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Note

High-performance liquid chromatographic determination of ranitidine in whole blood and plasma by using a short polymeric column

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Ranitidine (Fig. 1) is a new histamine H_2-receptor antagonist drug that is highly effective in the treatment of duodenal and stomach ulcers and Zollinger–Ellison syndrome [1–3]. It is known that certain minimal plasma or whole blood concentrations must be attained to achieve adequate inhibition of gastric acid secretion [4]. Many patients treated with ranitidine fail to show significant improvement. From a pharmacological point of view, this failure is related to therapeutic dosage. Therefore, in selected patients, the monitoring of plasma and whole blood ranitidine concentrations may provide a rational basis for dosage adjustment and insure optimum therapy.

High-performance liquid chromatography (HPLC) has been extensively used to separate and quantitate ranitidine from biological samples [5–13]. In these methods, the sample preparation has varied from the multiple-step extraction and evaporation procedures employing different organic solvents for pre-chromatography isolation of the drug. All of the methods available in the literature are either complex or time-consuming, or both.

This report describes a simple, rapid, sensitive, and selective reversed-phase HPLC (RP-HPLC) method for determining the ranitidine present in the plasma and whole blood of the subject that ingested the drug. This method utilizes an isocratic elution and a short column packed with polymeric reversed-phase particles.

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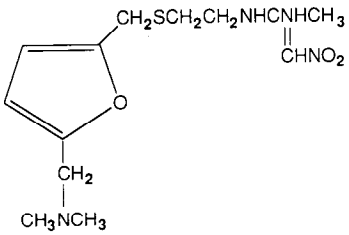

Fig. 1. Structural formula of ranitidine.

EXPERIMENTAL

Equipment

The analytical system consisted of a Waters M-6000 solvent delivery pump equipped with a U6K universal LC injector (Waters Assoc., Milford, MA, U.S.A.). A reversed-phase, 6 cm × 4.6 mm column packed with 10-μm polymeric reversed-phase particles (PRP-1) was used. A slurry packer was used to pack the analytical column (Micromeritics, Norcross, GA, U.S.A.). A Kratos Spectroflow 773 variable-wavelength (190 to 700 nm) UV–visible detector from Kratos Analytical Instruments (Ramsey, NJ, U.S.A.) was used. Data were recorded on a Houston Instrument Microscribe, TM 4500 strip chart recorder (Bausch & Lomb, Austin, TX, U.S.A.). An ultrasonic bath was used to degas the mobile phase (S & R Manufacturing Industries, Kearney, NJ, U.S.A.). An Eppendorf digital pipette (100–1000 μl) was used for all quantitative sampling (Cole-Parmer, Chicago, IL, U.S.A.). Deionized water was collected from a Sybron-Barnstead 60209 water purification system. A guard column, 4 cm × 2.0 mm, slurry-packed with 20–30 μm PRP-1 particles purchased from Hamilton (Reno, NV, U.S.A.) was also used.

Materials

Ranitidine, in the form of ranitidine hydrochloride (brand name Zantac®), was obtained in 2.0-ml single-dose vials from Glaxo (Research Triangle Park, NC, U.S.A.). Acetonitrile, methanol, isopropanol and *n*-propanol were all Gold Label and obtained from Aldrich (Milwaukee, WI, U.S.A.). Anhydrous potassium carbonate was ACS grade and was obtained from Mallinckrodt (St. Louis, MO, U.S.A.). Dibasic potassium phosphate and sodium 1-pentane sulfonate were reagent grade and were obtained from Aldrich. Zinc sulfate was obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). Styrene divinylbenzene polymeric stationary phase (PRP-1) was purchased from Hamilton. The pooled plasma and whole blood were obtained from the Blood Center of Southeastern Wisconsin (Milwaukee, WI, U.S.A.).

Chromatographic conditions

The mobile phase was solvent A–solvent B (18:82, v/v). Solvent A was 100% acetonitrile and solvent B was 5 m*M* dibasic potassium phosphate and 5 m*M* sodium 1-pentane sulfonate in deionized ultrapure water. After mixing, the pH of the mobile phase (mixture of solvents A and B) was adjusted to approximately

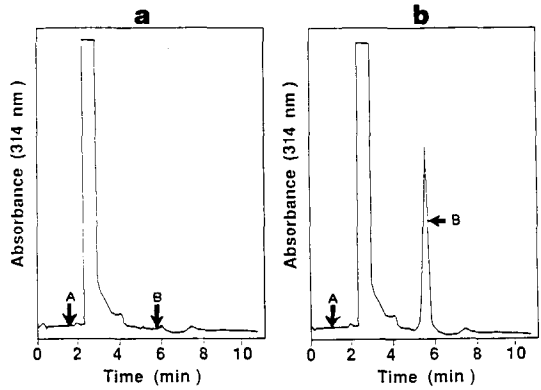

Fig. 2. Chromatograms of (a) an extract of plasma containing no ranitidine, detector sensitivity 0.02 a.u.f.s.; (b) an extract of plasma from a patient treated with ranitidine, concentration found was 285 ng/ml, detector sensitivity 0.004 a.u.f.s. B = ranitidine; A = injection point.

10 or above using a 0.1 *M* sodium hydroxide solution. The flow-rate was 1.0 ml/min. The detection wavelength was set at 314 nm with sensitivity from 0.20 to 0.002 a.u.f.s. The analytical and guard columns were operated at room temperature ($25 \pm 2°C$). Quantification was done by comparing the peak height of an unknown with a standard calibration curve.

Procedure and extraction

A stock solution of ranitidine was prepared by dissolving 50 mg of ranitidine hydrochloride in 100 ml of methanol. A 10-ml volume of this stock solution was diluted to 100 ml. This solution was used to prepare plasma, whole blood and water standards for the construction of the calibration curves.

The aged blood and plasma were kept frozen at $-10°C$ for several days, then thawed at room temperature. Thawed whole blood or plasma (1 ml) was pipetted into a borosilicate disposable test tube and was spiked with an aliquot of a standard stock solution of ranitidine. This was mixed on a vortex mixer for 30 s, and

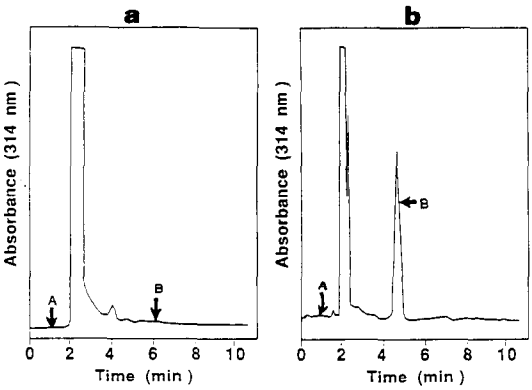

Fig. 3. Chromatograms of (a) an extract of whole blood containing no ranitidine, detector sensitivity 0.002 a.u.f.s.; (b) an extract of whole blood from a patient treated with ranitidine, concentration found was 140 ng/ml, detector sensitivity 0.003 a.u.f.s. B = ranitidine; A = injection point.

TABLE I

WITHIN-DAY AND DAY-TO-DAY ASSAY REPRODUCIBILITY

Actual concentration (ng/ml)	Concentration found (mean ± S.D., $n=5$) (ng/ml)	R.S.D. (%)
Within-day		
25.0	24.0 ± 0.80	3.3
50.0	50.6 ± 1.90	3.8
300	294 ± 16	5.4
1000	1085 ± 42	3.8
*Day-to-day**		
10.0	9.4 ± 0.80	6.4
100	98.6 ± 4.3	4.4
1000	1107 ± 33	2.9
5000	5148 ± 190	3.7

*Carried out every other day for a period of one week.

1.0 ml of methanol was added to the mixture. Zinc sulfate powder (15 mg) was added to the mixture, mixed on a vortex mixer for 30 s and centrifuged for 2 min at 800 *g*. The supernatant was decanted into a fresh culture test tube and was saturated with anhydrous potassium carbonate. This was again centrifuged for 1 min at 800 *g*. The salted-out methanol layer was transferred to another fresh test tube. A 100-μl aliquot of the methanol solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Chromatograms for the control plasma and whole blood were obtained using the chromatographic procedure described and these are shown in Figs. 2a and 3a. Typical chromatograms obtained following the extraction of plasma and whole blood from the subjects that ingested ranitidine are shown in Figs. 2b and 3b. The total time (starting from extraction until elution of ranitidine) was less than 15 min.

From the chromatograms of whole blood and plasma, it is clear that the ranitidine peak is well resolved and did not interfere with blood creatinine, other endogenous plasma or whole blood constituents. An internal standard was not used in the method. Instead, a linear calibration curve of peak height versus concentration was used ($r=0.998$ or better). Using this method eliminated the risk of any peak interference between the internal standard and the sample constituents.

From statistical calculations at the 98% confidence interval, the calibration curves that are obtained after extraction from pure water, plasma or whole blood are identical. Therefore, a calibration curve obtained by extraction of standards in water can be used to quantitate ranitidine in patients' plasma or whole blood.

Extraction efficiency was studied by adding known amounts of ranitidine to drug-free plasma or whole blood at high and low concentrations. After extraction

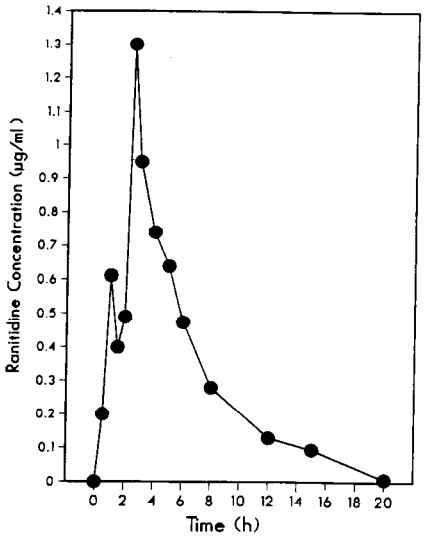

Fig. 4. Whole blood concentration versus time curve following an oral administration of 300 mg of ranitidine to a patient suffering from acute duodenal ulcer.

and injection into the chromatograph, the peak height obtained was compared with the peak height of a standard solution of ranitidine in pure methanol. Extraction efficiencies ranged from 90 to 96% ($n=8$ for each concentration).

The precision and accuracy of the method were evaluated by repetitive analysis of whole blood or plasma spiked with ranitidine. Four aliquots of pooled plasma spiked with ranitidine at different concentrations are shown in Table I. For the within-day analysis, the average relative standard deviation (R.S.D.) was 4.07%. Table I also shows the day-to-day precision of the assays (every other day over one week); the average R.S.D. is 4.35%. The linear range of the assay was found to be at least 7.0 ng/ml to 30 µg/ml. The R.S.D. of the slope of this linear curve was 4.5%. The limit of detection of the method is 0.70 ng/ml for a 100-µl injection.

The concentrations of ranitidine that were determined for whole blood and plasma on a given patient sample were very similar (whole blood, 152 ng/ml, plasma, 146 ng/ml). Hence, either whole blood or plasma can be used to quantitate ranitidine for effective dose adjustments.

In the analytical system, a guard column was used between the injector and the analytical column. The guard column needed to be repacked with new packing after about 200 injections of the sample. No deterioration of the efficiency of the analytical column was observed after at least 400 injections. Since the PRP-1 polymeric packing is very stable from pH 0 to 13, the alkaline mobile phase was not harmful to column. The percent of methanol salted-out by anhydrous potassium carbonate from an aqueous solution is $94 \pm 8\%$ ($n=10$) by volume [14]. Methanol was used as the extraction solvent because, compared to acetonitrile and isopropyl alcohol, it showed the best extraction efficiency and selectivity for the parent drug from whole blood and plasma. The extraction efficiencies for methanol, acetonitrile and isopropanol were 94, 79 and 83%, respectively. For all

quantitative calculations, the volume of methanol added for extraction was used. The mobile phase ratios used were found to be optimum for selectivity and sensitivity. Because the pK_a values of positively charged ranitidine are 2.7 and 8.2, it was necessary to elute the drug in its molecular form using the alkaline mobile phase for better chromatographic sensitivity and reproducibility [15].

Interferences of different drugs with the ranitidine peak were also studied. The drugs tested for interferences were acetaminophen, ampicillin and tetracycline. These drugs were chosen for testing because at least two of the patients ingesting ranitidine (and whose plasma samples were analyzed by this method) were ingesting these drugs simultaneously. None of these three drugs interfered with the ranitidine peak. They all eluted much earlier or later than the ranitidine peak under the experimental chromatographic conditions.

Using this analytical method, one can study the in vivo pharmacokinetics of ranitidine. The whole blood concentration of ranitidine was followed over a time course of 0 to 20 h in a patient suffering from an acute duodenal ulcer. After a single oral dose of 300 mg of drug, two peak concentrations of ranitidine were demonstrated (Fig. 4). This agrees with previous observations by Van Hecken et al. [16] and Miller [17]. After 20 h, the concentration of ranitidine in whole blood or plasma was less than 10 ng/ml. Because of high sensitivity of this method, a pharmacokinetic study can be done with levels as low as 3.0 ng/ml.

CONCLUSION

The ranitidine HPLC assay described is a significant improvement over those previously reported [5–13] in terms of sensitivity, ease of sample preparation, and simple extraction procedure. All of the previous ranitidine assays require multiple-step extractions with one or more evaporation steps for sample preparation before chromatography. In contrast, the assay described here involves a single protein precipitation followed by the salting-out of the extraction solvent. The evaporation step has been eliminated from the procedure, thus reducing the sample preparation time to less than 10 min. In addition to reducing time, this procedure also reduces manipulative errors. The ranitidine HPLC assay described here is highly reproducible and much faster than previously reported methods [5–13].

REFERENCES

1 T.S. Gaginella and J.H. Bauman, Drug Intell. Clin. Pharm., 17 (1983) 873.
2 J.B. Zeldis, L.S. Friedman and K.J. Isselbacher, N. Engl. J. Med., 309 (1983) 368.
3 R.N. Brogden, T.M. Heel and G.S. Avery, Drugs, 15 (1978) 93.
4 W. Domschke, G. Lux and S. Domschke, Gastroenterology, 76 (1979) 123.
5 P.F. Carey and L.E. Martin, J. Liq. Chromatogr., 2 (1970) 291.
6 G.W. Mihaly, O.H. Drummer, R.A. Smallwood and W. Louis, J. Pharm. Sci., 69 (1980) 155.
7 H.M. Vandenberghe, S.M. MacLeod, W.A. Mahon, P.A. Lebert and S.J. Soldin, Ther. Drug Monit., 2 (1980) 379.
8 P.F. Carey, L.E. Martin and P.E. Owen, Biochem. Soc. Trans., 9 (1981) 112.
9 J. Boutagy, D.G. Moore, I.A. Munro and G.M. Shenfield, J. Liq. Chromatogr., 7 (1984) 651.
10 P.F. Carey, L.E. Martin and P.E. Owen, J. Chromatogr., 225 (1981) 161.

11 M.S. Lant, L.E. Martin and J.J. Oxford, J. Chromatogr., 323 (1985) 143.
12 H. Kubo, Y. Kobayashi and K. Tokunaga, Anal. Lett., 18 (1985) 245.
13 G. Mullersman and D. Derendorf, J. Chromatogr., 381 (1986) 385.
14 A.M. Rustum, Ph.D. Dissertation, Marquette University, Milwaukee, WI, 1986, Table 2.8, pp 2-32.
15 L.E. Martin, J. Oxford and R.J.N. Tanner, J. Chromatogr., 251 (1982) 215.
16 A.M. Van Hecken, T.B. Tjandramage, A. Mullie, R. Verbesselt and P.J. De Schepper, Br. J. Clin Pharmacol., 14 (1982) 195.
17 R. Miller, J. Pharm. Sci., 73 (1984) 376.