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Determination of ranitidine in plasma by high-performance liquid chromatography

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Abstract: A high-performance liquid chromatographic method has been developed for the determination of ranitidine in plasma. Ranitidine was extracted with acetonitrile by adding it to the plasma and then salting it out with potassium carbonate. The chromatographic column was 5-μm ODS silica, the mobile phase being acetonitrile–7 mM triethylammonium ion in phosphoric acid (pH 3.00) (30:70, v/v). The ranitidine peak was monitored at a wavelength of 315 nm, the retention time for ranitidine being 4.6 min. A limit of detection of 3 ng ml^{-1} was obtained for a 100-μl injection of ranitidine. The method was found to be reproducible with a relative standard deviation (RSD) between 0.8–5.3% (n = 5) over the concentration range 25–80 ng ml^{-1} in plasma. The ranitidine concentration was determined in 18 different patients' plasmas. Ranitidine and its metabolites ranitidine *S*-oxide, ranitidine *N*-oxide and desmethylranitidine, were also studied for chromatographic resolution from each other. It was shown that a group of common drugs did not interfere with ranitidine determination.

Keywords: *Ranitidine; high-performance liquid chromatography; determination in plasma; ranitidine metabolites.*

Introduction

Ranitidine (Fig. 1) is a drug used to treat peptic and duodenal ulcers. It acts as a histamine H_2-receptor antagonist. In order to monitor plasma levels of ranitidine in critical care patients and to carry out pharmacokinetic studies of the drug, a rapid, sensitive and reproducible method for its determination is required. Others have used high-performance liquid chromatography (HPLC) for the determination of ranitidine in biological samples [1–6]. However, the methods previously published are time consuming. They require an evaporation step and use an internal standard which may be unstable [3].

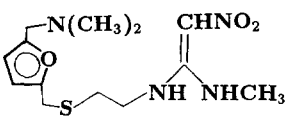

Figure 1
Structural formula of ranitidine.

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This report describes a useful method for ranitidine determination in plasma. Unlike others, it does not employ an evaporation step and is thus more rapid. The limit of detection is 3 ng ml^{-1} and this limit is comparable to the most sensitive method reported [6]. Its reproducibility is superior to that of methods previously reported.

Experimental

Apparatus

The chromatographic system consisted of a Waters M-6000A solvent delivery pump (Waters Associates, Milford, MA). A Rheodyne model 7125 injector with a sample loop of 200 μl (Cotati, CA) was used. The analytical column used was an Alltech (Deerfield, IL) 5-μm Econosphere ODS column (150 × 4.6 mm). A 2-cm guard column packed with 8-μm Rosil C_{18} stationary phase (Alltech) was used. The UV-visible detector used was a Kratos Spectroflow 773 variable wavelength detector obtained from Kratos Analytical Instruments (Ramsey, NJ). The recorder was a Houston Instrument strip chart recorder (Austin, TX). An ultrasonic water bath from L & R Manufacturing Industry (Kearny, NJ) was used to de-gas the mobile phase. For all volumetric transfers, a 100–1000 μl Eppendorf digital pipette from Cole Parmer (Chicago, IL) was used. A Sybron-Barnstead 60209 water purification system (Boston, MA) was used to prepare deionized water.

Materials

Ranitidine was obtained in 2-ml single dose vials from Glaxo Inc., (Research Triangle Park, NC). The metabolites of ranitidine were obtained from the Biomedical Pharmacology Division of Glaxo Group Research Limited (Ware, Hertfordshire, UK). Acetonitrile used was HPLC grade and was obtained from American Burdick & Jackson (Muskegon, MI). Potassium carbonate was obtained from Mallinckrodt Chemical Works (St. Louis, MO). Triethylamine was analytical grade and was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). The plasma was obtained from Milwaukee Blood Center (Milwaukee, WI) and Milwaukee County Hospital (Milwaukee, WI).

Preparation of the stock and the standard solutions

A stock solution of ranitidine was prepared in acetonitrile by adding 500 μl (12.5 mg ranitidine) of the original ranitidine solution to make 250 ml of acetonitrile solution. When not in use, the solution was kept in the freezer at −10°C.

The plasma was kept in a freezer at −10°C. Plasma was thawed to room temperature and 1.0 ml of it was placed in a disposable borosilicate tube and spiked with the necessary amount of ranitidine. Then the required amount of acetonitrile was added to make the total volume of acetonitrile 1.0 ml. In this way, eight standard solutions of ranitidine with concentrations of 25, 50, 100, 200, 300, 400, 600 and 800 ng ml^{-1} of plasma were prepared.

Extraction process

The plasma sample, together with the acetonitrile, was vortex mixed for 30 s. It was then centrifuged at 1200 ***g*** for 2 min. The supernatant was transferred to another culture tube and potassium carbonate was added together with vortex mixing until the organic layer was salted out from the aqueous layer. The mixture was centrifuged for 2 min. The organic layer was transferred to a test tube and 100 μl of it was injected into the chromatographic column.

Chromatographic conditions

The mobile phase was prepared by mixing 30%, by volume, of acetonitrile with 70%, by volume, of 7 mM triethylamine. The pH of the 7 mM triethylamine solution was adjusted to 3.00 by adding 85% phosphoric acid. The mobile phase was pumped through the column at a flow rate of 1.0 ml min^{-1}. The UV-visible detector was set at 315 nm with a sensitivity ranging from 0.02 to 0.002 AUFS. The volume of ranitidine solution injected into the column was 100 μl. A guard column was connected before the analytical column. The retention time of ranitidine was found to be 4.6 min ($k' = 2.5$).

Analysis of patients' plasma samples

Plasma samples from 18 patients were extracted with acetonitrile, and chromatographed in the same manner as used for the standard solutions above.

Results and Discussion

Bioanalysis of patients' samples

A typical chromatogram of ranitidine in plasma is shown in Fig. 2. Figure 3 shows the chromatogram of control plasma. The chromatogram showed no interfering peak at the ranitidine peak position. To construct a calibration curve, the peak heights of ranitidine were measured in duplicate, and plotted against their corresponding known concentrations. In this way, two different calibration curves for ranitidine were obtained; one for plasma and the other for pure water. Both these calibration curves were based on samples extracted as described above. A third calibration curve was made by using acetonitrile standard solutions without any extraction. All these calibration curves were found to be linear with correlation coefficients of 0.999. The statistical data for these calibration curves are shown in Table 1. At the 95% confidence level, the *t*-test calculations showed that these three curves had identical slopes. Because the intercepts are negligible, the identity implies that extraction was quantitative and that any one of these calibration curves could be used for the determination of ranitidine in plasma. The assay method developed was found to be reproducible with RSDs between 0.8–5.3%, over the concentration range examined (Table 2).

The concentration of ranitidine in the plasma of 18 different patients was determined. All these patients were critically ill and were given ranitidine either orally or as an injection. The ranitidine concentration in plasma was measured from the peak height in the chromatogram and the regression equation for the calibration curve. Most of the patients' plasma concentrations fell in the range of 200 ng ml^{-1} down to the limit of detection.

Study of potential interference

Concomitant drugs. A series of drugs normally used in the treatment of the critically ill patient was chosen to study their possible chromatographic interference with the ranitidine peak. Table 3 shows the drugs used in this study. None of these drugs was found to interfere with the ranitidine determination.

Metabolites. A study of the possible interference of the three metabolites of ranitidine was made. Under the chromatographic conditions described above, the order of elution of ranitidine and its three metabolites was ranitidine *S*-oxide ($k' = 1.0$), desmethylranitidine ($k' = 2.0$), ranitidine *N*-oxide ($k' = 2.2$) and ranitidine ($k' = 2.5$). The resolution

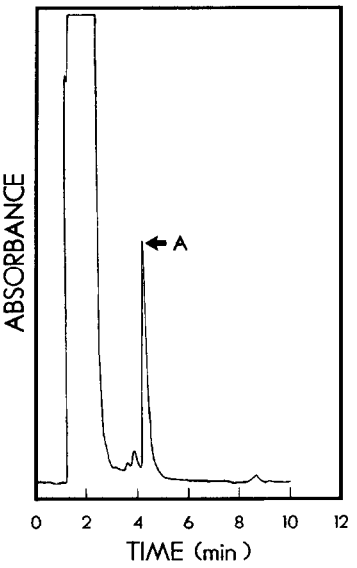

Figure 2
A typical chromatogram of the plasma extract of a patient after administration of ranitidine; A, ranitidine peak (280 ng ml^{-1}); detector sensitivity, 0.01 AUFS at 315 nm. For details of chromatography, see text.

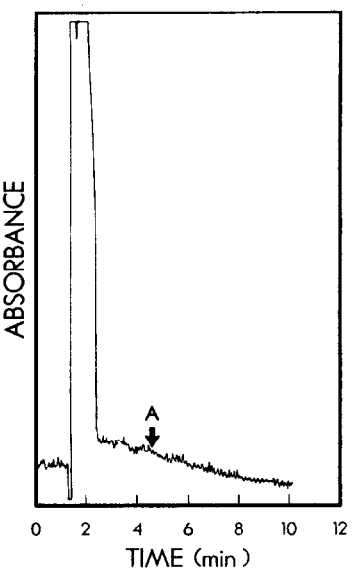

Figure 3
Chromatogram of control plasma extract. A, ranitidine peak; detector sensitivity, 0.002 AUFS at 315 nm. At the ranitidine peak position, no other interfering peak was found (for details, see text).

Table 1
Linear regression analysis of calibration curves*

Curve	Fluid extracted	Slope ± SE ($\times 10^4$)	Intercept ± SE ($\times 10^4$)	Identity of slopes† t_{calc}
A	Plasma	0.186 ± 0.001	−0.683 ± 0.284	0.2132 (A vs C)
B	Water	0.187 ± 0.002	−0.771 ± 0.798	0.6838 (A vs B)
C	No extraction	0.188 ± 0.002	−0.973 ± 0.590	0.2697 (B vs C)

*$y = mx + b$, where y is the peak height in absorbance units and x is concentration in ng ml^{-1}. For all curves $r = 0.999$ ($n = 9$).
†$t_{table} = 2.145$ ($P = 0.95$).

Table 2
Reproducibility of the method over a range of ranitidine concentrations

Mean concentration (ng ml^{-1})	SD ($n = 5$)	RSD (%)
28	1.2	4.3
49	2.6	5.3
99	5.0	5.0
198	5.6	2.8
302	4.6	1.5
402	5.0	1.2
598	6.2	1.0
808	6.7	0.8

Table 3
List of drugs used to study their potential chromatographic interference with ranitidine

Drugs used	Drugs used
Cefazolin sodium	Heparin sodium
Clindamycin phosphate	Hydroxyzine
Dexamethasone	6α-Methylprednisolone(21-succinate sodium)
Diazepam	Midazolam
Digoxin	Nafcillin sodium
Diphenhydramine	Phenobarbital
Furosemide	Phenytoin sodium
Gentamicin	Prochlorperazine
Haloperidol	Tobramycin sulphate

of these four peaks was also determined in a series of mobile phases, where acetonitrile was varied from 15 to 30% v/v. The results shown in Table 4 indicate that the highest resolution (R_s) between ranitidine and the closest potential interfering metabolite, ranitidine *N*-oxide, could be achieved with the mobile phase used in the proposed method. The poor resolution observed with other mobile phases was due to band broadening and peak tailing.

The *S*-oxide metabolite was never resolved from the strong endogenous constituent peaks (Fig. 2) in any of the patient samples. The desmethyl metabolite was found in most patient samples as a small peak preceding the ranitidine peak (Fig. 2) but of varying height depending on the sample. The ratio of its peak height to that of ranitidine was similar to that shown in Fig. 2, or smaller. The *N*-oxide metabolite was not observed in these patient samples.

Extraction, stability and reproducibility

The extraction efficiency of ranitidine from plasma was found to be 100% (Table 1). The extraction efficiency was expressed as the ratio of the calibration curve slope from the extracted fluid to that of the curve from the non-extracted standards. At a signal-to-noise ratio of 3:1, the limit of detection for ranitidine was found to be 3 ng ml^{-1}. This high sensitivity was achieved through injection of a relatively high volume, 100 μl. This relatively high injection volume of ranitidine in acetonitrile did not cause any peak deterioration. The retention of peak shape indicated the absence of an injection solvent-

Table 4
Relative resolution between ranitidine, ranitidine *N*-oxide, ranitidine *S*-oxide and desmethylranitidine* with different mobile phases†

Acetonitrile (%)	Resolution (R_s) Ranitidine/*N*-oxide	*N*-oxide/desmethyl	Desmethyl/*S*-oxide
15	0.4	0.3	2.1
20	0.5	0.4	2.2
25	0.6	0.5	2.3
30	1.0	0.7	2.3

*Retention times of ranitidine, the *N*-oxide, desmethyl and *S*-oxide were 4.6, 4.2, 3.9 and 2.6 min, with capacity factors of 2.5, 2.2, 2.0 and 1.0, respectively.
†All other chromatographic conditions were as used in the method described, except that the ratio of acetonitrile to aqueous solution was varied in the mobile phase.

strength effect on the ranitidine peak for that particular mobile phase [7]. The large volume of injection obviated any solvent evaporation step to concentrate the sample.

The stability of ranitidine at room temperature in plasma was also studied by spiking plasma with ranitidine to give a concentration of 800 ng ml^{-1}. The concentration of ranitidine in plasma was then determined repeatedly during a 48-h period. The reported level of plasma ranitidine was found to be highly stable over a period of at least 48 h.

In the great majority of cases, HPLC drug determination in biological fluids employs an internal standard. Others have drawn attention to the requirements and shortcomings of the use of an internal standard [8, 9]. For example, the error in measuring the peak heights of the internal standard and the analyte is greater than that involved in measuring the peak height of the analyte alone. Moreover, the chance of there being interfering peaks is greater when two peaks must be resolved instead of one. The internal standard method can be beneficial when transfers, evaporations, and solid phase extractions, or other experimental operations that can cause losses, are used since the ratio of internal standard to analyte remains constant. The method described herein does not use an internal standard, and was deliberately designed so that there would be no transfer losses affecting the outcome. The primary source of error that could be minimized by use of an internal standard was that involved in delivering the correct injection volume. Because the injection volume was large (100 μl) the volume reproducibility using the loop-valve injector was good (Table 2).

Conclusion

A sensitive and rapid HPLC method has been developed for the determination of ranitidine in plasma. The typical assay time is about 15 min. No internal standard is required and this simplifies the method. The analytical column was found to be stable after 500 injections. Because no interfering peak was found with common drugs used for critically ill patients, the method can be used to monitor the ranitidine level in clinical samples. The metabolites of ranitidine were also found not to interfere with the assay. The high stability of ranitidine in plasma at room temperature permits easy storage of samples and obviates the need for immediate analysis after drawing blood.

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