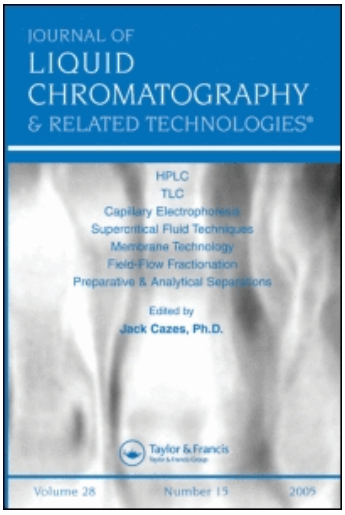

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Al-Khamis, K. I. , El-Sayed, Y. M. , Al-Rashood, K. A. and Bawazir, S. A.(1995) 'High Performance Liquid Chromatographic Determination of Ranitidine in Human Plasma', Journal of Liquid Chromatography & Related Technologies, 18: 2, 277 — 286
To link to this Article: DOI: 10.1080/10826079508009238
URL: http://dx.doi.org/10.1080/10826079508009238

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(2), 277-286 (1995)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF RANITIDINE IN HUMAN PLASMA

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Abstract

A rapid reversed phase HPLC method for determination of ranitidine in human plasma has been developed. The procedure involved extraction of the drug from alkalinized plasma spiked with the internal standard (procainamide) using 4% v/v isopropanol in ethylacetate. The extract was evaporated under nitrogen and the residue was reconstituted with methanol and injected onto U-Bondapak C_{18} column. The mobile phase is 8% v/v acetonitrile in 10 mM potassium phosphate buffer (pH 5.1); at a flow rate of 2.5 ml/min and UV detection at 330 nm. The efficiency of extraction was 90% with a detection limit of 20 ng/ml. The within-day coefficient of variations ranged from 4.09% to 5.81%, whereas those of between-day were from 7.5% to 9.51%.

Introduction

Ranitidine is a histamine H_2-receptor antagonist which is very effective in the treatment of gastric and duodenal ulcers [1].

Several reversed phase high-performance liquid chromatographic methods have been reported for determining ranitidine plasma concentrations [2-9]. These methods present various difficulties including, long and tedious extraction procedure [7], use of ion-pair chromatography [6, 9], high percentage of the organic modifier in the mobile phase [4, 5, 8], interferences of endogenous plasma substances [6, 3] and low sensitivity [3].

In contrast, the method described here is simple, less expensive, free of interferences, time-saving, sensitive and selective for the quantitation of ranitidine in human plasma, which makes its applicability in bioavailability studies valuable.

Materials and Methods

Apparatus:

A Waters HPLC Unit (Waters Associates, Milford, MA, USA) was used. It consisted of a system controller (M-720), a data module (M-730), an auto-injector (WISP-712), a solvent delivery system (M-501), UV detector (M-484), and a reverse phase U-Bondapak C_{18} column (10 um, 15 cm x 3.9 mm I.D.).

Chemicals and Reagents:

Ranitidine hydrochloride (Glaxo Ltd., Herts, U.K.) as well as procainamide hydrochloride (Smith Kline and French Laboratories Ltd., Herts, U.K.) were kindly gifted. Acetonitrile, methanol and ethylacetate (HPLC grade) were from E.

Merck (Darmstadt, Germany), potassium phosphate buffer (BDH Chemicals Ltd., Poole, U.K.) and isopropanol (Riedel-De Haen Ag, Seelze-Hannover, Germany) were used. All chemicals were of analytical grade.

Stock Solutions:

A stock solution of ranitidine in methanol was prepared at a concentration of 1 mg/ml. A stock solution of 1 mg/ml procainamide (internal standard) in methanol was prepared and diluted to a concentration of 10 mg/l in methanol for the assay.

Chromatographic Conditions:

A mobile phase containing 8% v/v acetonitrile in 10 mM potassium dihydrogen phosphate (pH 5.1) was used. The flow rate was 2.5 ml/min and the detector was set at a wavelength of 330 nm and a sensitivity of 0.005 AUFS. Experiments were conducted at ambient temperature.

Sample Preparation:

To 1.0 ml of human plasma, 25 ul of internal standard (10 mg/l) and 15 ul of 6N NaOH were added. Then 2.0 ml of 4% v/v isopropanol in ethylacetate was added. The tube was then mechanically shaken for 20 minutes, and then centrifuged at 3000 rpm for 10 minutes. The organic layer was transferred to a 5 ml centrifuge tube and evaporated under a stream of purified nitrogen to dryness at 40^{o}C. The residue was reconstituted in 100 ul methanol and 75 ul of the sample was injected onto the column.

Calibration and Recovery:

Calibration was performed by adding known amounts of ranitidine to blank human plasma to yield concentrations over a range of 0.05-1.0 mg/l, and these standards were then extracted according to the procedure described above.

Ranitidine recovery from plasma was determined by comparing the peak height ratios to those obtained from similar aqueous solutions.

The precision of analysis was assessed by 7 replicate analyses of human plasma spiked with ranitidine to give concentrations of 0.075, 0.35 and 0.8 mg/l, and then within-day variations were calculated. Day to day variations were also calculated for the above concentrations up to 10 days (Table 1).

Specificity

Other drugs (Cimetidine, metoclopramide, naproxen, diclofenac, ibuprofen, phenylbutazone, ketoprofen, lidocaine, verapamil, diazepam, glibenclamide, chlorpheniramine and brompheniramine) were tested for their possible interferences in the HPLC assay. All the investigated drugs were not detected within 15 minutes from the injection except lidocaine which eluted at 8.1 min.

Human Studies:

Pharmacokinetic studies on ranitidine involved its administration to a normal volunteer. Clinical investigations excluded cardiac, hepatic and renal diseases. The volunteer

Table 1: Within- and between-day precision of ranitidine in human plasma.

Within-day*			Between-day**		
Added conc. (mg/l)	Measured conc. (mg/l)	%Bias@	Added conc. (mg/l)	Measured conc. (mg/l)	%Bias@
0.075			0.075		
Mean	0.072	-4.00	Mean	0.061	-18.67
SD	0.0041		SD	0.0058	
CV%	5.64		CV%	9.51	
0.350			0.350		
Mean	0.344	-1.71	Mean	0.350	0.0
SD	0.020		SD	0.029	
CV%	5.81		CV%	8.29	
0.800			0.800		
Mean	0.881	10.13	Mean	0.841	5.13
SD	0.036		SD	0.063	
CV%	4.09		CV%	7.5	

* Mean values represent seven different plasma samples for each concentration.

** Mean values represent seven different plasma samples for each concentration assayed on different days over a period of 10 days.

@ Bias=100x(Measured concentration-Added concentration/added concentration

received 150 mg ranitidine tablet orally after fasting overnight, and 3 ml of blood samples were drawn in heparinized tubes prior to drug administration and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 hrs after administration. Heparinized blood samples were centri-

fuged at 3000 rpm for 10 min., and plasma samples were kept at -20°C until analysis.

Results and Discussion

Using the described procedure, the chromatograms of ranitidine and the internal standard are shown in Fig. 1. Ranitidine showed a peak (b) with a retention time of 5.83 minutes, internal standard showed a peak (a) and a retention time of 3.79 min.

Fig. 1 shows the chromatograms obtained from human plasma extract taken 4.0 hrs (B) and 7.0 hrs (C) following ranitidine administration.

Operation of the detector at 330 nm, rather than 229 nm [3, 6] resulted in a cleaner chromatogram since there was no interference from endogenous plasma constituents or concomitant drugs without loss of sensitivity.

The calibration curve for ranitidine was linear over the range investigated with a correlation coefficient of 0.99 (Fig. 2). The detection limit was 20 ng/ml.

The recovery of ranitidine from the human plasma using this method was 90% on the average, which compared favorably with those reported by other workers [2, 5, 6].

The overall precision of the method was measured on the basis of samples with three different concentrations (Table 1).

The effect of freezing and thawing of the plasma samples was studied over a period of two weeks at 0.075 and 0.35 mg/l. There was no significant differences in the

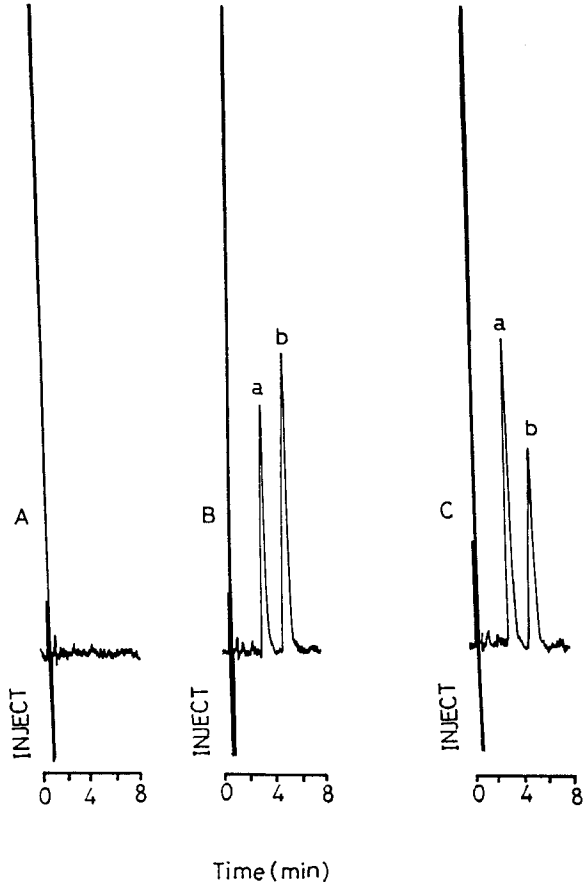

Fig. 1: Typical chromatograms of human plasma extract following oral administration of 150 mg ranitidine to a human volunteer. Blood samples were drawn prior to dose (A), 4 hrs (B) and 7 hrs (C) after administration of ranitidine. Peaks: (a) is internal standard and (b) is ranitidine. Chromatographic conditions are as in text.

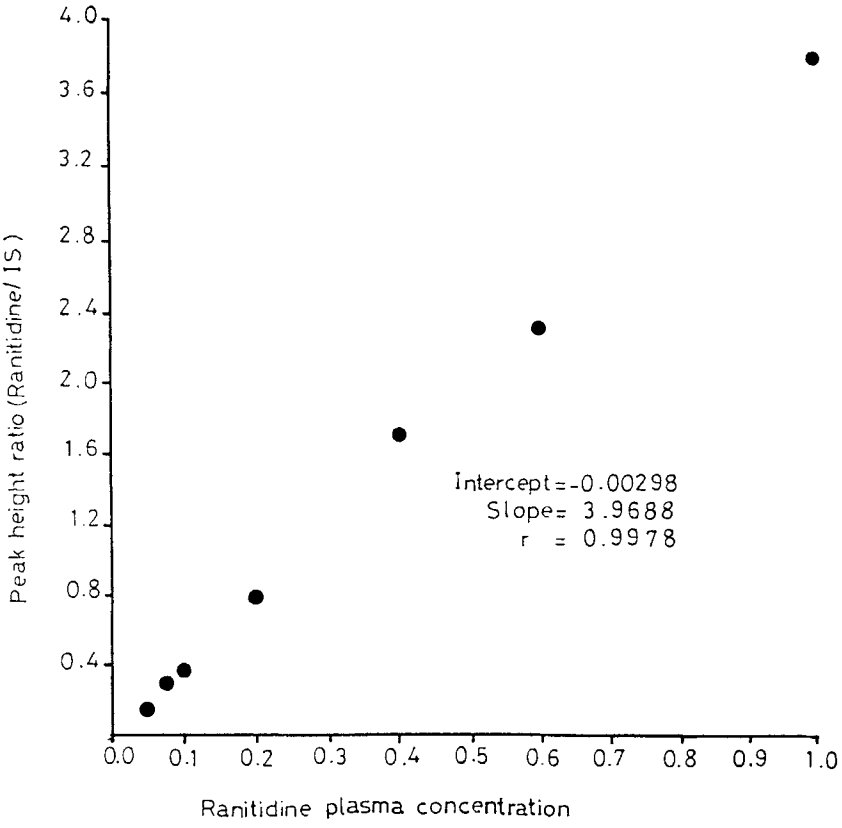

Fig. 2: Standard curve for ranitidine in human plasma. Least squares linear regression analysis resulted in: Y = -0.0029+3.9688x, r=0.9978.

results of the concentrations measured which assured that freezing and thawing of the sample had no effect on the estimation of ranitidine by the above procedure.

The applicability of the assay procedure is illustrated in Fig. 3 which shows the ranitidine plasma concentration-

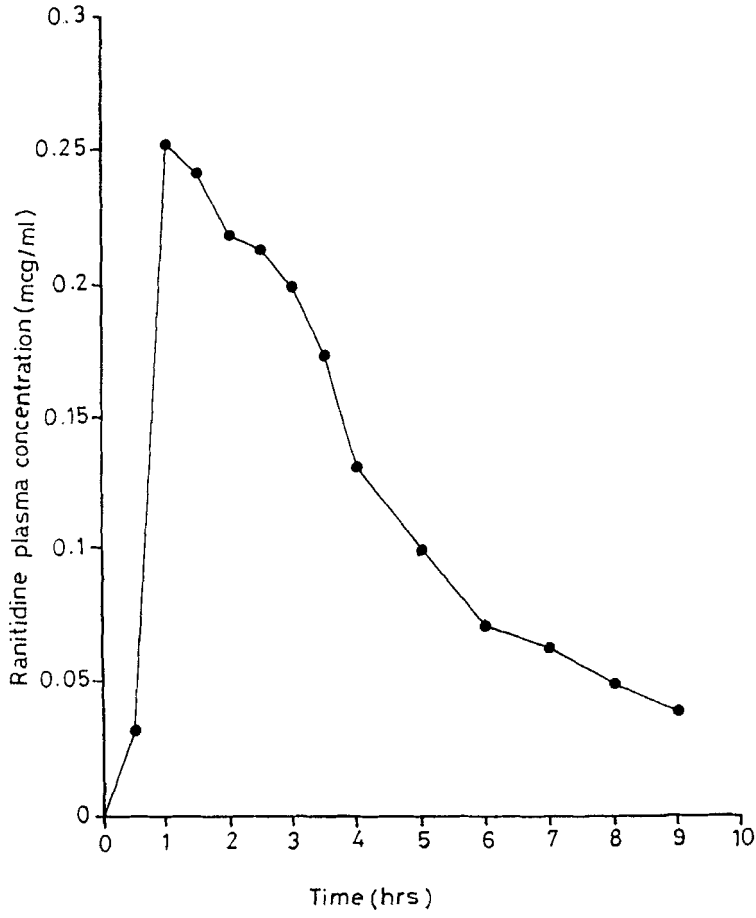

Fig. 3: Plasma level vs time following oral administration of 150 mg ranitidine to a human volunteer.

time profile following oral administration of ranitidine to a healthy subject.

The advantage of this assay is its simplicity, sensitivity, selectivity as well as its application in pharmacokinetic and bioequivalency studies of ranitidine.

Acknowledgement

The authors wish to thank M. Kamal M. Matar for his excellent technical assistance.

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Received: June 15, 1994
Accepted: June 27, 1994