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Automated liquid chromatographic determination of ranitidine in microliter samples of rat plasma

A. B. SEGELMAN[a],*
Department of Chemical Biology and Pharmacognosy, College of Pharmacy, Rutgers-The State University, Piscataway, NJ 08854 (U.S.A.)
and
V. E. ADUSUMALLI and F. H. SEGELMAN
Wallace Laboratories, Division of Carter-Wallace, Inc., Half Acre Road, Cranbury, NJ 08512 (U.S.A.)

ABSTRACT

An improved high-performance liquid chromatographic method with UV detection at 313 nm has been developed for quantitation of ranitidine in 100 μl of rat plasma over the range 25 to 1000 ng/ml. To each sample were added the internal standard (metiamide) and 2 *M* NaOH. After dichloromethane extraction, the nitrogen-dried extracts were reconstituted in the mobile phase of 0.01 *M* phosphate buffer–triethylamine–methanol–water (530:5:390:75, v/v). Chromatography on μBondapak C_{18} with quantitation by peak height ratios showed an analyte recovery of 97%; a limit of detection of 10 ng/ml; a precision of 1–10% and an accuracy of 1–5%. About 90 samples can be processed in 24 h.

INTRODUCTION

Ranitidine {N-[2-[[[5-[(dimethylamino)methyl]-2-furanyl]-methyl]thio]ethyl]-N^1-methyl-2-nitro-1,1-ethenediamine} is a H_2-receptor antagonist, effective in inhibiting histamine- and pentagastrin-induced secretion of gastric acid [1,2]. The drug is widely used for the management of peptic ulcer disease [3].

Several high-performance liquid chromatographic (HPLC) methods for measuring ranitidine in biological fluids have been reported [4–10]. Some of these methods require *ca.* 1 ml of plasma [4–7] to achieve a detection limit of 10–20 ng/ml. Varughese and Lee [8] reported a method for determining ranitidine by direct injection of the supernatant from protein precipitiation of plasma with acetonitrile. However, with this method we observed a relatively high limit of detection (50 ng/ml), endogenous interferences, and the need for a rigorous column clean-up schedule to maintain acceptable results. Vandenberghe *et al.* [9], using a variable-wavelength detector, described a method for ranitidine assay in 200 μl of human plasma with a detection limit of 10 ng/ml. However, this method would not be suitable for serial blood sampling typical of rat pharmacokinetic studies, since it would require at least 400 μl of

[a] Present address: Nature's Sunshine Products, Spanish Fork, UT 84660, U.S.A.

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whole blood to be withdrawn *ca.* 15 times over a period of 12 hours, thus compromising homeostasis of this small animal.

We describe here a sensitive HPLC method for the determination of ranitidine with UV detection at 313 nm in 100 μl of rat plasma obtained from *ca.* 225 μl of blood. Pursuant to our studies on tannin-drug interactions, we have routinely employed this procedure to analyze large numbers of rat plasma samples from single animals over 24 h without compromising the animal or the sensitivity of ranitidine quantitation.

EXPERIMENTAL

Chemicals

Ranitidine hydrochloride (Zantac® injectable, 25 mg of ranitidine/ml) was purchased from Glaxo (Research Triangle Park, NC, U.S.A.). The internal standard (I.S.), metiamide, was supplied by Smith, Kline & French Labs. (Philadelphia, PA, U.S.A.). All chemicals were purchased from the vendors indicated: sodium hydroxide, potassium phosphate monobasic, sodium phosphate dibasic (Fisher Scientific, Fair Lawn, NJ, U.S.A.); HPLC-grade methanol, acetonitrile, dichloromethane and 2-propanol (Burdick & Jackson, purchased through Baxter Healthcare, McGaw Park, IL, US.A.); triethylamine (Pierce, Paris, KY, U.S.A.); HPLC-grade water was obtained using a Milli-Q® water system (Millipore, Bedford, MA, U.S.A.). Blank rat plasma (heparinized) was obtained from normal, untreated male Sprague-Dawley rats.

Instrumentation

HPLC was performed on a Waters Assoc. (Milford, MA, U.S.A.) component system, equipped with a Model M4 pump, Model 710B WISP autoinjector, and a Model 441 fixed-wavelength detector (313 nm), which was interfaced with the HP3357 Laboratory Automation System (LAS) (Hewlett-Packard, Avondale, PA, U.S.A.). Using a 313-nm filter compared to those at 214 and 280 nm, there was no interference by plasma endogenates and excellent sensitivity of detection was achieved. The maximum absorptivity of ranitidine in the mobile phase occurred at 315 nm.

Chromatographic conditions

The mobile phase consisted of 0.01 *M* phosphate buffer [11] (pH 7.5)–triethylamine–methanol–acetonitrile (530:5:390:75, v/v). After the addition of triethylamine to the phosphate buffer, the pH was readjusted to 7.5 with phosphoric acid (85%, v/v), and the final solution was mixed with the organic components. Chromatography was performed at ambient temperature (22 $\pm$ 1°C) on a 300 $\times$ 3.9 mm I.D. Waters μBondapak C_{18} column, 10-μm particle size, protected with a Waters C_{18} Corasil Bondapak guard column. The flow-rate was 1.0 ml/min, and the eluent was monitored at 313 nm with the attenuation set at 0.01 a.u.f.s. Peak heights for ranitidine and the I.S. were measured using the HP3357 LAS. These data were used to generate peak height ratios (ranitidine/I.S.) for establishing standard curves. Retention times of I.S. (metiamide) and ranitidine were approximately 4.7 $\pm$ 0.5 and 6.6 $\pm$ 0.3 min, respectively. After about 90 analyses, the column was washed consecutively with 100 ml of each, water, acetonitrile and methanol before re-equilibration with mobile phase.

Standard solutions

Three ranitidine stock solutions (0.1 mg/ml) were prepared from individual vials of commercially available ranitidine injectable solution (25 mg/ml). Working standards in water and plasma containing 25, 50, 100, 250, 500 and 1000 ng/ml of ranitidine were prepared from each stock solution. HPLC analysis of the water samples as well as the plasma standards after extraction, was performed immediately to determine recovery of the drug from plasma and intra-day variability of the assay. Standards were kept frozen at $-15°C$ until used for the determination of inter-day assay variability as well as drug stability in rat plasma. The metiamide I.S. solution (20 mg/ml) was freshly prepared in 50% aqueous 2-propanol, since it has been reported to be unstable in aqueous solution [9].

Extraction procedure

To 100 μl of each fortified blank plasma sample in a 75 × 12 mm glass tube was added 100 μl of 2 *M* sodium hydroxide, 0.5 ml of the I.S. solution and 2.0 ml of dichloromethane. The sample was extracted by vortex mixing for 30 s and then centrifuged for 5 min at 600 *g* to give well-separated phases. The upper organic phase was aspirated off, and the lower organic phase (*ca.* 1.7 ml) was transferred to a clean 75 × 12 mm glass tube for evaporation under nitrogen at room temperature. The dried residue was reconstituted in 100 μl of mobile phase by vortex mixing, and 80 μl was injected for HPLC analysis.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram, (A) without and (B) with triethylamine in the

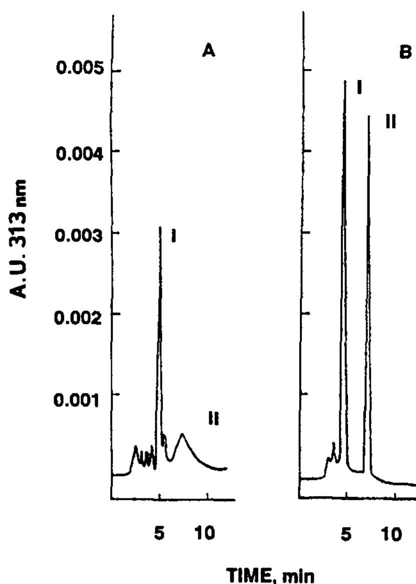

Fig. 1. Chromatography of rat plasma extracts from plasma containing 200 $\mu g/ml$ metiamide I.S. (I) and 500 ng/ml ranitidine (II) with a mobile phase (A) containing no triethylamine and (B) containing triethylamine.

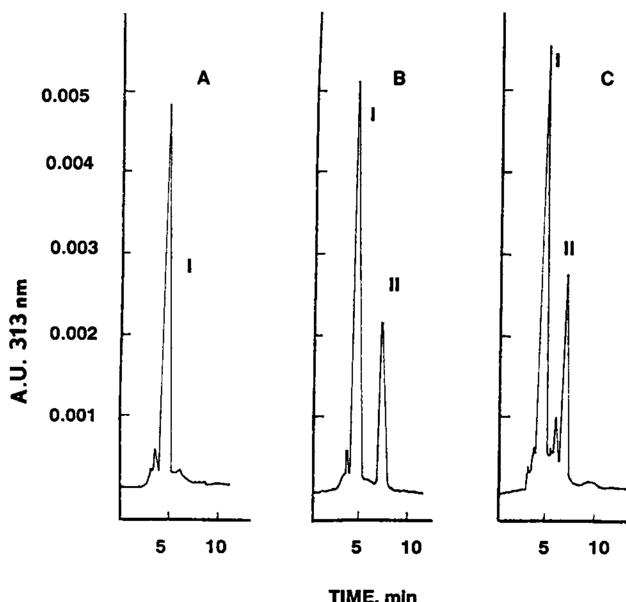

Fig 2. Typical chromatograms, representing extracts of: (A) blank rat plasma with 200 μg/ml I.S. (I), (B) plasma standard with 250 ng/ml ranitidine (II) and (C) plasma from a rat, 2 h after an oral dose of 10 mg/kg ranitidine.

mobile phase. Unique to our ranitidine assay method, with the triethylamine added, the ranitidine peak sharpness improved markedly and thereby increased the assay sensitivity by peak height measurements. Typical chromatograms are shown in Fig. 2, representative of a plasma blank, standard, and test sample.

Linearity of detector response for ranitidine in rat plasma standards was achieved over the concentration range 25 to 1000 ng/ml, as evidenced by the correlation coefficient ($r^2 > 0.999$). The assay precision, measured by the coefficient of variation (C.V.), and the assay accuracy, measured by the relative mean error (R.M.E.) were calculated from the with-in day sets of plasma standards, prepared from three separate ranitidine stock solutions (Table I). The precision of the method ranged from 1 to 10% and the accuracy from 1 to 5%. The average absolute recoveries of the drug and the I.S. were 97 and 87%, respectively. This was determined by comparison of the peak heights obtained from fortified plasma standards with those from directly injected standard solutions at each concentration level. The inter-day reproducibility of the assay was determined periodically over 52 days (Table I). The limit of detection (D) was found to be 10 ng/ml, as calculated from the formula $D = xb + fs$, where xb represents the mean determined values for a series of blank plasma samples, s is the standard deviation for these blank determinations, and f is a factor associated with the number of replicates [12].

Storage of the drug plasma standards at −15°C did not alter the amount of ranitidine recovered at days 4, 17, 30 and 52 *versus* day 0 as tested by two-way ANOVA calculation, $F_{0.05}$ (4,5) < 5.19.

Application of the method was demonstrated by measuring plasma levels of

TABLE I

PRECISION AND ACCURACY OF ASSAY FOR RANITIDINE IN RAT PLASMA

Ranitidine added (ng/ml)	Mean ranitidine determined (ng/ml)	Coefficient of variation (%)	Relative mean error (%)	Absolute recovery (%)
Intra-day (n = 3)				
25	24	9.6	−5.2	118
50	50	2.6	0.1	104
100	99	2.1	−0.6	98
250	256	0.5	2.4	96
500	491	1.0	−1.8	89
1000	1008	1.3	0.8	89
Inter-day (n = 5)				
25	25	13.2	−0.8	
50	52	7.2	3.8	
100	97	3.9	−2.7	
250	253	3.0	1.1	
500	495	2.3	−1.1	
1000	1005	0.9	0.5	

ranitidine in rats ($n = 8$) after oral administration of 10 mg/kg of drug (Fig. 3). In order to facilitate the analyses, a fully automated and computerized HPLC method was developed. The HPLC analysis time per sample was *ca.* 10 min. About 90 samples could be extracted and analyzed in a 24-h period. At least 1500 samples have been assayed per column with proper column care.

As previously stated, other reported methods for ranitidine quantitiation, were

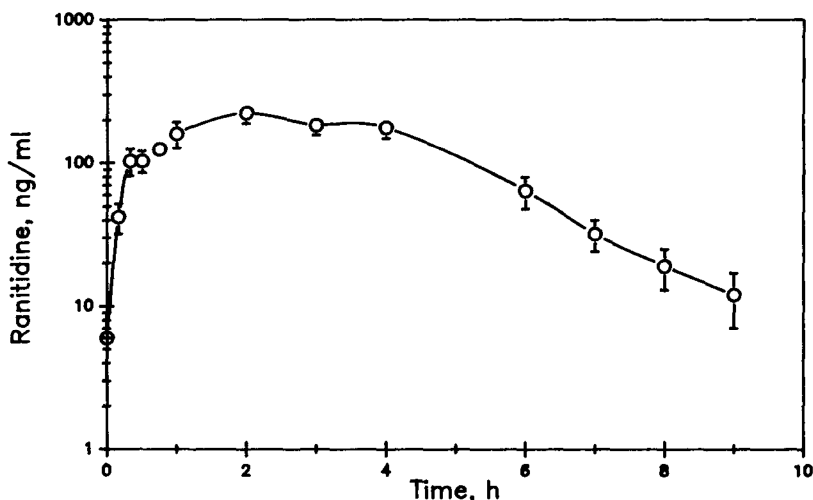

Fig. 3. Plasma concentrations of ranitidine following a 10 mg/kg oral administration of the drug to rats ($n = 8$).

not suitable for pharmacokinetic studies in rats, since relatively large amounts of blood would have to be taken at each of many serial blood samplings, and the total of which would compromise the animal. Although one reported method [7] used smaller amounts of sample, it was found to have two major disadvantages, namely, low sensitivity and a tedious column clean-up procedure. The facile and efficient extraction and HPLC procedure reported here permits the rapid, accurate and sensitive quantitation of ranitidine in 100 μl of rat plasma.

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