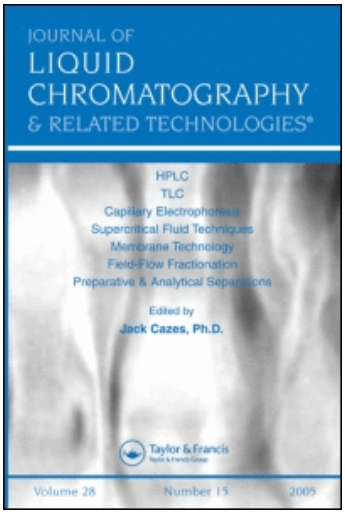

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RAPID REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY METHOD FOR RANITIDINE HYDROCHLORIDE IN DOSAGE FORMS

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ABSTRACT

A HPLC method with photometric detection has been developed for the rapid assay of ranitidine hydrochloride in dosage forms and samples from tablet dissolution testing. This method also separates ranitidine from its related compound ranitidine S-oxide. Analyses were carried out on a Microsorb-MV C18 column, with a (1:1) mixture of methanol-0.01 M Na_2HPO_4 (pH 7.0) as the mobile phase, and detection at 320 nm. At a flow rate of 1.0 mL/min, typical retention times for ranitidine and its S-oxide compound were 3.50 min and 1.95 min, respectively. Detector responses were linearly related to on column concentrations of ranitidine and ranitidine S-oxide in the ranges 0.035-9.000 μg and 0.005-0.320 μg, respectively. Recoveries of ranitidine from spiked synthetic formulations simulating tablets, injections and syrups ranged from 99.7 ± 0.5% to 100.5 ± 0.5% of the added amount (n = 2). For assay purposes, tablets were extracted into or liquid samples (injections, syrups) were quantitatively diluted with methanol-water (1:1), and the solutions were injected onto the column. Samples from tablet dissolution tests required no preliminary preparation. Assay values by the proposed method were found to agree closely with those obtained using methods in the USP XXII.

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INTRODUCTION

Among analytical techniques suggested for the determination of the quality and purity of the H_2-receptor antagonist ranitidine hydrochloride as a raw material, in commercial dosage forms and in extemporaneously-made parenteral solutions, HPLC is probably the one that has received the most extensive use (1-8). For example, the stability of ranitidine hydrochloride in total parenteral nutrition (TPN) solutions has been verified with a reversed phase (RP) HPLC method entailing a C_2 column, various ratios of acetonitrile-phosphate buffer (pH 6.8) as the eluent, and detection in the ultraviolet range (1). By switching to a C_{18} column and adding an ion-pairing reagent to the mobile phase, this method was subsequently modified to monitor the stability of ranitidine in TPN solutions (2) and in frozen and refrigerated solutions for injection (3). For both methods, however, neither full experimental details nor their applicability to the analysis of commercial dosage forms or of ranitidine related compounds have been described. A later, simpler approach has been based on the use of a C_{18} RP-HPLC column, a methanol-water mobile phase and detection at 221 nm. Although this method permitted a complete analysis of ranitidine in tablets and injections to be completed in less than 3 minutes, it required the column to be maintained at 50°C throughout the experiment to ensure resolution of the analyte from the recommended internal standard; in addition, the ability of this method to separate ranitidine

from any of its related compounds has not been documented (4). An alternative method, possessing both quantitative and stability-indicating capabilities, has relied on the use of an nonpolar column and photometric detection (5), but the utility of this approach in the analysis of dosage forms other than tablets and injections or of related compounds has not been investigated.

More recently, two HPLC methods have been described for the quantification of ranitidine and ranitidine related compounds in raw material samples (6,7) and tablets (7). One of these methods (7), requiring the use of a methanol-0.1 M aqueous ammonium acetate mobile phase, an ODS column, and detection at 322 nm, has been adopted by the USP XXII (8) for the assay of ranitidine and its tablets and injections. In addition, this method permits the separation of ranitidine from related compound C (ranitidine S-oxide), reported to occur both as a degradation product (6) and as a metabolite (9). For optimal resolution between the ranitidine peak and the ranitidine S-oxide peak, it is specified that the ratio of the mobile phase components as well as the flow rate be kept within certain limits. In our experience, this method is specific and accurate; but changing the brand of C_{18} column used may, in some cases, necessitate the composition ratio and flow rate of the original mobile phase to be significantly altered in order to maintain baseline resolution, reasonable elution times and minimal peak tailing. The purpose of this report is to describe a simple and specific isocratic RP-HPLC method for the determination of ranitidine

hydrochloride in pharmaceutical samples. This method separates ranitidine from its S-oxide compound in less than 5 minutes, shows good reproducibility from column to column, and is well suited for the assay of ranitidine in commercial liquid and solid dosage forms.

EXPERIMENTAL

Samples and Materials

Ranitidine hydrochloride and ranitidine S-oxide were a generous gift from the manufacturer (Glaxo Pharmaceuticals, Research Triangle Park, NC). They were dried to constant weight prior to use. Several lots of ranitidine hydrochloride tablets (150 and 300 mg), injections (25 mg/mL), and syrups (15 mg/mL) were obtained from local commercial sources. Solvents for chromatographic analysis were of HPLC grade (J.T. Baker); the methanol for dilutions, H_3PO_4, and Na_2HPO_4 were of analytical reagent grade (Mallinckrodt).

Sample Preparations

a. Ranitidine hydrochloride standard preparation - An accurately weighed quantity of ranitidine hydrochloride (about 75 mg) was dissolved in and quantitatively diluted with methanol-water (1:1), to obtain a preparation containing 75 μg/mL of ranitidine hydrochloride. The standard preparation for tablet dissolution testing assays was prepared in identical manner, except that the final concentration of ranitidine hydrochloride was 150 μg/mL.

b. Ranitidine hydrochloride and ranitidine S-oxide preparation - Accurately weighed quantities of ranitidine hydrochloride and ranitidine S-oxide were dissolved in methanol-water (1:1), to yield a preparation containing these compounds in concentrations of about 75 μg/mL and 2 μg/mL, respectively.

c. Tablet preparation - A group of 10 ranitidine hydrochloride tablets was weighed and ground to a fine powder. An accurately weighed quantity of powder, equivalent to about 150 mg of ranitidine hydrochloride, was transferred to a 100 mL volumetric flask, extracted with about 50 mL of methanol-water (1:1) with the aid of sonication for 10 min, and diluted to volume with the same solvent. The suspension was filtered, and a 5 mL portion of the filtrate was quantitatively diluted with methanol-water (1:1) to 100 mL.

d. Liquid dosage form (injection, syrup) preparation - An accurately measured volume of injection or syrup, equivalent to about 75 mg of ranitidine hydrochloride, was transferred to a 50 mL volumetric flask, diluted to volume with methanol-water (1:1), and mixed. A 5 mL portion of this solution was transferred to a 100 mL volumetric flask, diluted with methanol-water (1:1) to volume, and mixed.

e. Tablet dissolution testing preparation - Six tablets from each lot were tested according to the dissolution method for ranitidine hydrochloride tablets of USP XXII, i.e., apparatus 2, 900 mL of water as the dissolution medium, 37°C, and stirring at 50 rpm for 45 min. A

portion of the dissolution medium was filtered through a 0.45 μm membrane filter prior to HPLC analysis.

Synthetic Preparations

a. Synthetic tablet preparation - Prepared by sonicating ranitidine hydrochloride (75 or 150 mg), a (1:1:1) mixture of lactose-starch-magnesium stearate (250 mg) and 50 mL of methanol-water (1:1) in a 100 mL volumetric flask for 10 min. After dilution to volume with the same solvent, and mixing, the suspension was filtered. A portion of the filtrate was quantitatively diluted with methanol-water (1:1), to give a preparation containing a concentration of ranitidine hydrochloride similar to that of the standard preparation.

b. Synthetic injection preparation - Prepared in a volumetric flask with water, to contain 25 mg of ranitidine hydrochloride, 5 mg of phenol, 2.4 mg of Na_2HPO_4, and 0.96 mg of KH_2PO_4 in each mL. A portion of this solution was quantitatively diluted with methanol-water (1:1) to a final concentration of ranitidine hydrochloride similar to that of the standard preparation.

c. Synthetic syrup preparation - Prepared in a volumetric flask, by dissolving ranitidine hydrochloride in simple syrup to a concentration of 15 mg/mL. A portion of this solution was quantitatively diluted with methanol-water (1:1), to give a preparation containing a concentration of ranitidine hydrochloride similar to that of the standard preparation.

HPLC Method

a. Apparatus - Consisting of a Series 10 liquid chromatograph and a LC 90 UV spectrophotometric detector (Perkin-Elmer Corporation), connected either to a strip chart recorder (Knauer) or a ChromJet electronic integrator (Spectra-Physics). Samples were introduced through a sample injection valve fitted with a 20 μL sample loop (Rheodyne).

b. Chromatographic conditions - Separations were performed on a 15 cm x 4.6 mm i.d., Microsorb-MV C18, 5 μm, column (Rainin). Elutions were carried out with a (1:1) mixture of methanol and 0.01 M Na_2HPO_4 (previously adjusted to pH 7.0 with H_3PO_4), filtered and degassed prior to use. The flow rate was 1.0 mL/min, and the detection wavelength was 320 nm.

c. Calculations - The quantity of ranitidine hydrochloride in the sample preparation analyzed was calculated using one of the following equations:

$$\text{mg/tablet} = (R_{sp}/R_{st}) \times C \times 2 \times (A/W)$$

$$\text{mg/mL liquid dosage form} = (R_{sp}/R_{st}) \times (C/V)$$

$$\% \text{ dissolved} = (R_{sp}/R_{st}) \times S \times M \times (0.1/D)$$

where R_{sp} and R_{st} = the peak responses for the sample preparation and the standard preparation, respectively; C = the amount of ranitidine hydrochloride in the standard preparation, i.e., 75 μg/mL; A = the average tablet weight, mg; W = the weight of sample taken for the assay, mg; V = the volume of injection or syrup taken for the assay, mL; S = the amount of ranitidine in the standard preparation for the tablet dissolution test assay, i.e.,

150 μg/mL; M = the total volume of dissolution medium used, mL; and D = the amount of ranitidine hydrochloride declared per tablet, mg.

RESULTS AND DISCUSSION

The spectrophotometric detection of ranitidine and ranitidine S-oxide in HPLC analysis has been performed usually at 320-322 nm (1-3,5-8), but also at 220-228 nm (4,9). Although the present study verified peak responses at 320 nm not to be significantly different from those obtained at 228 nm, the former wavelength was preferred over the latter to ensure greater selectivity in the presence of potentially interfering peaks from excipients or additives in the dosage form. Moreover, elutions could also be monitored at 254 nm but with a 2-fold (ranitidine S-oxide) to 2.7-fold (ranitidine) decrease in sensitivity.

The effects of certain mobile phase-related factors such as pH of the buffer component, the ratio of the organic component to the buffer component, and the flow rate on resolution, R, and peak tailing, T, were systematically evaluated to achieve optimum resolution between ranitidine and its S-oxide compound. Ranitidine S-oxide is a degradation product of ranitidine which has also been used as part of the system suitability test for the HPLC analysis of ranitidine in pharmaceutical samples (7,8). As shown in Figure 1, by maintaining the ratio of methanol to phosphate buffer in the mobile phase constant at (1:1) and increasing the pH of the phosphate buffer in a step-

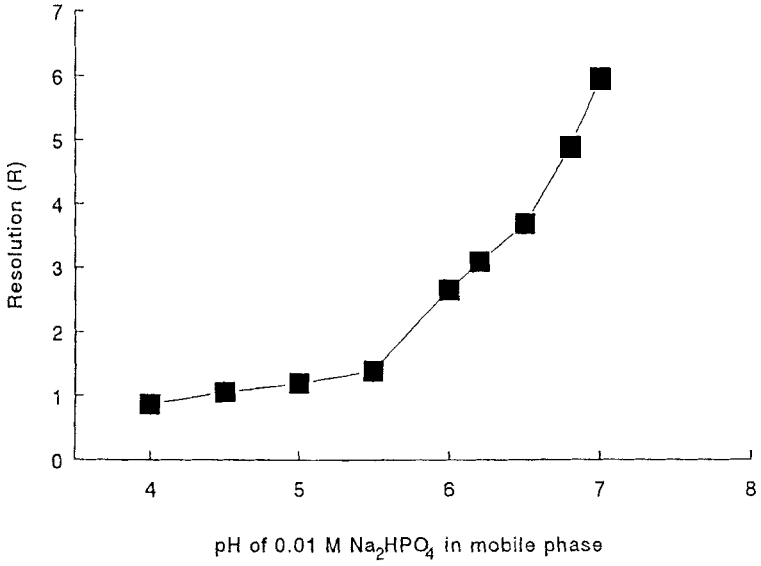

Figure 1. Effect of the pH of the 0.01 M Na_2HPO_4 solution on resolution, R, between ranitidine and ranitidine S-oxide peaks. All experiments were conducted using a (1:1) methanol-0.01 M Na_2HPO_4 mixture flowing at 1.0 mL/min.

wise manner, R increased with increasing pH of the phosphate component, to become ≥ 3.0 at pH ≥ 6.0 and maximal at pH 7.0. The effect of the concentration of methanol on R was studied using ratios of methanol to phosphate buffer (pH 7.0) in the mobile phase between (40:60) and (65:35). In this manner, separations with R > 2.5 and requiring less than 5 min were obtainable with a mobile phase composed of methanol-0.01 M Na_2HPO_4 (pH 7.0) in a (1:1 to 3:2) ratio (Figure 2) and flowing at the rate of 1.0-2.0 mL/min. The use of lower ratios resulted in elutions that were much slower and in some peak tailing. Assays were

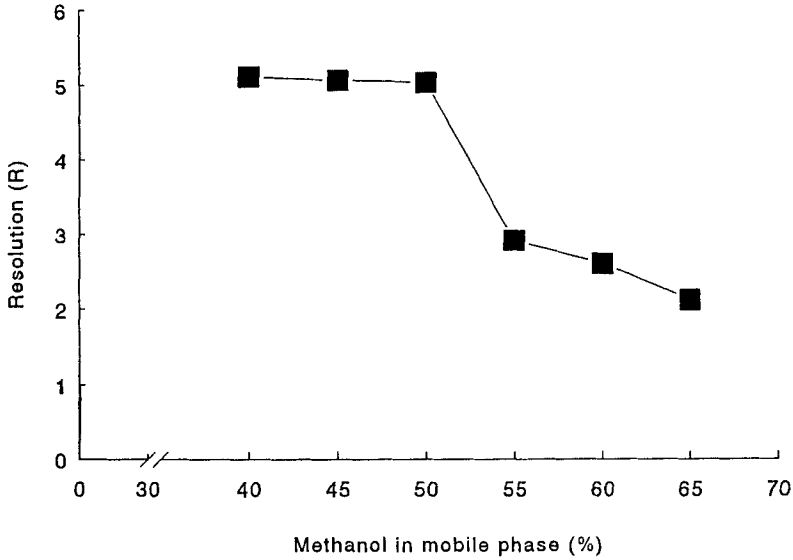

Figure 2. Effect of the percentage of methanol in the mobile phase on resolution, R, between ranitidine and ranitidine S-oxide peaks. All experiments were conducted at a flow rate of 1.0 mL/min.

routinely performed using a methanol to 0.01 M phosphate buffer (pH 7.0) ratio of (1:1) and a flow rate of 1.0 mL/min. These conditions were found to produce equivalent results when the analysis was repeated on either a μBondapak C_{18} (Waters) or an Econosphere C_{18} (Alltech) column. Typical chromatograms, obtained using the recommended conditions, are shown in Figure 3.

To verify the linearity of the proposed method, peak responses (heights or areas) at 320 nm were assessed using serial dilutions of stock solutions of ranitidine hydrochloride (450 μg/mL) and ranitidine S-oxide (16 μg/ mL) in methanol-water (1:1). Peak responses were found to be

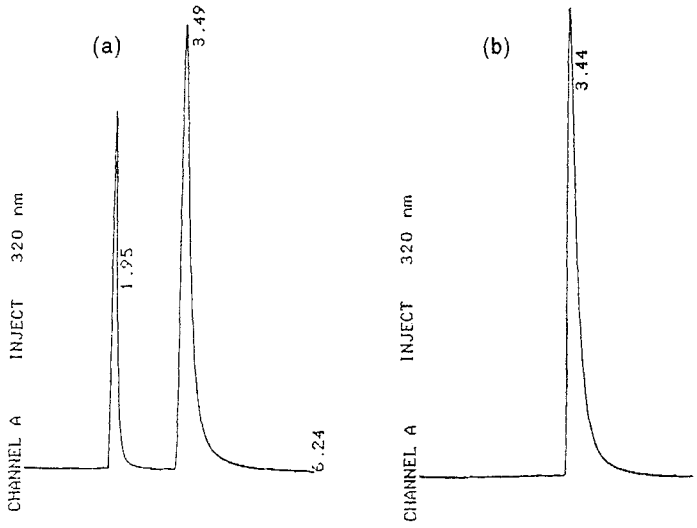

Figure 3. Typical high-performance liquid chromatograms of (a) a standard preparation of ranitidine plus ranitidine S-oxide, and (b) a tablet sample. Flow rate was 1.0 mL/min; for comparative purposes, a chromatogram recorded at 1.5 mL/min is shown in the inset. Ranitidine is the slower eluting peak.

linearly related to on column concentrations of ranitidine and ranitidine S-oxide between 0.035-9.000 μg (n = 2, r = 0.999) and 0.005-0.32 μg (n = 2, r = 0.999), respectively, with the curves passing through the origin. The precision of the proposed method was evaluated by measuring peak heights and peak areas following multiple injections of a standard preparation containing about 56 μg/mL of ranitidine and 1 μg/mL of ranitidine S-oxide. Based on peak heights from 6 consecutive injections, the RSD values for ranitidine and its S-oxide were 0.92% and 0.02%, respectively; the RSD values for the corresponding areas

were 1.40% and 1.26%, respectively. Accuracy was evaluated by spiking synthetic formulations, prepared to simulate tablets, injections and syrups, with known amounts of ranitidine and subjecting the samples to the assay procedure described for commercial dosage forms. As shown in Table 1, mean recoveries (n = 2) of ranitidine hydrochloride from the various synthetic formulations were, in all cases, not less than 99.0% of the added amount.

The application of the proposed method to the assay of commercial dosage forms yielded the results summarized in Table 2. These results, representing the mean values of duplicate determinations, were in turn compared with those obtained by the HPLC method of USP XXII (8). Since the syrup sample is not listed in the official compendium, this dosage form was analyzed in the same manner as the injections. Intermethod differences for 150 and 300 mg tablets ranged from 0.7-2.6% of declared; for injections and syrups, the differences were about 1.45% and 1.35% of declared, respectively. All samples complied with the official requirements for drug potency, and were found to be free of contamination with the S-oxide or any other reported related compound (6,8). Furthermore, the proposed method was also useful to measure the amount of ranitidine released during the course of the USP XXII tablet dissolution test. The assay results for two sets of 150 mg tablets and one set of 300 mg tablets are shown in Table 3. A comparison of these results with those derived using the official spectrophotometric method indicated the existence of a close agreement, with the intermethod differences

Table 1

Results of recovery studies of ranitidine hydrochloride from synthetic dosage forms by the proposed HPLC method

Synthetic formulation	Ranitidine hydrochloride found, % of added			
	Run 1	Run 2	Mean	SD
Tablet, 150 mg	99.0	100.0	99.5	± 0.5
Tablet, 300 mg	100.5	99.5	100.0	± 0.5
Injection, 25 mg/mL	99.0	98.9	98.9	± 0.2
Syrup, 15 mg/mL	101.0	100.0	100.5	± 0.5

ranging from 0.2-1.7% of dissolved in 45 min. All tablets conformed to the USP XXII dissolution requirements. Tablet excipients or additives did not interfere with the proposed assay method.

In conclusion, the HPLC method presented here will permit the analysis of ranitidine hydrochloride in commercial solid and liquid dosage forms and in tablet dissolution samples in a rapid, specific and quantitative manner, and by using uniform experimental conditions. In comparison with compendial HPLC methods for tablets, injections and tablet dissolution samples of the title drug, in the proposed method all samples for analysis were prepared in methanol-water rather than in the mobile phase, the new chromatographic conditions met all of the official

Table 2

Determination of ranitidine hydrochloride in commercial dosage forms by proposed HPLC method and USP XXII HPLC method[a,b]

Lot No.	Ranitidine hydrochloride. % of declared					
	Proposed method			USP XXII method		
	Run 1	Run 2	Mean	Run 1	Run 2	Mean
	Tablets, 150 mg/tablet					
1	99.5	101.3	101.3	99.3	98.7	99.0
2	103.0	103.7	103.7	101.0	100.4	100.7
	Tablets, 300 mg/tab					
1	102.9	101.8	102.3	102.3	100.5	101.4
2	98.9	99.9	99.4	100.1	100.1	100.1
	Injections, 25 mg/mL					
1	108.6	108.6	108.6	107.6	106.8	107.2
2	106.0	106.0	106.0	105.0	104.0	104.5
	Syrups, 15 mg/mL					
1	100.0	102.4	101.2	101.7	102.5	102.6
2	103.2	102.6	102.9	102.5	100.7	101.6

[a]USP XXII ranges for tablets and injections = 90.0 to 110.0%
[b]The syrup is not listed in USP XXII.

Table 3

Assay results (% found) for tablet dissolution test samples by proposed HPLC method and USP XXII spectrophotometric method[a]

Tablet No.	150 mg (Lot 1)		150 mg (Lot 2)		300 mg	
	HPLC	USP	HPLC	USP	HPLC	USP
1	99.5	99.8	94.4	97.6	93.4	93.1
2	90.5	91.9	90.0	93.0	101.7	99.0
3	92.8	92.6	98.9	99.7	104.2	102.6
4	100.4	101.8	92.8	94.9	96.1	95.5
5	98.0	94.8	94.7	94.7	99.5	99.7
6	97.8	96.5	95.7	94.0	99.5	98.6
Mean	96.4	96.2	93.9	95.6	99.1	98.1
SD	3.91	3.96	3.41	2.50	3.86	3.33

[a]USP XXII tolerances: not less than 80% of the labeled amount of ranitidine is dissolved in 45 min.

system suitability requirements for the HPLC assay of ranitidine, and peak elution times at similar flow rates were shorter. Moreover, the proposed method yielded assay results that agreed well with those obtained using methods in the USP XXII.

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