ELSEVIER

Journal of Chromatography B, 751 (2001) 291–296

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Rapid determination of ranitidine in human plasma by high-performance liquid chromatography without solvent extraction

Abolhassan Ahmadiani*, Hossein Amini

Department of Pharmacology, Faculty of Medicine, Shaheed Beheshti, University of Medical Sciences, Tehran, I.R. Iran

Received 24 August 1999; received in revised form 21 July 2000; accepted 28 August 2000

Abstract

A simple high-performance liquid chromatographic procedure was developed for the determination of ranitidine in human plasma. The method entailed direct injection of the plasma samples after deproteination using perchloric acid. The chromatographic separation was accomplished with an isocratic elution using mobile phase consisting of 21 m*M* disodium hydrogen phosphate–triethylamine-acetonitrile (1000:60:150, v/v), pH 3.5. Analyses were run at a flow-rate of 1.3 ml/min using a μbondapak C_{18} column and ultraviolet detection at a wavelength of 320 nm. The method was specific and sensitive, with a quantification limit of approximately 20 ng/ml and a detection limit of 5 ng/ml at a signal-to-noise ratio of 3:1. The mean absolute recovery was about 96%, while the within- and between-day coefficient of variation and percent error values of the assay method were all less than 8%. The linearity was assessed in the range of 20–1000 ng/ml plasma, with a correlation coefficient of greater than 0.999. This method has been used to analyze several hundred human plasma samples for bioavailability studies.

Keywords: Ranitidine

1. Introduction

Ranitidine, *N*,*N*-dimethyl-5-[2-(1-methylamino-2-nitrovinylamino)ethylthiomethyl]furfurylamine, is a histamine H_2 receptor antagonist, which inhibits completely and reversibly the interaction of histamine with H_2 receptors [1]. Many high-performance liquid chromatographic (HPLC) techniques have been reported for the determination of ranitidine in plasma or serum [2–9]. All of these methods involve time-consuming sample preparation, which complicates routine analysis. Most of the reported methods require liquid–liquid extraction with organic solvents [2–6] or solid-phase extraction [7–9], which are not economically feasible for routine use in pharmacokinetic studies where numerous samples should be analyzed. This paper describes a one-step sample preparation using perchloric acid that simplifies the analysis of ranitidine in plasma. Consistent recovery of ranitidine and lack of volume transfers eliminate the need for an internal standard. Although this method is really simple, its

*Corresponding author. Fax: +98-21-240-3154.
E-mail address: aaz@farabi.hbi.dmr.or.ir (A. Ahmadiani).

0378-4347/01/$ – see front matter
PII: S0378-4347(00)00487-4

sensitivity seems to be sufficient for pharmacokinetic studies.

2. Experimental

2.1. Chemicals and reagents

Ranitidine–HCl was supplied by Shasun chemicals and drugs company (99.88%, Cuddalore-607 005, India). HPLC-grade methanol and acetonitrile, and analytical-grade triethylamine (TEA), disodium hydrogen phosphate, sodium hydroxide, concentrated phosphoric acid and perchloric acid (PCA), were obtained from Merck (Darmstadt, Germany).

2.2. Instrumentation

The apparatus used for this work was an LC-6A solvent delivery pump equipped with an SPD-6A UV–Vis detector and a C-R6A integrator (all from Shimadzu, Kyoto, Japan). The detector was set to 320 nm. The samples were applied by a Rheodyne 7125 loop injector with an effective volume of 50 μl. A μbondapak C_{18} column (250×4.6 mm I.D.; 10 μm), fitted with a refillable guard column, packed with μbondapak C_{18} (6×5 mm I.D.; 10 μm) (both from Waters, Milford, MA, USA), was used for the chromatographic separation. The mobile phase comprised 21 m*M* disodium hydrogen phosphate–triethylamine–acetonitrile (1000:60:150, v/v), adjusted to pH 3.5 with concentrated phosphoric acid.

Analyses were run at a flow-rate of 1.3 ml/min at room temperature and the samples were quantified using peak height.

2.3. Assay standards

Standard solutions of ranitidine–HCl were prepared by dissolving the required amount of ranitidine–HCl in methanol to get 0.1 mg/ml of ranitidine base and the solution was stored at −20°C.

2.4. Sample preparation

To a 250 μl volume of plasma, 20 μl of 60% PCA was added, vortex-mixed for 30 s and was centrifuged for 5 min at 11 300 *g*. The supernatant was transferred to another microcentrifuge tube, and a 50 μl volume was injected into the chromatograph.

2.5. Assay validation

Standard calibration curves were constructed by spiking drug-free plasma with a known amount of ranitidine in the concentration range of 20–1000 ng/ml. The plasma standards were also used to determine the within- and between-day precision and accuracy ($n=6$) of the method. In addition, the absolute recovery ($n=6$) was estimated by comparison with direct injection of aqueous drug solutions of corresponding concentrations.

2.6. Application

The assay was used for a comparative bioavailability study of two tablet preparations containing 150 mg ranitidine–HCl (Zantac, Glaxo, UK) and a generic ranitidine preparation (Lorestan Pharmaceutical Co., Lorestan, Iran).

Fourteen healthy volunteers participated in the study. The study was conducted using a two-way crossover design, as a single dose, randomized, trial. The two formulations were administered on two treatment days, separated by a washout period of 7 days, to fasted subjects who received a single oral dose of 150 mg of one study medication of ranitidine. Food and drink were not allowed until 3 h after ingestion of the tablet. Multiple blood samples (3 ml) were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h post dosing. The plasma was immediately separated by centrifugation and stored frozen at −20°C until analysis.

3. Results and discussion

Plasma deproteination for the determination of drugs is commonly accepted as the simplest method of sample preparation. These methods usually have high reproducibility and do not require the use of an internal standard. Previously developed HPLC procedures for the determination of ranitidine in plasma are based on liquid–liquid extraction or solid-phase extraction of ranitidine from plasma samples [2–9].

The method applied in our study involved the direct injection of the plasma samples after precipitation with PCA.

Although HPLC is now considered to be a mature separation method, there are still a number of areas in which problems are encountered, and where further research is desirable. One of the most important of these is in the analysis of basic compounds, which can interact undesirably with the column surface of silica-based reversed-phase materials, giving rise to tailing peaks, poor quantification, irreproducible retention times and even complete retention of some solutes [10]. Small basic molecules may be adsorbed to the stationary phases used in reversed-phase liquid chromatography due to their hydrogen-binding and/or ion-exchange interaction with uncapped silanols in the support [10,11]. Additionally, interactions with metallic impurities may occur, or, alternatively, silanophilic interactions may be enhanced by the presence of these impurities [11]. The extent of these interactions is variable depending upon the source and quality of the packing material [10,11].

Review of the literature indicated that ranitidine, as a basic drug, may show interactions with uncapped silanols in the support. However, the extent of the interaction is variable, depending on the reported method. Salem et al. [4] reported that using an ion-pairing reagent and a mobile-phase with a low pH improved the shape and symmetry of the ranitidine peak. The low pH [3,4,9] or the high pH value [6] of the mobile phase used in some assays, or the use of TEA [7] or organic sulfonates [4,5], may be due to the problem of ranitidine interaction with uncapped silanol.

Moreover, the sample preparation method may also enhance the occurrence of such interactions. Yuen and Peh [12] reported that when the amount of PCA used for deproteinizing 250 μl of plasma was increased from 10 to 30 μl, the metformin–HCl (another basic drug) peak became skewed, non-symmetrical and broad. Because we used PCA for deproteinizing plasma, the interaction of ranitidine with the column support may be enhanced and it may explain why the addition of a silanol-blocking agent to the mobile phase was necessary in our method. TEA was chosen as the base additive for silanol-blocking since triamines exhibit the lowest attack on the silica matrix [13]. Without the addition of TEA, ranitidine from plasma samples, when treated with PCA, did not give a sharp peak and produced either no bands or broad peaks. However, with the addition of TEA, ranitidine gave sharp and symmetrical peak that were well separated from endogenous compounds.

Chromatograms obtained with blank plasma, plasma spiked with ranitidine and a sample from a volunteer after dosing with ranitidine are shown in Fig. 1. The ranitidine peak, which had a retention time of 6.7 min, was well resolved and free of

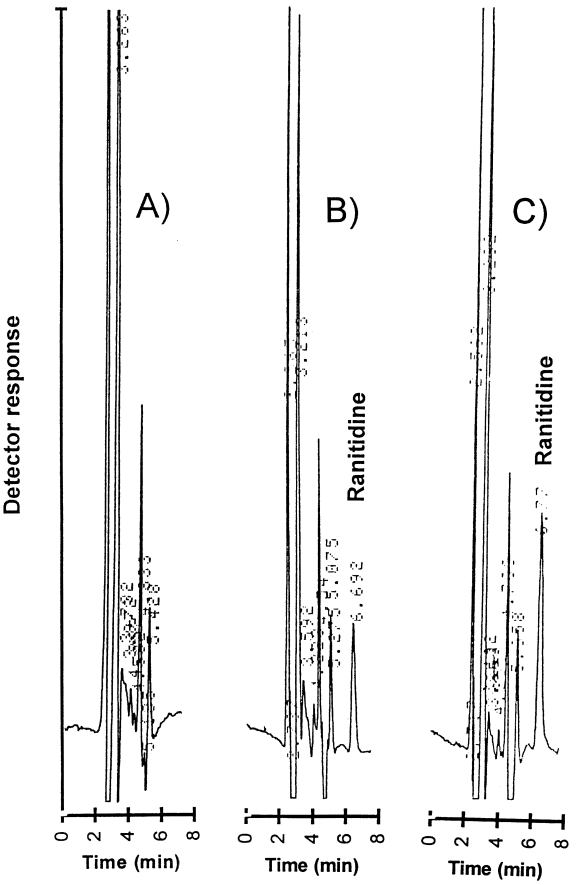

Fig. 1. Chromatograms of ranitidine in plasma. (A) Blank plasma, (B) plasma spiked with 100 ng/ml of ranitidine, (C) a plasma sample from a volunteer, containing 206.8 ng/ml, 1 h after taking a 150 mg tablet of ranitidine. The peak at 6.7 min=ranitidine. (Attenuation=0; chart speed=3 mm/min).

interference from endogenous compounds in the plasma. The absolute recovery, within-day and between-day accuracy and precision values are presented in Table 1. The average absolute recovery value was approximately 96%. The coefficient of variation (C.V.) of within- and between-day precision and accuracy, with percent error values, were found to be less than 8%. The standard calibration curves ($n=6$) were found to be linear over the concentration range of 20–1000 ng/ml, with correlation coefficients of greater than 0.9992. The limit of detection of ranitidine was 5 ng/ml while the limit of quantification was 20 ng/ml, which was comparable to the results (15 ng/ml) obtained by Wong et al. [2]. The limit of the method described here can also be reduced to 10 ng/ml by increasing the injection volume from 50 to 100 μl. However, a quantification of 20 ng/ml is sufficient to perform bioavailability studies.

The effects of varying the amounts of TEA and buffer salt and of varying the pH were investigated (Fig. 2). Other columns tested for validity were Nucleosil C_{18}, 250×4.6 mm I.D., 5 μm (HiChrom, Berkshire, UK) and CSC-Sil ODS-2, 150×4.6 mm I.D., 5 μm (Chromatography Sciences Company, Canada). There was a difference in the amount of TEA required in the mobile phase for the different columns. This problem may be overcome by utilizing a silanol-deactivated column in future work, which could probably lead to the elimination of TEA from the mobile phase, as this strategy was successfully applied for diltiazem–HCl by Rutledge et al. [14]. Freeze–thaw stability and the stability of ranitidine plasma samples at −20°C has been reported previously [7]. Also, it has been reported that ranitidine stock solutions prepared in acetate or phosphate buffer at pH 3 are stable when stored at 4–6°C for several months [5,15]. However, to demonstrate the stability of ranitidine under highly acidic conditions, the stability of ranitidine in the deproteinized plasma sample was monitored at room temperature over 1 day after treatment with PCA, at ranitidine plasma concentrations of 500 and 50 ng/ml. It was found that ranitidine is stable over this period.

The guard column should be replaced every 200 injections. However, the analytical column showed no performance problems after about 1000 injections.

This method probably can be further advanced by employing midbore HPLC column technology, which can reduce the mobile-phase requirements. It has been reported that using a midbore column did not necessitate the modification of the conventional HPLC system [7].

The present method was used successfully for a comparative bioavailability study of two different ranitidine–HCl tablet preparations. Fig. 3 shows the individual plasma concentration–time profile of the volunteers, obtained using two preparations of ranitidine.

In conclusion, the present method is simple, precise and sensitive for the determination of ranitidine in plasma. The method used a simple plasma deproteination step instead of extraction and was suitable for bioavailability studies.

Table 1
Absolute recovery, within-day and between-day precision and accuracy ($n=6$)

Concentration (ng/ml)	Recovery		Within-day		Between-day	
	Mean	C.V.	Precision	Accuracy	Precision	Accuracy
	(%)	(%)	(C.V.%)	(%)	(C.V. %)	(%)
20	93.7	6.4	5.9	6.9	6.7	7.5
50	98.1	6.6	6.4	5.1	7.9	5.3
100	95.4	1.4	1.5	7.8	4.7	4.9
250	96.8	2.9	3	−6.25	3.7	−4.5
500	96.6	4.7	4.3	−2	5.9	1.3
750	93.1	1.7	2.3	−1.8	3.2	−2.4
1000	97.5	1.8	1.8	−2.8	2.1	1.9

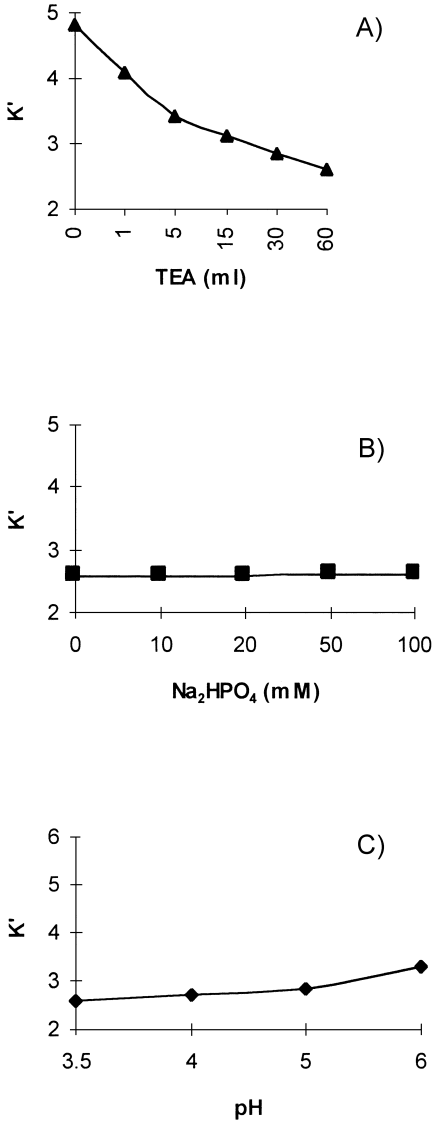

Fig. 2. Dependence of retention time of ranitidine on: (A) TEA addition to the mobile phase, which consisted of 21 m*M* hydrogen phosphate buffer–acetonitrile (1000:150, v/v), pH 3.5. (B) Ionic strength with TEA additive. Mobile phase: hydrogen phosphate buffer–acetonitrile–TEA (1000:150:60, v/v), pH 3.5. (C) pH with TEA additive. Mobile phase: 21 m*M* hydrogen phosphate buffer–acetonitrile–TEA (1000:150:60, v/v). Column: μBondapak C_{18} (250×4.6 mm I.D., 10 μm).

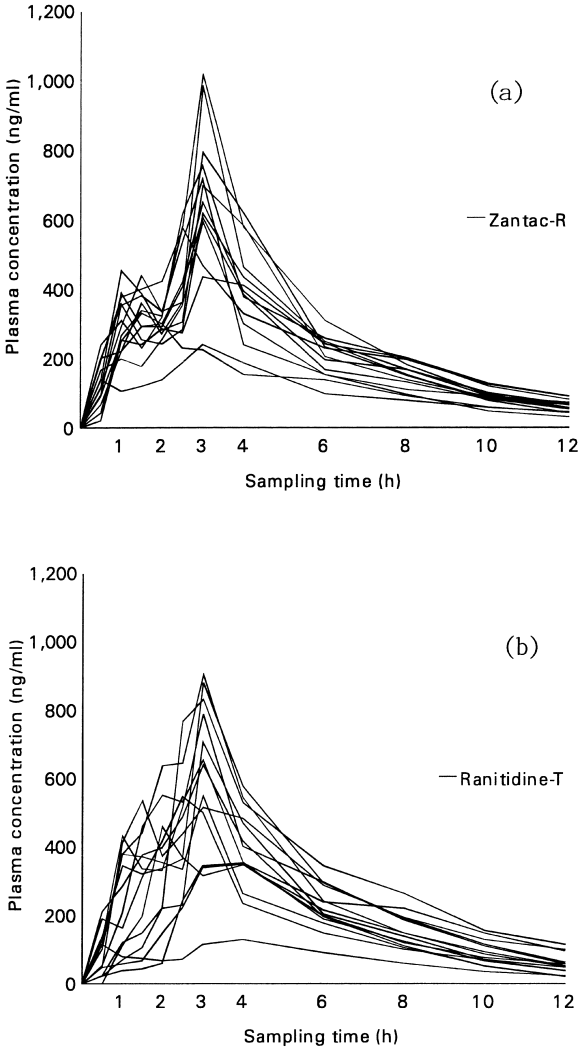

Fig. 3. Plasma concentration–time profiles of ranitidine in 14 healthy male volunteers following oral administration of 150 mg of Zantac (a) and Ranitidine (b) in a crossover study.

References

[1] S.M. Grant, H.D. Langtry, R.N. Brogden, Drugs 37 (1989) 801–870.
[2] C.F. Wong, K.K. Peh, K.H. Yuen, J. Chromatogr. 718 (1998) 205–210.
[3] C. Lopez-Calull, L. Garcia-capdevila, C. Arroyo, J. Bonal, J. Chromatogr. 693 (1997) 228–232.
[4] M.S. Salem, A.M. Gharaiebeh, H.N. Alkaysi, A. Badwan, J. Clin. Pharm. Ther. 13 (1988) 351–357.

[5] G. Mullersman, H. Deredorf, J. Chromatogr. 381 (1986) 385–391.
[6] G.W. Mihaly, O.H. Drummer, A. Marshal, R.A. Smallwood, W.J. Louis, J. Pharm. Sci. 69 (1980) 1155–1157.
[7] D. Farthing, K.L. Brouwer, I. Fakhry, D. Sica, J. Chromatogr. 688 (1997) 350–353.
[8] T.L. Lloyd, T.B. Perschy, A.E. Gooding, J.J. Tomlinson, Biomed. Chromatogr. 6 (6) (1992) 311–316.
[9] H.T. Karnes, K. Opong-mensah, D. Farthing, L.A. Beightol, J. Chromatogr. 422 (1987) 165–173.
[10] D.V. McCalley, R.G. Brereton, J. Chromatogr. A 828 (1998) 407–420.
[11] W.A. Moats, J. Chromatogr. 386 (1987) 69–78.
[12] K.H. Yuen, K.K. Peh, J. Chromatogr. 710 (1998) 243–246.
[13] A. Wehrli, J.C. Hildenbrand, H.P. Keller, R. Stampfli, J. Chromatogr. 149 (1978) 199–210.
[14] D.R. Rutledge, A.H. Abadi, L.M. Lopez, C.A. Beaudreau, J. Chromatogr. 615 (1993) 111–116.
[15] H.M. Vandenberghe, S.M. Macleond, W.A. Mahon, P.A. Labert, S.J. Soldin, Ther. Drug Monit. 2 (1980) 379–384.