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Short communication

Simple high-performance liquid chromatographic method for the determination of ranitidine in human plasma

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Abstract

A simple high-performance liquid chromatographic method was developed for the determination of ranitidine in human plasma. Prior to analysis, ranitidine and the internal standard (metoprolol) were extracted from alkalinized plasma samples using dichloromethane. The mobile phase was 0.05 *M* potassium dihydrogenphosphate–acetonitrile (88:12, v/v) adjusted to pH 6.5. Analysis was run at a flow-rate of 1.3 ml/min and at a detection wavelength of 229 nm. The method is sensitive with a detection limit of 1 ng/ml at a signal-to-noise ratio of 3:1, while the quantification limit was set at 15 ng/ml. The calibration curve was linear over a concentration range of 15–2000 ng/ml. Mean recovery value of the extraction procedure was about 90%, while the within-day and between-day coefficients of variation and percent error values of the assay method were all less than 15%.

Keywords: Ranitidine

1. Introduction

Ranitidine is a potent histamine H_2-receptor antagonist used in the treatment of duodenal ulcer, gastric ulcer, and Zollinger–Ellison syndrome [1–3]. Various high-performance liquid chromatographic (HPLC) methods [4–8] have been reported for the determination of ranitidine in biological fluids. Carey et al. [4] described a method involving a two-step extraction procedure in the sample pre-treatment which appeared to be tedious and time-consuming. A method using a single extraction procedure was reported by Mihaly et al. [5], but the assay validation was conducted at one concentration only. In addition, the mobile phase has a pH value of 8, which may lead to rapid deterioration of the silica-based column. Another method which also involved a one-step extraction procedure was reported by Mullersman and Derendorf [6]. This method however used 10 ml of organic extraction solvent and 2 ml of plasma sample. Moreover, no recovery value was reported for the extraction procedure. Meanwhile, Rustum et al. [7] reported a method involving protein precipitation and salting out procedures in the sample pretreatment which appeared to be tedious and complicated. Furthermore, this method was limited to the use of polymeric base reversed-phase column as the pH of the mobile phase was above 10. On the other hand, Prueksaritanont et al. [8] used a combination of two solvents for extraction in order to obtain a satisfactory recovery.

In this paper, we report a relatively simple, sensitive, and easy to operate HPLC method using UV detection for the determination of ranitidine in

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human plasma. This method involves a one-step single solvent extraction procedure in the sample preparation. We also demonstrated the applicability of this method in a bioavailability study.

2. Experimental

2.1. Materials

Potassium dihydrogenphosphate and sodium hydroxide pellets, AR grade were purchased from Merck (Darmstadt, Germany). Metoprolol tartrate standard was obtained from National Pharmaceutical Control Bureau (Kuala Lumpur, Malaysia). Ranitidine HCl standard was obtained from the US Pharmacopeia (MD, USA). All other solvents used were of AR grade or of HPLC grade purchased from Mallinckrodt (Kentucky, USA).

2.2. Instrumentation

The HPLC system comprised a Jasco PU-980 pump (Jasco, Tokyo, Japan), a Jasco UV—975 UV–Vis detector (Jasco) equipped with a Hitachi D-2500 Chromato-integrator (Hitachi, Tokyo, Japan) and a Rheodyne 7161 sample injector fitted with a 100-μl sample loop. A Supelcosil LC-CN (Supelco, Bellefonte, PA, USA) column (5 μm, 250×4.6 mm I.D.) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) packed with Spherisorb® CN (5 μm, pore: 80 Å) powder (Keystone Scientific, Bellefonte, PA, USA) was used for the chromatographic separation. The mobile phase consisted of 0.05 *M* potassium dihydrogenphosphate–acetonitrile (88:12, v/v) adjusted to pH 6.5 using 2.5 *M* sodium hydroxide solution and delivered at a flow-rate of 1.3 ml/min. The detection wavelength was set at 229 nm with a sensitivity range of 0.005 a.u.f.s.

2.3. Sample preparation

A 1-ml volume of plasma sample was accurately measured into a 10-ml glass tube with a PTFE-lined screw cap, followed by the addition of 50 μl of 40 μg/ml metoprolol tartrate internal standard solution, 100 μl of 10 *M* sodium hydroxide solution and 5 ml of dichloromethane extracting solvent. The mixture was vortexed for 90 s and centrifuged at 2000 *g* for 15 min. The organic layer was transferred into a reactivial and evaporated to dryness at 45°C under a gentle stream of nitrogen gas. The residue was reconstituted with 125 μl of mobile phase and 100 μl was injected onto the column. Samples were quantified using peak height ratio of ranitidine over the internal standard.

2.4. Assay validation

The ranitidine stock solution was prepared by dissolving 100 mg of ranitidine in 1000 ml of distilled water to achieve a concentration of 100 μg/ml. The working ranitidine standard solutions were then prepared by serial dilutions of the stock solution with distilled water. Standard calibration curves were constructed at the following concentrations, 15, 31, 62, 125, 250, 500, 1000 and 2000 ng/ml. This was carried out by first spiking 50 ml of pooled blank plasma with 4 ml of 25 μg/ml drug solution to give a concentration of 2000 ng/ml. Subsequent concentrations were obtained by serial dilutions of this plasma sample with blank plasma. These plasma standards were also used to determine the extraction recovery, within-day and between-day precision and accuracy ($n=6$) of the method. The recovery of the extraction procedure for ranitidine and the internal standard was calculated by comparing the peak height obtained after extraction with that of aqueous drug solution of corresponding concentration without extraction. The accuracy was expressed as percentage error, obtained by calculating the percentage of difference between the measured and spiked concentration over that of the spiked value whereas the precision was denoted using the coefficient of variation.

2.5. Stability of standard drug solutions

Aqueous solutions of ranitidine HCl containing 1 and 10 μg/ml and of internal standard containing 2 and 16 μg/ml were prepared in distilled water and kept at room temperature (25°C) and in the refrigerator (4°C). The stability study was conducted by injecting the drug solutions onto the HPLC

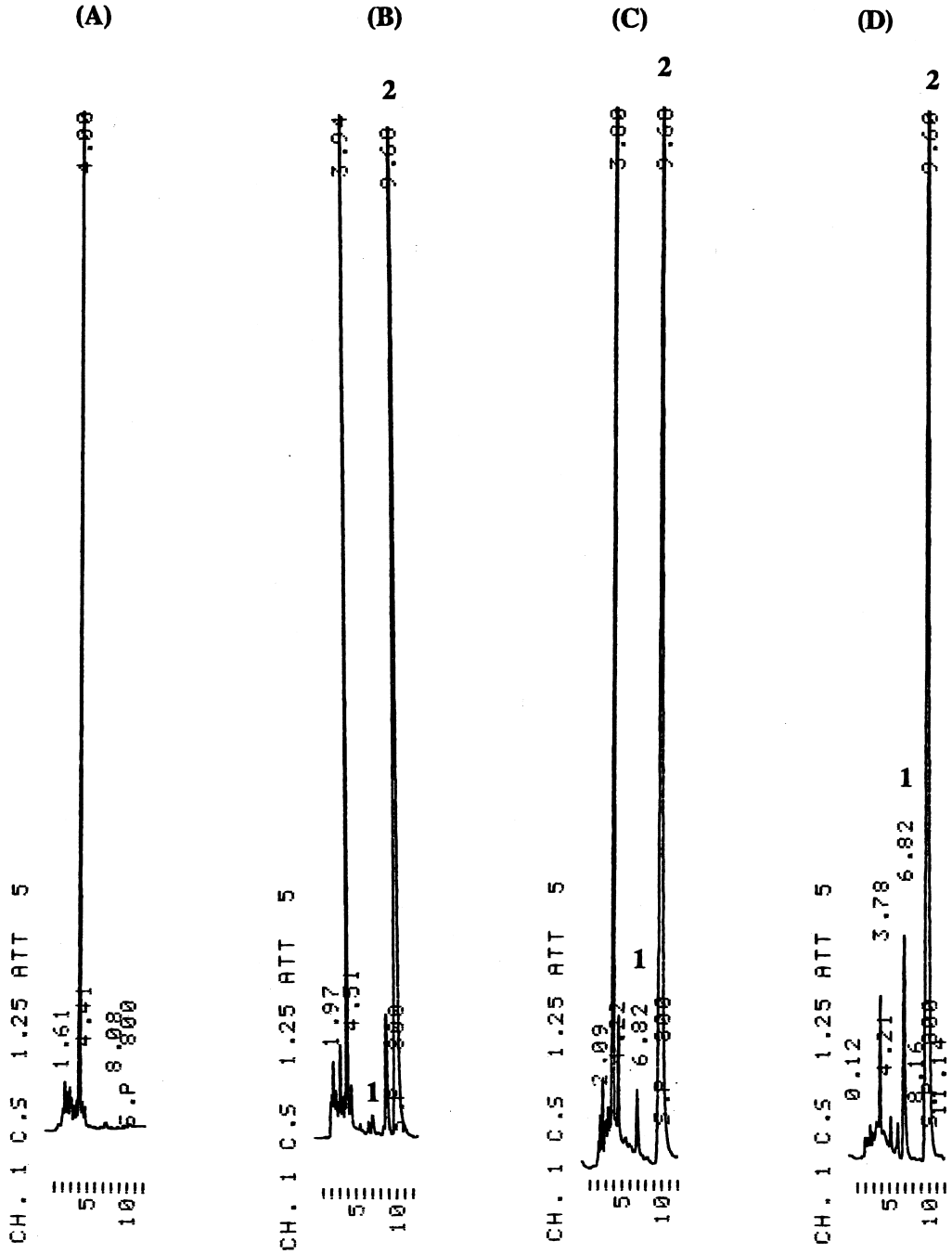

Fig. 1. Chromatograms for the analysis of ranitidine in plasma. (A) Blank plasma; (B) plasma spiked with 40 μg/ml of metoprolol and 1 ng/ml of ranitidine; (C) plasma spiked with 40 μg/ml of metoprolol and 15 ng/ml of ranitidine; (D) a volunteer plasma containing 52 ng/ml of ranitidine 12 h after oral administration of 300 mg of ranitidine. (*Y*-axis: attenuation=5; *X*-axis: chart speed=1.25 mm/min. 1=ranitidine, 2=metoprolol).

column and comparing the peak height obtained over time.

3. Results and discussion

During assay development, several solvents namely, dichloromethane, ethyl acetate and chloroform were used to extract ranitidine from plasma samples. Dichloromethane was found to provide the cleanest chromatogram and also satisfactory recovery values of approximately 90%. Mihaly et al. [5] reported a recovery value of 84% for ranitidine even though these workers used a larger volume of dichloromethane (10 ml) for extracting the same volume of plasma (1 ml) as used in the present study.

The molarity of NaOH used was found to be critical in achieving good recovery. When the molarity of NaOH was below 10 *M*, there was a correspondingly decrease in the recovery of ranitidine with a decrease in the molarity of NaOH. The recovery of ranitidine dropped to below 75% when 5 *M* NaOH was employed. Hence 10 *M* of NaOH was found to be optimum.

Different internal standards which included cimetidine, famotidine and metoprolol tartrate were employed in sample preparation. It was found that the recovery values for both cimetidine and famotidine were less than 70% when extracted using dichloromethane. Furthermore, these two compounds interfered with the endogenous compounds in the plasma during analysis. On the other hand, metoprolol achieved a better recovery of approximately 88% and was free of interference from endogenous compounds in the plasma.

Chromatograms obtained from blank plasma, plasma spiked with internal standard and ranitidine at concentrations of detection limit and quantification limit, as well as a healthy volunteer after dosing with 300 mg of ranitidine are shown in Fig. 1A–D. The peaks of ranitidine and internal standard were well resolved and free of interference from endogenous compounds, with retention times of 6.82 and 9.60 min, respectively. The total run time for each sample was 12 min.

Our mobile phase consisted of buffer and acetonitrile which is quite similar to those of Mullersman and Derendorf [6] and Rustum et al. [7]. However, no ion-pairing reagent was used in our mobile phase, whereas octanesulfonic acid was used by Mullersman and Derendorf [6] and sodium pentane sulfonate by Rustum et al. [7]. Furthermore, the mobile phase employed in the method of Rustum et al. [7] has a pH of above 10 and thus was only limited to the use of polymeric base column.

Calibration curves were obtained by plotting peak height ratio of ranitidine over metoprolol versus ranitidine HCl standard concentrations. The standard calibration curves ($n=6$) were linear over the concentration range used, with a correlation coefficient of 0.9999, a slope of $2.24 \cdot 10^{-3}$ and an intercept of -0.0336 as shown in Fig. 2. The extraction recovery, within-day and between-day accuracy and precision values are presented in Table 1. The coefficient of variation (C.V.) and percent error values of both the within-day and between-day precision and accuracy were all less than 15%. A detection limit of 1.0 ng/ml was obtained at a signal-to-noise ratio of 3:1, while the quantification limit was set at 15.0 ng/ml being the lowest concentration used in constructing the standard curve. The detection limit value was comparable to those reported by Rustum et al. [7] and Mullersman and Derendorf [6] but more sensitive than those reported by Mihaly et al. [5] and Prueksaritanont et al. [8].

The peak height versus time profiles of ranitidine and metoprolol aqueous solutions are shown in Fig. 3. It can be seen from the plots that both ranitidine (at concentrations of 1 and 10 μg/ml) and metoprolol (at concentrations of 2 and 16 μg/ml) were

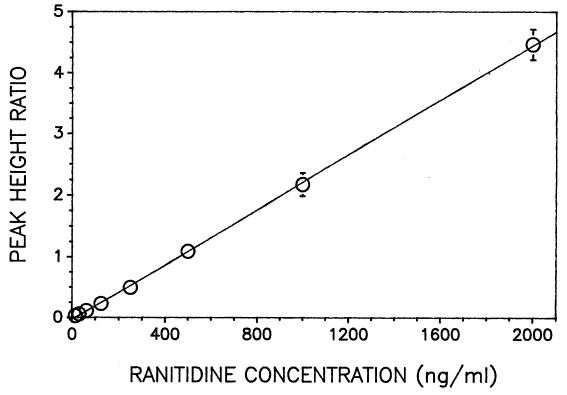

Fig. 2. Standard calibration curves ($n=6$).

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

Concentration (ng/ml)	Recovery		Within-day		Between-day	
	Mean (%)	C.V.%	Precision (C.V.%)	Accuracy (% error)	Precision (C.V.%)	Accuracy (% error)
15	93.0	4.5	12.1	14.6	13.5	13.3
31	90.0	3.9	5.7	14.3	12.1	7.6
125	88.0	2.9	4.5	4.9	9.0	5.7
500	90.0	3.3	9.8	6.7	7.9	8.1
1000	88.0	5.0	7.4	4.9	9.5	5.8
2000	91.0	4.5	8.7	1.4	5.4	2.9

found to be stable for at least 1 month when stored at room temperature (25°C) and in the refrigerator (4°C). There was no apparent decrease in the peak height of the samples on repeated injections. Moreover, no extra peak was observed in the chromatogram.

The present method was applied to analyze plasma samples of 12 healthy adult male volunteers from a comparative bioavailability study of two different ranitidine HCl tablet preparations, namely, Zantac and X'tac, the latter being a generic preparation. A total of 450 plasma samples was obtained from the comparative bioavailability study. A batch size of 50 plasma samples was analyzed per day and 350 samples were assayed per week by one analyst. Fig. 4 shows the mean plasma concentration–time profiles of the volunteers obtained with the two preparations after dosing with 300 mg of ranitidine. It can be seen from the plasma profiles of both preparations that ranitidine could still be detected up to 18 h and the last detectable concentration was less than 5% of the peak plasma concentration.

In conclusion, this paper describes a very simple and sensitive HPLC method for the determination of ranitidine HCl, suitable to monitor plasma concentrations during clinical studies.

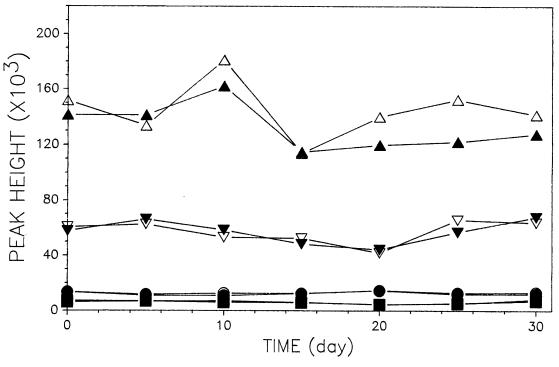

Fig. 3. Peak height versus time profiles of ranitidine (1 μg/ml: ○=4°C, ●=25°C; 10 μg/ml: △=4°C, ▲=25°C) and metoprolol (2 μg/ml: □=4°C, ■=25°C; 16 μg/ml: ▽=4°C, ▼=25°C) aqueous solutions.

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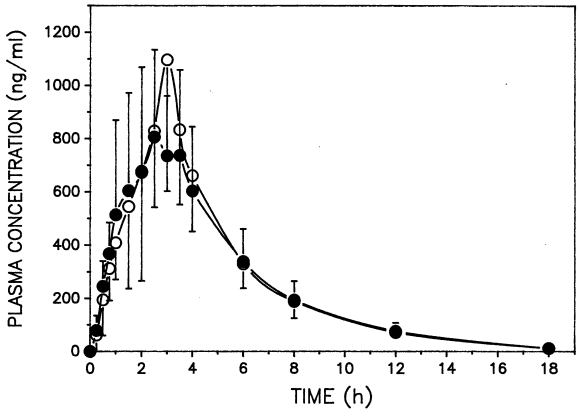

Fig. 4. Mean plasma ranitidine concentration versus time profiles from 12 volunteers following the oral administration of 300 mg of Zantac and X'tac (○=Zantac, ●= X'tac).

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