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Short communication

Sensitive high-performance thin-layer chromatography method for detection and determination of ranitidine in plasma samples

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Abstract

A high-performance thin-layer chromatographic procedure has been developed for the determination of ranitidine, a H_2-receptor antagonist, in plasma. The detection and quantification were performed without using internal standards. A single-stage extraction procedure was followed for extracting ranitidine from plasma, and a known amount of the extract was spotted on precoated silica gel F254 plates. Ranitidine was quantified using a Shimadzu CS930 dual-wavelength TLC scanner. The method provides a direct estimate of total ranitidine present in the plasma.

Keywords: Ranitidine

1. Introduction

Ranitidine, N-[2-[[-5-[(dimethylamine)methyl]-2-furanyl]methyl]thio]ethyl]-N′-methyl]-2-nitro-1,1-ethenediamine (Fig. 1), is a histamine H_2-receptor antagonist which inhibits gastric acid secretion.

A previously published method for the estimation of ranitidine by high-performance liquid chromatography (HPLC) [1–6] employed internal standards. This paper describes the HP thin-layer chromatography (TLC) technique for easy, economical and sensitive estimation of total plasma concentration of ranitidine (both free and protein bound) without the need for internal standards. The method was also used to obtain comparative bioavailability information about the drug in healthy volunteers for four marketed tablet preparations.

2. Experimental

2.1. Reagents

Ranitidine was obtained from Cheminor Drugs (Hyderabad, India). Four marketed preparations of 150-mg ranitidine tablets were used for comparative pharmacokinetic studies. Sodium hydroxide (5 *M*) and dichloromethane (analytical grade) were used for extraction, and methanol, ethylacetate and ammonia (25%) (analytical grade) were used for developing TLC plates (Silica gel 60 F254, Art 5554, DC-Alyfolien, Kieselgel 60 F254, E. Merck, Darmstadt, Germany).

2.2. Preparation of standards

A stock solution of ranitidine was prepared in methanol at a concentration of 0.1 mg/ml. Standard solutions were obtained by diluting the stock solu-

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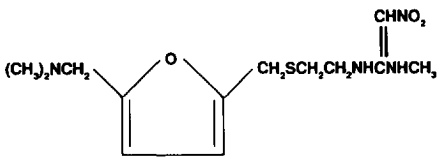

Fig. 1. Chemical structure of ranitidine.

tions to concentrations ranging from 50 to 1000 ng/ml.

2.3. Preparation of plasma samples

Analysis was performed on plasma containing known amounts of the drug, drug-free plasma and plasma containing an unknown quantity of the drug. A 1-ml sample of plasma was transferred to a 20-ml glass centrifuge tube and mixed with 100 μl of 5 *M* NaOH, by vigorous vortex-mixing. This alkaline plasma was extracted with dichloromethane (2×10 ml). The combined dichloromethane extract was dried completely at 45°C in a water bath.

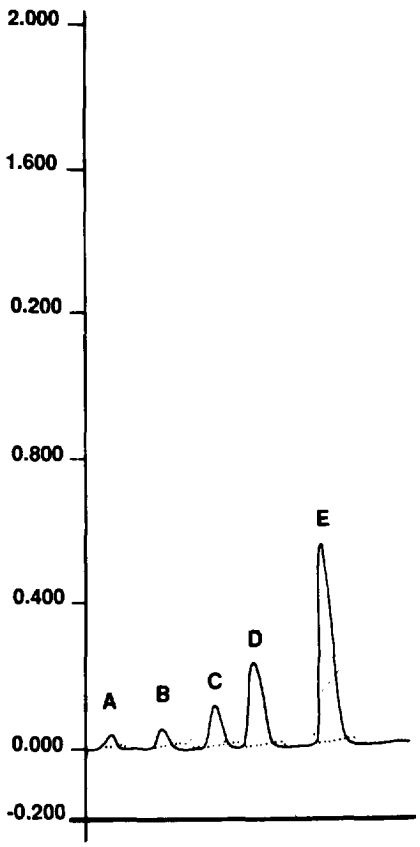

Fig. 2. Linear response of ranitidine (A=50 ng, B=100 ng, C=200 ng, D=500 ng, E=1000 ng).

2.4. Instrumentation and chromatographic conditions

All residues were redissolved in 100 μl of chilled methanol by vigorous vortex-mixing, and 35-μl aliquots of the samples were spotted on TLC plates with the help of a Camag Linomat IV autosampler. Ranitidine (100 ng, 500 ng) reference standard was separately spotted on each TLC plate as an external standard. The TLC plates were developed (10 cm) in a glass chamber (25×25×12 cm), first with ethylacetate, followed by methanol–ammonia–ethylacetate (90:1:10, v/v) up to 5 cm, where the spots were clearly separated as concentrated zones. The chambers were saturated with solvent system before development, and the TLC plates were dried completely by hot air after development in both solvent systems. The spots of ranitidine were visualized under UV (λ_{max} 324 nm). It was observed that ranitidine moved at R_F 0.36, and the spots remained stable for 24 h when kept in the dark. Determination of ranitidine was done by scanning the quenching of fluorescence of the TLC plates with a Shimadzu dual wavelength scanner (Model CS930, Shimadzu, Kyoto, Japan).

2.5. Quantitation

Calibration curves were obtained daily by plotting the area under the peak of ranitidine against the concentrations over the range 50–1000 ng. The areas under the peaks of unknown samples were compared with calibration curves of standards. The lowest detection limit of ranitidine was found to be 5 ng/ml of plasma.

2.6. Method validation

The recovery of ranitidine from plasma was determined by comparing peak areas obtained from plasma spiked with ranitidine at concentrations of 20, 50, 100, 200 and 500 ng/ml with the peak areas obtained from standards. The inter-day precision was

completed by analysing plasma samples in triplicate spiked with ranitidine at 50, 200 and 500 ng on the same day. The intra-day precision was determined by analysing 20, 100 and 200 ng standards simultaneously with unknown plasma daily for five days and also by comparing with the calibration curve. The linearity of the detector response was tested by spotting standards in triplicate for each concentration ranging between 20 and 500 ng.

2.7. Pharmacokinetic studies

Six healthy male human volunteers were selected for the study. Four brands of ranitidine (150 mg) tablets [A: Aciloc (Cadila); B: Histac (Ranbaxy); C: Ranitine (Torrent); D: Zinetac (Glaxo)] were bought on the market. These were administered orally in a single-blind, 4×6 latin-square, crossover design. The protocol was approved by the local ethical committee, and volunteers were included in the study only after obtaining written informed consent. The volunteers were fasted overnight before drug administration and continued fasting until 4 h post-dose but were allowed free access to water. Venous blood samples (5 ml) were collected from an antecubital vein into heparinised tubes before and at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h after drug administration. All blood samples were immediately centrifuged at 1000 *g* for 20 min, and the plasma samples were separated and frozen at −20°C until analysis. All volunteers were maintained on standard diet during the experiment under ideal conditions at our clinical pharmacology unit. A one-week washout period was allowed between each treatment.

3. Results

The peak area was observed to be dependent on the amount of standard ranitidine, and a linear relationship (r=0.999) was found between the peak areas of ranitidine at various concentrations over the range 50–1000 ng (Fig. 2). The accuracy, precision and reliability of the procedure were ascertained by adding known concentrations of the drug to drug-free plasma and analysing three samples of each concentration by the method described for extraction (Table 1). The concentration and peak-height ratios

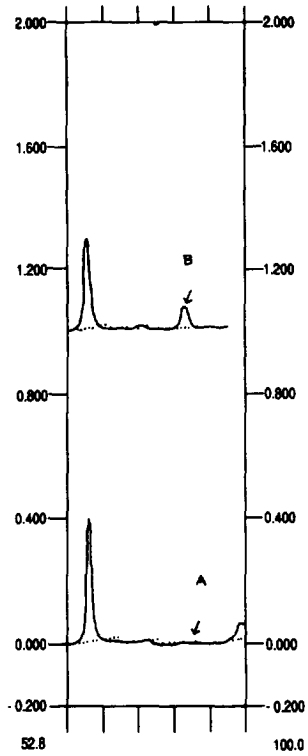

Fig. 3. Scanning profile of a volunteer's sample taken at 0 h (A) and 3 h (B) post-dose of 150 mg ranitidine administration.

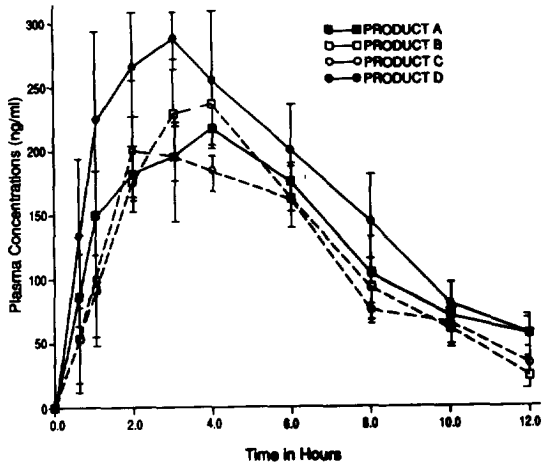

Fig. 4. Plasma concentration after oral administration of 150 mg ranitidine from four marketed products determined by HPTLC. Each point represents mean±S.E. (n=8, crossover design).

Table 1
Accuracy and precision of HPTLC method for determination of ranitidine in plasma

Concentration added (ng/ml)	Concentration detected (mean±S.D., n=5) (ng/ml)	C.V.[a] (%)	Accuracy[b] (%)
20	18.20±1.13	6.20	97.85
50	45.84±2.50	5.45	98.58
100	94.69±4.91	5.19	101.82
200	186.28±8.85	4.75	100.15
500	475.30±22.22	4.67	102.21

[a] Coefficient of variation.
[b] After correction for recovery.

(standard drug to spiked standard) over the range 20–500 ng were linearly related (slope= 0.9523±0.004, intercept −1.638±0.14 and correlation coefficient r=0.999). The recovery of ranitidine in the extraction procedure from 1 ml of plasma was found to be 93.11±1.59% (n=5). The intra-day and inter-day precisions are given in Table 2. The solvent system used for development of the plates produced no interference peaks in the area under the curve, and all other compounds were distinctly separated.

Table 2
Precision data of the HPTLC assay for ranitidine

Concentration added (ng)	Peak area[a] (mean±S.D.)	C.V. (%)
Inter-day		
50	10085.53±550.04	5.45
200	40982.33±1948.24	4.75
500	104566.00±4889.73	4.68
Intra-day		
20	4005.47±250.72	6.26
100	20833.27±1081.61	5.19
200	39259.00±2057.04	5.24

[a] Calculated for total concentration (integrated value).

The R_F value of ranitidine under the conditions used was found to be 0.36±0.06, and spots were quantified at λ_{max} 324 nm. The spot of ranitidine in plasma is distinctly separated and in volunteer plasma is clearly identified in comparison with blank plasma (Fig. 3).

The mean concentration of ranitidine at various time points for different marketed preparations is shown graphically in Fig. 4. The mean maximum concentration (C_{max}) and mean area under the plasma concentration curve ($AUC_{0\rightarrow\infty}$~h) for all four preparations were comparable and were statistically not significantly different (Table 3). The T_{max} for all preparations ranged between 2.75 and 3.75 h.

4. Discussion

The proposed HPTLC method can measure the total concentration of plasma ranitidine at a therapeutic dose of 150 mg per oral single-dose administration.

A comparison of the proposed HPTLC method with a previously published HPLC method [1]

Table 3
Pharmacokinetic parameters of ranitidine (150 mg) in human volunteers

Parameters	Product A (n=6)	Product B (n=6)	Product C (n=6)	Product D (n=6)	p Value
C_{max} (ng/ml)	242.16±11.83	245.24±22.68	255.67±44.48	322.42±50.87	NS
T_{max} (h)	3.25±0.48	3.75±0.25	2.75±0.48	2.75±0.63	NS
AUC (0→12 h) (ng ml^{-1} h^{-1})	1569.04±137.21	1498.24±196.68	1432.62±118.86	1748.25±253.88	NS
AUC (0→∞) (ng ml^{-1} h^{-1})	2002.32±274.52	1570.53±222.44	1607.00±135.82	1958.04±220.57	NS
K_{el} (h^{-1})	0.182±0.042	0.280±0.031	0.262±0.038	0.263±0.043	NS
$T_{1/2}$B (h)	4.53±1.066	2.58±0.33	2.83±0.45	2.83±0.41	NS

Data obtained by crossover design and presented as mean±S.E.M.; n=numbers of volunteers; NS=not significant (p>0.05).

was performed by using the plasma samples from the recovery studies. Two methods were tried. First, the sample was processed by the HPLC method and subsequently spotted onto the TLC plate. Second, the sample was processed by the proposed HPTLC method and then injected onto the HPLC column and eluted with the reported solvent system. Neither method succeeded in quantifying the drug. This is because a large number of interfering peaks and poor separation are observed with both methods. However, when processing by the proposed HPTLC method, it is possible to quantify the levels of ranitidine in plasma by spotting and detecting the samples without using an internal standard.

A previously published paper showed that six ranitidine brands available in India were not bioequivalent by HPLC [7]. However, by the method described herein, the four products tested were found to be bioequivalent. The proposed method was used to accurately determine ranitidine in tablets without interference from the excipients.

5. Conclusions

The proposed HPTLC method for the estimation of ranitidine in plasma has certain advantages over other reported methods. For example: (1) it gives a clear picture of total drug present after absorption and thus has direct clinical relevance; (2) it is economical and faster than previously published methods: on a single plate at least 10–12 samples can be analysed in 5–6 h; (3) unlike earlier methods, this method does not require an internal standard and quantification can be done using a reference drug as the external standard; (4) the recovery of the drug is improved compared with the HPLC method (93.11 ± 1.78); (5) the method described is a sensitive and specific assay for ranitidine in plasma and is suitable for pharmacokinetic studies after therapeutic doses.

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