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SIMULTANEOUS DETERMINATION OF RANITIDINE AND ITS METABOLITES IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A sensitive high-performance liquid chromatographic method was developed for the simultaneous determination of ranitidine and its metabolites, ranitidine N-oxide, ranitidine S-oxide and desmethylranitidine, in human plasma and urine. For the plasma analysis, 1-ml plasma samples spiked with phenylpyramidol as the internal standard were extracted at basic pH with acetonitrile-ethyl acetate (3:2, v/v). After evaporation and reconstitution, the samples were chromatographed on a cation-exchange column, with a mobile phase of 0.1 M sodium acetate buffer (pH 5)–acetonitrile–tetrahydrofuran (56.5:36:7.5, v/v) and ultraviolet detection at 320 nm. The extraction recoveries were 99.8, 30.4, 74.2 and 80.2% and the detection limits were 5, 15, 10 and 4 ng/ml for ranitidine, ranitidine N-oxide, ranitidine S-oxide and desmethylranitidine, respectively. For the urine analysis, a simple deproteinization with an equal volume of acetonitrile was satisfactory for sample preparation. The applicability of this method for the pharmacokinetic study of ranitidine following oral administration was demonstrated.

INTRODUCTION

Ranitidine, a potent histamine H_2 receptor antagonist, has been widely used for the treatment of duodenal and gastric ulceration. It is the preferred drug

for patients with the Zollinger–Ellison syndrome [1]. Earlier studies have indicated that it is mainly metabolized by oxidation to give ranitidine N-oxide, ranitidine S-oxide and desmethylranitidine [2,3]. For complete characterization of ranitidine pharmacokinetics, it is usually essential that a specific method for the quantitation of ranitidine and its metabolites in biological fluids be developed.

Many methods have been reported for the determination of ranitidine in biological fluids. They include radioimmunoassay [4], ion-selective electrodes [5] and high-performance liquid chromatography (HPLC) [2,6–12]. However, relatively few methods have been reported for the simultaneous analysis of ranitidine and its metabolites: these were liquid chromatography–mass spectrometry [13,14] and HPLC [2,7,8]. The former was rather complicated, and the latter had some drawbacks. One of the HPLC methods was only applicable for detecting ranitidine and the desmethyl metabolite [7]. The ion-pair chromatography successfully developed for the simultaneous determination of these four compounds, however, required several hours of equilibration of the HPLC system before the analysis, and also that the column temperature be maintained at 45°C during the analysis [2,8]. In addition, the sensitivity of the method was somewhat low, with detection limits of 20 ng/ml for each compound. The column performance also deteriorated with prolonged use, necessitating the substitution of a new ternary-solvent HPLC system for routine analyses of urine samples [8].

This paper reports a sensitive, selective and easy-to-operate HPLC method for the simultaneous determination of ranitidine and its metabolites in both human plasma and urine. The method involved the use of a cationic exchange column, one-step extraction of plasma and simple deproteinization of urine samples.

EXPERIMENTAL

Chemicals and reagents

Ranitidine hydrochloride, ranitidine N-oxide, ranitidine S-oxide and des-methylranitidine were from Glaxo (U.K.). Phenylpyramidol hydrochloride, used as internal standard, and all drugs tested for potential interferences were obtained from the Drug Analysis Division, Department of Medical Sciences (Bangkok, Thailand). HPLC-grade methanol, acetonitrile and tetrahydrofuran were obtained from Chameleon (Osaka, Japan). Ethyl acetate, 2-propanol (Carlo Erba, Italy), sodium acetate trihydrate (E. Merck, F.R.G.), sodium hydroxide (Eka Kemi, Sweden) and glacial acetic acid (E. Merck) were of analytical-reagent grade.

Stock solutions (20 μg/ml to 2 mg/ml) of ranitidine, the metabolites and phenylpyramidol were prepared separately in distilled water. All solutions were stored at 4°C and appeared to be stable for at least one month.

HPLC instrumentation and operating conditions

A Hitachi Model 655 A-11 high-speed liquid chromatograph (Tokyo, Japan), equipped with a Hitachi Model 655 A variable-wavelength UV monitor operating at 320 nm, a Rheodyne Model 7125 manual injector valve, with a 20-μl loop, and a Hitachi Model 655-71 data processor b were used. A 250 mm × 4.6 mm I.D. column packed with 10-μm strong cation-exchange resin (Partisil SCX) was obtained from Phenomenex (CA, U.S.A.). The mobile phase, pumped at a flow-rate of 1.9 ml/min, was acetonitrile–tetrahydrofuran–0.1 M sodium acetate buffer acidified with glacial acetic acid (pH 5) (36:7.5:56.5, v/v). All experiments were carried out at ambient temperature.

Sample preparation

Plasma samples. Human plasma (1 ml) was pipetted into a 15-ml screw-capped glass tube. The screw-cap was lined with a piece of aluminium foil to prevent possible leaching of chemicals and adsorption of the drugs onto the cap. After the addition of 1 ml of internal standard solution in acetonitrile, 40 μl of sodium hydroxide (10 M) and 4 ml of acetonitrile–ethyl acetate (1:1, v/v), the tube was vortex-mixed for 75 s and centrifuged at 800 g for 10 min. About 4.7 ml of the organic (upper) phase were removed and evaporated to dryness at 45°C under reduced pressure (Rotary evaporator, Model RE-15, Yamato Scientific, Japan). The residue was reconstituted in 60 μl of the mobile phase and vortex-mixed for 10 s. A 20-μl aliquot was then injected onto the HPLC column.

Urine samples. A 0.2-ml aliquot of human urine was mixed with 0.2 ml of internal standard solution in acetonitrile. After vortex-mixing for a few seconds, a 10- to 20-μl aliquot of this solution was injected onto the column.

Volunteer study

Two healthy volunteers (male, 24 years, 52 kg, and female, 30 years, 48 kg) were given single oral doses of 300 mg of ranitidine (Zantac tablets 300 mg, Glaxo) with 200 ml of water after an overnight fast. Venous blood samples were withdrawn and plasma fractions were separated immediately prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8 and 10 h after dosing. Urine samples were collected from each volunteer at periodic intervals for up to 24 h. The plasma and urine samples were stored frozen until analyses.

RESULTS AND DISCUSSION

Chromatography

Chromatograms from blank samples of human plasma and urine, samples spiked with known concentrations of ranitidine, the metabolites and the internal standard and plasma and urine samples from one of the volunteers are shown in Figs. 1 and 2. All the peaks were well resolved and did not interfere
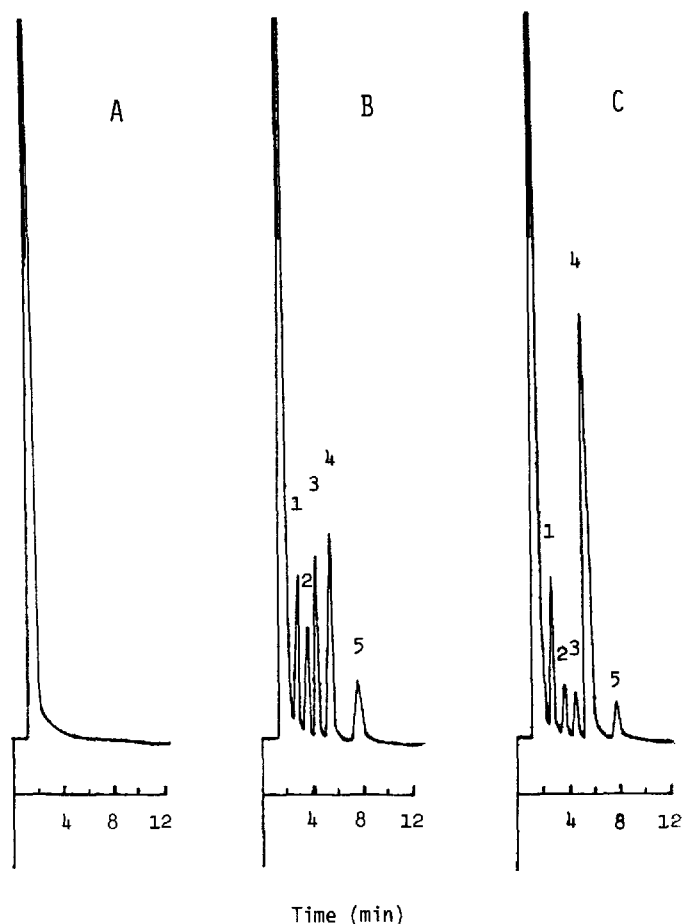
Fig. 1. Chromatograms of extracts from (A) blank human plasma, (B) human plasma spiked with 220 ng/ml ranitidine, 270 ng/ml ranitidine N-oxide, 125 ng/ml ranitidine S-oxide, 160 ng/ml desmethylranitidine and 250 ng/ml internal standard and (C) human plasma collected 6 h after an oral dose of 300 mg of ranitidine to subject 1. Peaks: 1 = internal standard; 2 = ranitidine N-oxide; 3 = desmethylranitidine; 4 = ranitidine; 5 = ranitidine S-oxide. Sensitivity setting = 0.004 a.u.f.s.

with endogenous substances. Under the conditions described, the retention times of ranitidine, ranitidine N-oxide, ranitidine S-oxide, desmethylraniti- dine and the internal standard were 5.3, 3.6, 7.5, 4.5 and 2.8 min, respectively. Variation of the pH of the mobile phase within ±0.2 units was not found to have significant effect on the resolution of the peaks or retention volumes. At pH higher than 5.3, the S-oxide metabolite was eluted a few minutes later. A wavelength of 320 nm was used for the detection since it provided maximum response for all compounds under investigation in the mobile phase used (Fig.
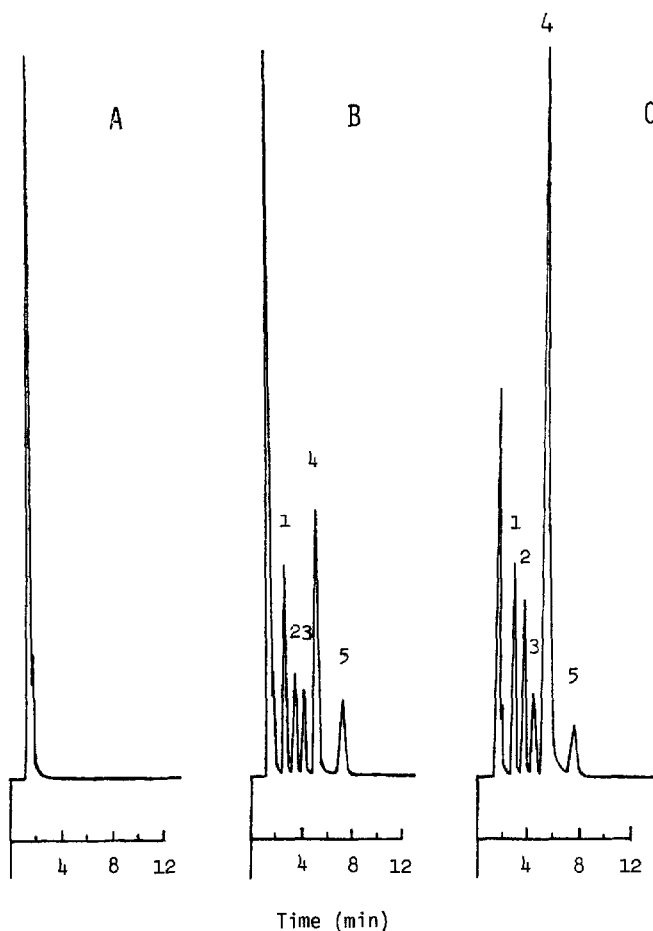
Fig. 2. Chromatograms of (A) blank human urine, (B) human urine spiked with 39.0 μg/ml ranitidine, 10.0 μg/ml ranitidine N-oxide, 11.5 μg/ml ranitidine S-oxide, 7.4 μg/ml desmethyl-ranitidine and 4.8 μg/ml internal standard and (C)human urine collected 4–8 h after an oral dose of 300 mg of ranitidine to subject 1. Peaks: 1 = internal standard; 2 = ranitidine N-oxide; 3 = desmethylranitidine; 4 = ranitidine; 5 = ranitidine S-oxide. Sensitivity setting = 0.016 a.u.f.s.

3). The selection of phenylpyramidol as the internal standard was based on the availability of the compound and its chromatographic and extraction behaviour.

The use of a cationic exchange column for the separation of ranitidine and its metabolites, all possessing protonated charges at acidic pH, was apparently distinctive. The equilibration between the stationary phase and the mobile phase could readily be achieved within 1 h. Under the present conditions, no deterioration of the column efficiency was observed after everyday use for several months for at least 300 injections. The selectivity of this method was also
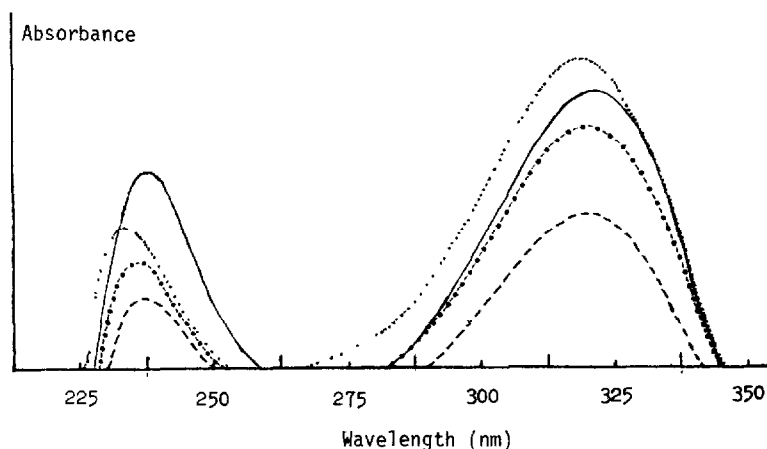
Fig. 3. UV spectra of ranitidine and its metabolites in the mobile phase (9 μg/ml solution). (·–·–·–·) Ranitidine; (- - - - -) ranitidine N-oxide; (·····) ranitidine S-oxide; (———) desmethylranitidine.

shown by the interference study. Among drugs tested (amitriptyline hydrochloride, dimenhydrinate, pheniramine maleate, diphenhydramine hydrochloride, oxyphencyclimine hydrochloride, propranolol, paracetamol, theophylline, tinidazole, ornidazole, diazepam, hydrochlorothiazide, tripelenamine hydrochloride, orphenadrine citrate and cimetidine), only propranolol and tripelenamine hydrochloride, with the retention times of 3 and 4.8 min, respectively, potentially interfere, with the internal standard and the desmethyl metabolite, respectively.

Extraction procedure

Several solvent systems were investigated for extraction of the compounds from plasma samples. They included methylene chloride, ethyl acetate and mixtures of various proportions of methylene chloride–acetonitrile, ethyl acetate–acetonitrile and methylene chloride–2-propanol. It was found that methylene chloride or ethyl acetate alone offered complete extraction for ranitidine but not for its metabolites. Increasing the polarity of the extraction solvents by adding acetonitrile or 2-propanol to the solvent system enhanced the recovery of the metabolites as well as of interfering substances. In the methylene chloride systems (methylene chloride–acetonitrile and methylene chloride–2-propanol), a small amount (less than 5%) of the two oxide metabolites could be extracted, while in the ethyl acetate–acetonitrile systems, only the N-oxide was recovered in a small amount. Ethyl acetate–acetonitrile (3:2, v/v) was selected as the extracting solvent because it provided satisfactory extraction efficiency for most compounds under study with modest interfering peaks.

The optimum volume of the extraction solvent for extraction from 1 ml of

TABLE I

EXTRACTION RECOVERIES OF RANITIDINE AND ITS METABOLITES FROM PLASMA

Compound	Spiked concentration (ng/ml)	Recovery (mean ± S.D., $n=4$) (%)
Ranitidine	55.0	100.36 ± 3.43
	1500.0	99.32 ± 1.37
Ranitidine N-oxide	60.0	31.05 ± 2.33
	875.0	29.76 ± 1.19
Ranitidine S-oxide	50.0	73.41 ± 2.58
	800.0	74.95 ± 3.85
Desmethylranitidine	26 0	80.70 ± 3.54
	650.0	79.70 ± 2.28
Internal standard	250.0	98.20 ± 4.53

plasma was found to be 5 ml. This should be adjusted according to the volume of plasma analysed, especially when extracting samples of more than 1 ml.

Table I shows the results of extraction recoveries from plasma obtained by comparing the peak heights of the drugs after extraction against corresponding peak heights from aqueous stock solutions. The recovery of ranitidine in the present study was virtually complete (99.8%) and the mean recoveries of the N-oxide, S-oxide and desmethyl metabolites were 30.4, 74.2 and 80.2%, respectively. In a previous study [8], extraction efficiencies of 47, 75, 100 and 83% were obtained for ranitidine, the N-oxide, S-oxide and desmethyl metabolites, respectively. This lower recovery of ranitidine may, in part, explain the inferior sensitivity of 20 ng/ml reported from that study.

Linearity, precision and sensitivity

Standard curves were constructed for each compound by plotting peak-height ratios versus standard concentrations. For the plasma analysis, the standard curves were linear over the ranges studied (40–4000 ng/ml for ranitidine and 20–1250 ng/ml for the metabolites), with correlation coefficients of 0.998 or higher. For the urine analysis, linear standard curves were obtained for up to 350 μg/ml for ranitidine, 100 μg/ml for ranitidine N-oxide and 60 μg/ml for the S-oxide and desmethyl metabolites, with the correlation coefficients of higher than 0.999.

Intra-day precision was evaluated by replicate analysis ($n=6$) of a pooled plasma or urine sample containing each of these drugs at various concentrations. Inter-day precision was similarly evaluated over one week ($n=5$). The results of intra-day and inter-day precision were presented in Tables II and III.

It is of interest to note that without the use of the internal standard, this method may still be used with excellent reproducibility, especially in analysing

TABLE II

INTRA-DAY VARIABILITY ($n=6$)

Compound	Plasma			Urine		
	Spiked concentration (ng/ml)	Calculated concentration (ng/ml)	C.V. (%)	Spiked concentration (μg/ml)	Calculated concentration (μg/ml)	C.V. (%)
Ranitidine	42.0	43 2 ± 0.76	1.76	1.74	1.71 ± 0.08	4.68
	505.0	499.4 ± 26 0	5.21	53 50	54.35 ± 2.30	4.23
	2012.0	2042.1 ± 70.7	3 46	316.80	330.04 ± 7 92	2.40
Ranitidine N-oxide	50.0	53.6 ± 2.97	5.54	0.69	0.70 ± 0.31	4.43
	303.0	291.7 ± 7.88	2.70	13.10	12.58 ± 0.42	3.34
	1045.0	1021.1 ± 57.7	5.65	98.30	100.59 ± 2.50	2.49
Ranitidine S-oxide	38.5	38.0 ± 3.50	9.21	0.69	0.66 ± 0.31	4.70
	296.0	304.3 ± 10.1	3.32	12.30	12.66 ± 0.74	5.85
	1050.0	1041.8 ± 50.0	4.80	40.80	41.03 ± 1.46	3.56
Desmethylranitidine	19 8	20.0 ± 1.77	8.85	0.54	0.52 ± 0.025	4.81
	201.0	198.4 ± 12.7	6.40	9 10	9.30 ± 0.40	4.30
	830 0	822.8 ± 30.1	3.66	40.60	42.77 ± 1.03	2.41

TABLE III

INTER-DAY VARIABILITY ($n=5$)

Compound	Plasma			Urine		
	Spiked concentration (ng/ml)	Calculated concentration (ng/ml)	C.V. (%)	Spiked concentration (ng/ml)	Calculated concentration (μg/ml)	C.V. (%)
Ranitidine	85.0	82.5 ± 6.11	7.41	2 94	2.97 ± 0 056	1.89
	1504 0	1540.4 ± 77.3	5.02	85.50	85 01 ± 2.00	2.35
Ranitidine N-oxide	52.0	51.9 ± 2.23	4.30	1.34	1.40 ± 0.04	2.86
	442 0	447.2 ± 30.9	6 91	24.10	23.85 ± 0.49	2.06
Ranitidine S-oxide	50 0	52.8 ± 2.86	5.42	1 34	1 36 ± 0.03	2.21
	450.0	446.0 ± 30.4	6.82	24 40	23.37 ± 1.20	5 14
Desmethylranitidine	22.5	23.4 ± 1.40	5.98	0.86	0.85 ± 0.024	2.82
	260.0	255.5 ± 15.46	6.05	17.40	17.98 ± 0.38	2.11

urine samples [with coefficients of variation (C.V.) of not more than 5%]. In the plasma analysis, where several steps requiring an accurate quantitation were involved, the coefficients of variation for all compounds ranged from 3.5 to 9.2%.

The lower limits of sensitivity of the present method, based on a signal-to-noise ratio of 3, were 5, 15, 10 and 4 ng/ml of plasma for ranitidine and the N-

oxide, S-oxide and desmethyl metabolites, respectively. In urine, the detection limits were higher than those obtained in plasma: 0.15 μg/ml for ranitidine and the N-oxide and desmethyl metabolites and 0.2 μg/ml for the S-oxide metabolite. These sensitivities appear to be sufficient for the determination of the drug levels usually encountered in the pharmacokinetic study of ranitidine.

Pharmacokinetic study

Fig. 4 shows the plasma concentration–time profiles of ranitidine and its metabolites following single oral doses of 300 mg of ranitidine to two healthy volunteers. The maximum plasma concentrations of ranitidine and the N-oxide, S-oxide and desmethyl metabolites were reached ca. 3 h after dosing and found to be 1113.5, 206.5, 98.3 and 65.6 ng/ml in subject 1 and 1366.8, 249.4, 66.0 and 81.0 ng/ml in subject 2, respectively. After 10 h, the concentrations of desmethylranitidine in both subjects were below 15 ng/ml. These plasma profiles of the three metabolites have never been previously illustrated.

Urinary recoveries of all compounds collected during the 24 h after drug administration are shown in Table IV. In plasma and urine from both subjects,
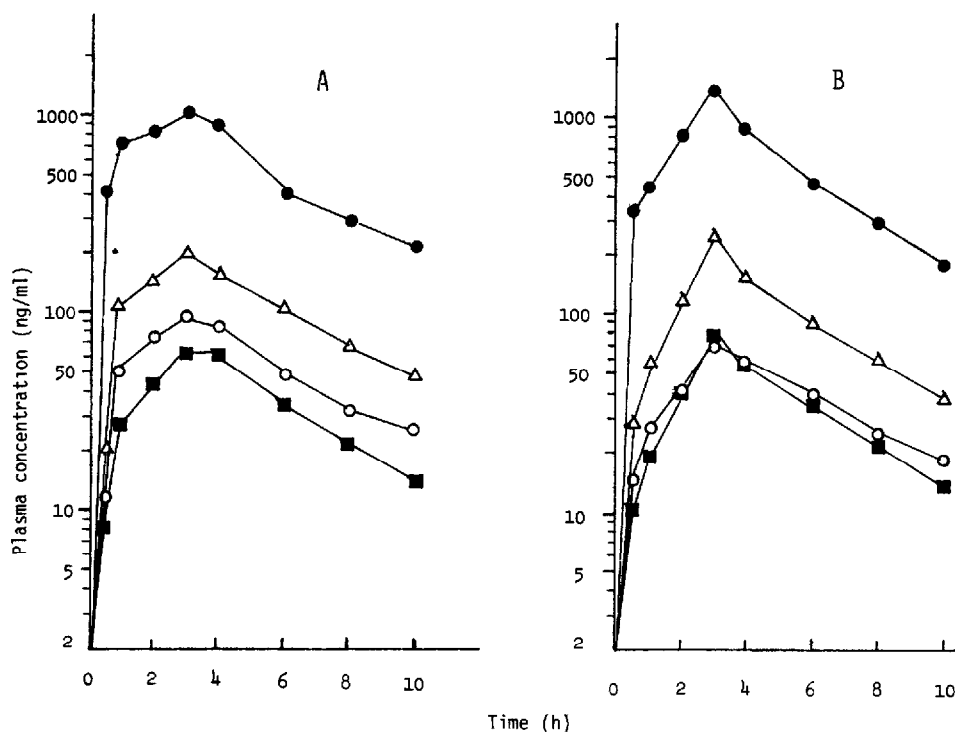

Fig. 4. Plasma concentration–time profiles of (●) ranitidine, (△) ranitidine N-oxide, (○) ranitidine S-oxide and (■) desmethylranitidine in subjects 1 (A) and 2 (B) following oral doses of 300 mg of ranitidine.

TABLE IV

URINARY RECOVERIES OF RANITIDINE AND ITS METABOLITES COLLECTED FROM TWO HEALTHY VOLUNTEERS DURING 10 h AND 24 h FOLLOWING ORAL ADMINISTRATION OF 300 mg OF RANITIDINE

	Cumulative urinary excretion (% of dose)							
	Ranitidine		Ranitidine N-oxide		Ranitidine S-oxide		Desmethylranitidine	
	10 h	24 h	10 h	24 h	10 h	24 h	10 h	24 h
Subject 1	41.03	43.41	6.52	6.85	1.42	1.59	2.74	2.90
Subject 2	34.62	37.12	4.78	5.24	1.92	2.07	2.08	2.26

ranitidine was present at relatively high levels compared with the metabolites. Among the metabolites, the N-oxide was found to be the major component. The elimination half-lives of ranitidine in these two subjects were 3.2 and 2.9 h, and the renal clearances, estimated by dividing urinary excretion by the area under the plasma concentration–time curve during 0–10 h, were 363.6 and 341.3 ml/min for subjects 1 and 2, respectively. These results are in good agreement with those reported earlier [2,15].

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