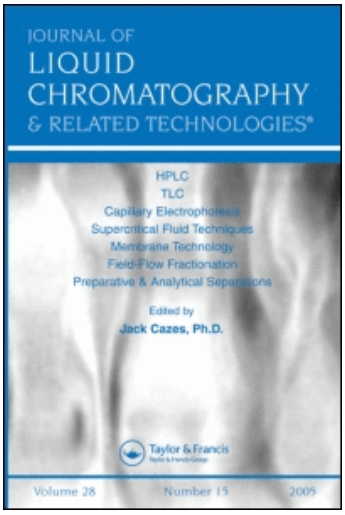

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A Sensitive Stability Indicating Assay for the H_2 Blocker Ranitidine

G. L. Hoyer[a]; J. Ledoux[a]; P. E. Nolan JR.[a]
[a] Department of Pharmacy Practice, College of Pharmacy and University Heart Center University of Arizona Tucson, Arizona

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A SENSITIVE STABILITY INDICATING ASSAY FOR THE H_2 BLOCKER RANITIDINE

GIFFORD L. HOYER, JANE LEDOUX, AND PAUL E. NOLAN, JR.*
Department of Pharmacy Practice
College of Pharmacy
and
University Heart Center
University of Arizona
Tucson, Arizona 85721

ABSTRACT

A stability indicating high performance liquid chromatography assay for ranitidine has been developed for the study of ranitidine in intravenous solutions. The assay is based on an isocratic mobile phase, a C_{18} reverse phase column, UV detection and utilizes an internal standard. The assay is shown to be accurate and precise over a range of ranitidine concentration commonly found in IV solutions. The mean inter- and intra-assay variability is low and retention times are stable. The assay separates ranitidine form ranitidine decomposition products produced at acidic and basic pH's.

INTRODUCTION

Ranitidine is a selective histamine H_2-receptor antagonist used in the treatment of peptic ulcer [1], reflux esophagitis [2] and dyspepsia [3]. Ranitidine is also prescribed in the

prophylaxis and treatment of stress ulcer [4]. For this latter clinical indication, ranitidine is administered intravenously (IV) often in combination with other drugs. Therefore, it is necessary to establish that ranitidine is stable under the conditions encountered during the coadmixing and administration of IV solutions.

High performance liquid chromatography (HPLC) is a commonly utilized assay methodology in studying the stability of ranitidine in IV solutions. Das Gupta and coworkers has published a study of the stability of ranitidine in IV admixtures [5]. The HPLC assay utilized in this study was purported to be stability-indicating although the documentation for this assertion was not adequately presented. Additional studies of the stability of ranitidine hydrochloride in intravenous infusion fluids and parenteral mixtures have been performed [6,7]. Both of these reports cited Evans et. al. [8] and the United States Pharmacopeia [9] as the basis for the HPLC assays utilized in the studies. The Pharmacopeia method cited is a TLC method which is stability indicating. Evans et. al. [8] developed an HPLC assay "for the assay of ranitidine content (i.e. strength).". Although aspects of the Evans assay resemble stability-indicating assays, the method does not establish stability-indicating capability as outlined by Trissel et.al. [10]. The ranitidine was not subjected to stresses that would potentially degrade ranitidine, therefore, the assay did not have the opportunity to separate ranitidine from potentially unknown degradation products. The only statement by Evans et. al. regarding the stability of ranitidine as related to this assay was that ranitidine was stable in the mobile phase as based on peak height ratio. In addition, Evans et. al. chose a wavelength of detection that reduced interference from related compounds but did

not provide maximum sensitivity. Therefore, several previous [6,7] studies of ranitidine stability used assays that may not have been stability-indicating.

The purpose of this study is to establish and document a stability-indicating assay for ranitidine. The assay is able to detect and separate ranitidine and ranitidine degradation products that are formed when ranitidine is subjected to stresses such as extremes in temperature and pH. The method developed is isocratic, utilizes low concentrations of organic solvents, and does not require either ion-pairing reagents or organic modifiers.

MATERIALS AND METHODS

Injectable ranitidine hydrochloride solution, analytical grade ranitidine HCl powder, and analytical grade ranitidine-S-oxide powders were supplied by Glaxo Pharmaceuticals, Inc., Research Triangle Park, NC. The internal standard was analytical grade caffeine (Sigma Chemical Co., Gaithersburg, MD). Analytical grade potassium phosphate monobasic (KH_2PO_4) (Mallinckrodt, Paris, KY) was used to for the mobile phase the pH was adjusted using phosphoric acid or sodium hydroxide. HPLC grade acetonitrile (EM Science, Gibbstown, NJ) was used as the organic component of the mobile phase. The water used in the buffer and mobile phase was glass distilled, treated by ion exchange, charcoal filtered and subsequently filtered through a 0.45 μg pore nylon filter.

Instrumentation and Chromatographic Conditions:

The HPLC system utilized for this assay consisted of a Beckman Ultrasphere-ODS C_{18} column 0.46 cm x 25 cm (Beckman Instruments Inc., San Ramon, CA), a Spectra-Physics Isochem LC pump, a Spectra-

Focus detector, a SP8880 Autosampler, and a ChromJet integrator (Spectra-Physics, San Jose, CA).

The chromatographic separation utilized in this assay was based on a mobile phase consisting of 0.01M monobasic potassium phosphate (KH_2PO_4) adjusted to pH 5.0 (using phosphoric acid and/or sodium hydroxide), and acetonitrile (92:8 v/v). The mobile phase was delivered at a constant flow rate of 2 mL/min. The column and mobile phase were maintained at ambient temperature. The deuterium lamp was lit and mobile phase was pumped through the column for 30 minutes prior to analytical assays to allow for equilibration of the lamp and column. In addition, a conditioning injection containing 50 μg each of ranitidine, ranitidine-S-oxide and caffeine was applied to the column prior to analytical assays. The detector was set to measure U.V. absorbance at 262 nm.

Preparation of Standard Solutions and Samples:

Standards for the standard curve were made from a stock solution of 5 mg/mL of ranitidine hydrochloride. The standard concentrations were 50, 100, 250, 500, 1200 μg/mL. A stock solution of caffeine 1 (mg/mL) was diluted with water to make a working standard of 200 μg/mL. One hundred microliters of the standards or unknown solutions were mixed with 50 μL of the internal standard caffeine and 850 μL of water and pipetted into duplicate, labelled conical tubes. The tubes were vortexed briefly and 50 μL was injected onto the HPLC column for analysis. All standards and unknowns were analyzed in duplicate.

A standard curve consisting of 5 standard concentrations ranging from 0.05 to 1.2 μg/mL was performed during each analytical assay. In addition to the standards, duplicate blank solutions, consisting of 950 μL H_2O and 50 μL internal standard, and spiked

samples were also analyzed, as zero and accuracy controls respectively, during every analytical investigation. The spiked samples were prepared by a third party to concentrations unknown to the HPLC operator. Solutions for the study of ranitidine stability were treated in the manner previously outlined for the standards. All standards, controls, and unknowns were analyzed in duplicate. The concentrations of spiked solutions and unknown solutions were determined by back calculating from the best fit line as established by linear regression based on x = standard concentrations and y = ranitidine/internal standard peak area ratios.

Assay Validation:

During each analysis the standard curve, blanks, and spiked samples with concentrations of 200 and 750 μg/mL were run. Intra-assay variation was estimated by calculating the mean and standard deviation of the concentration of 8 samples of each spiked standard assayed on the same day. Inter-assay variation was estimated from the concentration of spiked samples determined in 5 consecutive chromatographic assays. The results of intra- and inter-assay variation are listed in Table 3.

The accuracy and precision of the assay was determined by comparing five consecutive chromatographic assays. A comparison of calculated concentrations versus actual concentration of the standards spanning the range of the standard curve 50 through 1000 μg/mL was made to determine accuracy. The precision of the assay is demonstrated by the inter-assay coefficient of variation of the calculated concentrations of the standards.

To demonstrate that the assay was stability-indicating it was necessary to subject ranitidine to extreme conditions to cause

TABLE 1
Stability Indicating Nature of Ranitidine Assay

Treatment	Mean Peak Area	%Δ in Peak Area
Original Solution	0.74060	
Refrigerated 7 days	0.73536	- 0.7%
Freezing 7 days	0.74334	+ 0.4%
Room Temperature 24 hr	0.75041	+ 1.3%
Room Temperature 48 hr	0.74041	0.0%
Heating 75°C 24 hr	0.72822	- 1.7%
Heating 75°C 48 hr	0.68062	- 8.1%
1 M NaOH 24 h	< detection limit	> - 95%
1 M NaOH 48 hr	< detection limit	> - 95%
1 M HCl 24 hr	0.40304	- 43.6%
1 M HCl 48 hr	0.32721	- 55.1%

degradation of ranitidine. The assay must then separate the parent compound from the degradation products. The conditions consisted of subjecting ranitidine (50 μg/mL) to refrigeration, below freezing temperatures (-15°C), heat (75°C), 1M HCL (pH 2), 1M NaOH (pH 11) and room temperature with fluorescent light exposure. Table 1 lists the average temperature recorded in room air, the heating block and the freezer. A decrease in the peak area ratio of 10% was considered a significant decrease in the parent compound ranitidine (see Table 2).

RESULTS

The temperatures of the heating block, refrigerator and room temperature ranitidine were monitored hourly for ten hours per day for three days. The average room temperature was 22°C ± 1.0°C, the

TABLE 2
Intra-assay and Interassay Variability of Ranitidine Assay

Intra-assay Variation (n=8)

Spiked Concentration (μg/mL)	Mean Calculated Concentration (μg/mL)	Standard Deviation	% Coefficient of Variation
200	198	3.46	1.7%
750	758	7.31	0.97%

Interassay Variation (n=5)

Spiked Concentration (μg/mL)	Mean Calculated Concentration (μg/mL)	Standard Deviation	% Coefficient of Variation
200	194	8.89	4.6%
750	730	50.3	6.9%

TABLE 3
Precision and Accuracy of Ranitidine Assay

Actual Concentration (μg/mL)	Mean Calculated Concentration (μg/mL)	% Coefficient of Variation	Δ of Means Actual versus Calculated
50	50.9	0.0699	1.72%
100	98.3	0.0359	1.70%
250	249.7	0.0099	0.12%
500	501.9	0.0325	0.38%
1000	999.3	0.0071	0.07%

average temperature for the heating block was 75°C ± 0.5°C and the average freezer temperature was -15°C ± 0.5°C. Table 1 shows the ratio of the peak areas of ranitidine divided by the internal standard caffeine. Ranitidine proved to be very stable at room temperature, in the refrigerator and in the freezer. Heating appears to have decreased the ranitidine concentration, but it was less than a 10% change and is not considered significant. The only significant decreases in ranitidine were noted after exposure to 1M NaOH (pH 11) and 1M HCL (pH 2). After exposure to either acid or base, the concentration of ranitidine fell to less than 10% of the original concentration of ranitidine. The chromatograph of ranitidine exposed to NaOH showed the break-down products

The inter- and intra-assay variability of the assay are listed in Table 2. The intra-assay variability was calculated from concentrations of 200 and 750 ug/mL spiked samples of ranitidine using the concentrations determined for 8 samples that were analyzed during the same analysis. The mean calculated concentration was 198 μg/mL for the 200 μg/mL spiked sample and 758 μg/mL for the 750 μg/mL spiked sample. The percent coefficient of variation was 1.7% and 0.97% for the 200 and 750 μg/mL spiked sample respectively.caused by exposure to strong base (see Figure 1).

The interassay variability was calculated from the concentration of ranitidine determined during 5 assays run on consecutive days. The mean calculated concentration of 200 μg/mL and 750 μg/mL spiked samples was 194 μg/mL and 730 μg/mL, respectively. The percent coefficient of variation was 4.6% and 6.9% for the 200 μg/mL and 750 μg/mL, respectively.

The accuracy and precision of the assay are shown in Table 3. The accuracy of the assay was the greatest at the 1000 μg/mL

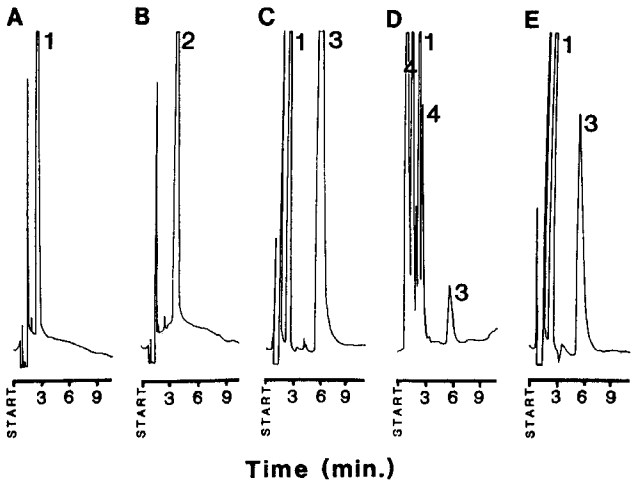

Figure 1: Chromatograms of standards and treated ranitidine solutions. The chromatograms presented represent chromatography of solutions containing; A) caffeine, B) ranitidine-S-oxide, C) internal standard and ranitidine, D) ranitidine in 2 M NaOH for 72 hrs., E) ranitidine stored frozen for 72 hrs. The numbered peaks represent; 1) caffeine, 2) ranitidine-S-oxide, 3) ranitidine, 4) unidentified ranitidine breakdown products.

concentration and least accurate at the 50 μg/mL concentration. However, the percent difference in means (actual versus calculated) concentration never exceeded 1.72%. The precision of the assay was estimated with the coefficient of variation. The greatest precision was with the 1000 μg/mL standard and the least with 50 μg/mL. This is expected when using a non-weighed linear curve.

DISCUSSION

This assay is stability-indicating by the ability to separate of the parent compound, ranitidine, from its breakdown products

produced by exposure to 1M NaOH, 1M HCL and elevated temperature. The assay shows excellent interassay variability with a percent coefficient of variability less than 2%. The intra-assay variability had a percent coefficient of variability of less than 7%. The accuracy and precision of the assay has a percent change in mean of less than 2%.

In Figure 1 ranitidine, ranitidine-S-oxide and caffeine have retention times of 5.67, 3.67 and 2.32 minutes, respectively. The peaks have good symmetry and demonstrate little tailing. Also shown in Figure 1 the degradation products caused by 1M NaOH (pH 11) and 1M HCL (pH 2) all have retention times less than 3 minutes. This assay results in separation of ranitidine from all degradation products created during this study.

Our lowest level of detection was achieved utilizing the 50 μg/mL standard. 100 μL of the standard was diluted to 10% and a 50 μL sample of the dilution was injected onto the column. The amount of ranitidine injected was 250 ng. This assay was designed to study the stability and compatability of ranitidine in IV solutions. The concentration of these solutions generally range from 0.1 to 1.0 mg/mL. Therefore, the range of the standard curve was established between 0.05 and 1.2 mg/mL. During analytical evaluations utilizing this standard curve, it was necessary to use a relatively high attenuation setting on the detector and integrator. These settings could be lowered to achieve a limit of detection of 5 ng injected on column. Based on the previous data we feel that this assay is very suitable for stability studies of ranitidine and may be suitable for quantitating patient blood concentrations of the drug.

This assay has demonstrated the accuracy, precision, and low variability desired in an HPLC assay utilized for the quantitation of unknown samples. In addition, the assay has shown the ability

to separate ranitidine degradation products from ranitidine. These findings support the use of this assay for stability studies of ranitidine. The simplicity of the chromatographic conditions, isocratic solvent delivery, low concentration of organic solvent needed in the mobile phase, and dearth of organic modifiers or ion pairing agents also make this an appealing assay for conducting stability studies involving ranitidine.

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