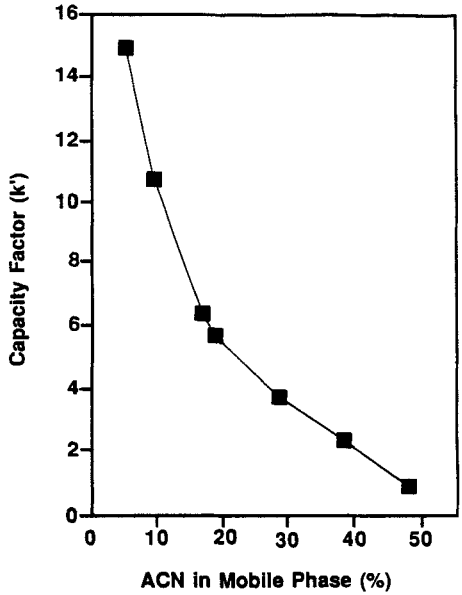


FIGURE 6: Effect of mobile phase ACN on the k' value of ranitidine. Twenty ng of ranitidine was injected at each interval.

those conditions and measuring the peak height accurately would be very difficult.

The retention time and capacity factor (k') of ranitidine increased and the peak height decreased with decreasing amounts of ACN in the mobile phase. The decrease in peak height was due to the chromatographic band-broadening of the ranitidine peak. Figure 6 shows the plot of k' and ACN (%) in the mobile phase. It is clear from the figure that the k' decreases sharply with an increase of ACN, and then becomes fairly constant as it approaches a value of one or less. It was found that the k' value of ranitidine should be at least 4.5 in order to achieve adequate resolution of the ranitidine peak from the plasma peaks. The mobile phase ratios used in this experiment gave a k' value of approximately 5.5.

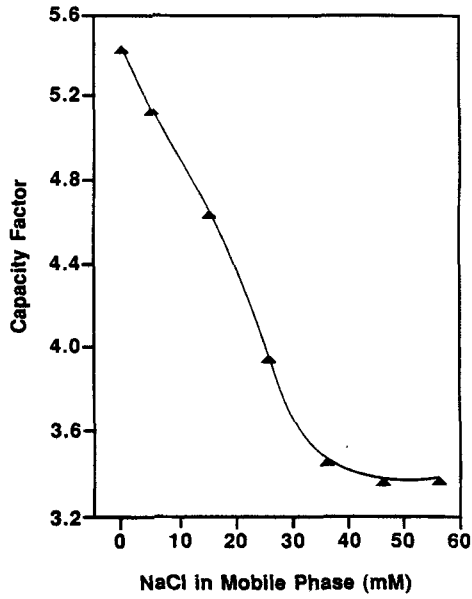


FIGURE 7: Effect of mobile phase ionic strength on the k' value of ranitidine. Other chromatographic conditions are as described in Figure 4.

Another experiment was done to investigate the ionic strength of the mobile phase on the retention time and k' value of ranitidine. Figure 7 shows the plot of the results of this experiment. Inspection of Figure 7 shows that k' decreases with the increase of the ionic strength of the mobile phase. This effect levels off as the k' value of ranitidine approaches a value of approximately 3. Therefore, extra care must be taken in sample injection so that the ionic strength of the mobile phase stays reasonably unchanged after the sample injection. Irreproducible retention time and peak height is a good indication that the ionic strength of the mobile phase was altered during injection of the sample solution.

Several columns were tested for selectivity and sensitivity of the ranitidine peak. It was found that the silica-based reversed-phase columns

gave higher theoretical plates/meter than the polymeric-based reversed-phase columns. Because the mobile phase was alkaline and the silica-based stationary phases were unstable under alkaline pH, the pH stable (1-13) PLRP-S column was used.

The recovery of ranitidine from patients' plasma samples was determined by "spiking" an aliquot of a standard ranitidine solution into a sample to give twice the concentration of the drug that had been determined earlier. After "spiking", the concentration was determined by injecting the sample into the chromatographic system and comparing the peak height with a standard calibration curve. The following equation was used:

$$\text{Percent concentration} = \frac{\text{Concentration determined after "spiking"}}{\text{Concentration expected after "spiking"}} \times 100$$

The results of the recovery experiment of plasma samples of patients having high and low concentrations of ranitidine are in Table 4.

Inspection of Table 4 shows that the recovery of ranitidine from patients' plasma varied from 92% to 105%. However, the recovery of ranitidine from plasma samples "spiked" with standard ranitidine solution to give concentrations of 20 ng/mL or less varied from 85% to 110%.

The *in vivo* pharmacokinetics of ranitidine in a patient who ingested a single oral dose of 150 mg of ranitidine was studied using this method. The

TABLE 4

Recovery of Ranitidine in Patients' Plasma Samples

Mean Concentration (μg/mL) (n = 2)			
In Plasma	Expected After "Spiking"	Determined After "Spiking"	Percent Recovery
0.035	0.70	0.67	96
0.28	0.56	0.59	105
1.4	2.8	2.6	93
0.76	1.52	1.4	92
2.3	4.6	4.5	98
8.7	17.4	17.7	102

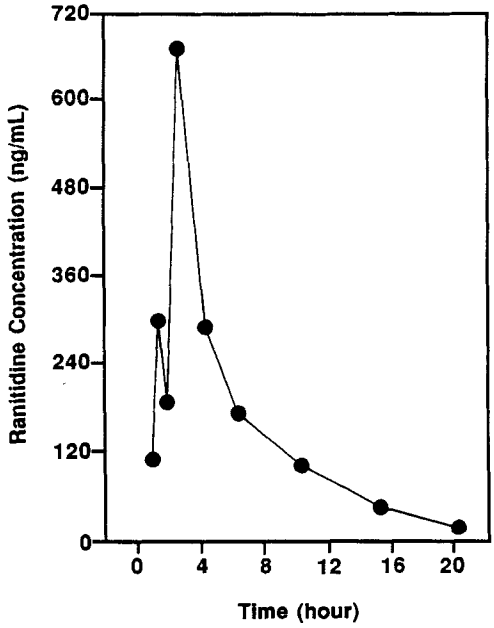


FIGURE 8: Plasma concentration versus time curve following a single dose oral administration of 150 mg of ranitidine to a patient suffering from duodenal ulcer.

patient was suffering from duodenal ulcer and has been administered the drug (150 mg twice daily) for more than 6 weeks. Figure 8 shows the ranitidine concentration in plasma with respect to time. The elimination rate constant (k_e) was obtained by an open two-compartment model with first-order absorption and elimination from the central compartment. The data were analyzed by linear regression. The elimination half-life ($t_{1/2}$) was determined from the equation $t_{1/2} = -0.693/k_e$ and was found to be 2.92 hours. The time required to reach the maximum concentration (t_{max}) of ranitidine was approximately 2.5 hours. The maximum plasma concentration (C_{max}) was found to be approximately 678 ng/mL.

From Figure 8 it is clear that ranitidine gave two peak concentrations when the drug was ingested orally. Van Hecken and Miller also observed two peak concentrations of ranitidine in the samples of a subject that ingested the drug (13, 14). The double peak could be due to an initial absorption of the drug in the stomach, since ranitidine is a weakly-basic compound and the pH values of the stomach and intestine are significantly different. This double peak could also be related to the dosage form. A prerequisite of drug absorption when administered orally is to bring the drug into solution at the site of absorption. Some pharmacotechnical factors may alter the biological performance of a drug by changing the rate and extent of drug release from the dosage form upon administration. Therefore, drug disintegration products having different release and dissolution characteristics will result in different blood or plasma level curves.

Initial disintegration products of ranitidine may generate small particles and larger granules. Rapid dissolution of ranitidine from the smaller particles may have generated the smaller peak and a larger contribution from the granules may have produced the additional, larger predominant peak. This hypothesis can be tested by administering the drug in liquid form.

CONCLUSION

The analytical method presented in this paper provides a simple, sensitive, and rapid method for the estimation of ranitidine in plasma specimens of patients who ingested the drug. By following the *in vivo* pharmacokinetics and measuring the concentration of ranitidine of patients' plasma, rationalization of dose and therapy may be optimized, especially for patients who are elderly and have multiple organ disfunction.

An analytical method is a prerequisite in determining the above mentioned clinical parameters. Ranitidine assays described by other authors in the literature require multiple-step extraction and evaporation for sample preparation before chromatography. In this method, the extraction and

evaporation steps have been eliminated from the procedure, which reduces the time of sample preparation and increases the reproducibility of the assay significantly. A straight calibration method was used instead of an internal standard method because of the high precision of the method. The linear dynamic range of the method is at least four orders of magnitude with a %RSD of less than 6.

The sensitivity of an HPLC method depends on many factors, such as means of detection, volume of sample injected, background signal from a blank sample, analytical recovery, and performance of the analytical column. With the conditions described in this method, the limit of quantification (LOQ) was found to be 5 ng/mL with a signal:noise ratio of 3.

Addition of tetraethylammonium hydroxide in the mobile phase increased the symmetry and shape of the ranitidine peak significantly. This was probably due to deactivation of some of the polar sites of the stationary phase, the ranitidine molecule, or both. The sharpness of the peak also increased the sensitivity and the resolution of ranitidine.

Acetaminophen, cimetidine, creatinine, tetracycline, ampicillin, ranitidine S-oxide, ranitidine N-oxide, and desmethylranitidine were tested for chromatographic peak interference with the ranitidine peak and were all found to be negative. The method described in this paper was demonstrated to be more cost-effective, sensitive, rapid, and simple than any method described in the literature.

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