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RAPID ANALYSIS OF RANITIDINE IN BIOLOGICAL FLUIDS AND DETERMINATION OF ITS ERYTHROCYTE PARTITIONING

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SUMMARY

A reversed-phase ion-pair high-performance liquid chromatographic assay is described for the rapid and sensitive quantitation of the H_2-receptor antagonist ranitidine in human plasma and urine. The method involves a single-step extraction of the alkalinized sample with methylene chloride and analysis of the evaporated extract on a cyano column. Detection was performed by ultraviolet absorbance monitored at 318 nm. The overall run time of the assay was 5 min at a flow-rate of 2.0 ml/min. The limit of sensitivity was 1 ng/ml ranitidine in human plasma.

Urine and plasma samples collected from a subject after administration of an oral dose of 150 mg of ranitidine were analyzed by this method. Furthermore, the procedure was applied to determine the red blood cell partition coefficient of ranitidine in a concentration range up to 10 μg/ml.

INTRODUCTION

Ranitidine (I, Fig. 1), a potent histamine H_2-receptor antagonist, is a substituted aminoalkylfuran which unlike cimetidine does not contain an imidazole ring. It has been reported to be four times more active on a molar basis than cimetidine in inhibiting gastric acid secretion in man [1].

Several high-performance liquid chromatographic (HPLC) techniques for quantitation of ranitidine in biological fluids have been reported and applied in pharmacokinetic studies [2—6]. The combination of liquid chromatography and mass spectrometry has also been applied for analysis of ranitidine [7, 8]. These assays imply a reversed-phase HPLC system using a C_8 or C_{18} silica gel column. Different mobile phases have been used in each case and the methods of drug extraction prior to analysis varied widely. For routine investigations a

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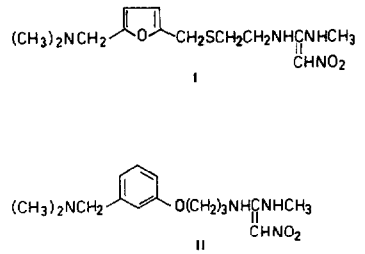

Fig. 1. Chemical structures of ranitidine (I) and the internal standard (II).

simple and rapid sample preparation procedure with a reproducible and effective extraction of ranitidine is desirable. Also the chromatographic system should provide a fast establishment of equilibrium between the mobile and the stationary phase, a stable column performance even after prolonged usage, as well as a relatively short run time for the assay.

However, none of the analytical methods that have been proposed so far have met all the above requirements. Furthermore, in following plasma ranitidine concentrations after therapeutic doses for more than 12 h, there is need to determine blood levels in the low nanogram range. Carey and Martin [2] and Vandenberghe et al. [4] described HPLC methods reaching a detection limit of 10 ng/ml of plasma. Only one HPLC assay has been reported [3] where a sensitivity as low as 5 ng/ml could be achieved. However, rapid column deterioration may result from continuous use of the basic mobile phase used in this assay.

This paper describes an approach for a simple assay method for ranitidine that is both rapid and highly sensitive employing a one-step single solvent extraction of the alkalinized sample. No further sample preparation was necessary and analysis was performed by a reversed-phase ion-pair HPLC system with a cyano column.

EXPERIMENTAL

Materials

Ranitidine hydrochloride and the internal standard (AH 20480) (II, Fig. 1) were gifts from Glaxo (Greenford, U.K.) and used as supplied. Acetonitrile (HPLC grade, Fisher Scientific, Fairlawn, NJ, U.S.A.) was used. All other reagents and solvents used were of analytical grade.

Instrumentation

The following instruments were used for the HPLC assay: high-pressure pump, Constametric III (LDC/Milton Roy, Riviera Beach, FL, U.S.A.), variable-wavelength UV detector (LDC/Milton Roy), manual injection valve 190 (Negretti Southampton, Hampshire, U.K.) with a 20-μl or 50-μl loop, integrator Model 3390A (Hewlett-Packard, Palo Alto, CA, U.S.A.) and a Du Pont Zorbax CN column, 15 cm × 4.6 mm I.D. (6 μm particle size) with a Du Pont 5 cm × 4.6 mm I.D. guard column filled with Spherisorb CN, (Phase Separations, Hauppage, NY, U.S.A.).

Assay conditions

The mobile phase was acetonitrile—0.05 *M* KH_2PO_4 (15:85) containing 0.005 *M* octanesulfonic acid. The flow-rate was 2.0 ml/min. Absorbance was monitored at 318 nm. The chart-speed was 0.5 cm/min.

Standard solutions and stability

Aqueous stock solutions of ranitidine (I) and internal standard (II) containing each 2 mg/ml were prepared in distilled water, 0.05 *M* sodium acetate solution (pH 8), phosphate buffer (pH 7) and acetate buffer (pH 3). For the red blood cell partitioning studies a stock solution of 2 mg/ml ranitidine in modified isotonic Hank's solution [9] was prepared. All solutions were stable when stored at 6°C for several months.

Calibration curves

Blank plasma and urine were spiked with various amounts of the appropriately diluted aqueous stock solutions of ranitidine and an according volume of internal standard solution. Blank plasma was obtained from Civitan Blood Bank (Gainesville, FL, U.S.A.). Urine was collected from a drug-free, caffeine-free volunteer.

Calibration curves were obtained by plotting peak-height ratios of ranitidine to the internal standard (II) versus concentrations of ranitidine. Their equations were calculated by the least-squares method using linear regression.

The ranitidine concentrations were chosen to cover a range of 0.5 ng/ml to 10 μg/ml in plasma and 5—500 μg/ml in urine.

Sample preparation

The spiked plasma samples (2 ml) were alkalinized with 200 μl of 5 *M* sodium hydroxide and vortexed for a few seconds. Methylene chloride (10 ml) was added. The tubes were shaken 10 min on a vortex mixer and then centrifuged for 10 min at 1200 *g*. The aqueous upper layer was aspirated off and discarded, and the organic layer transferred to a clean tube to achieve complete separation from proteins and traces of aqueous phase. The methylene chloride was evaporated to dryness under nitrogen in a water bath at 40°C. Prior to chromatography the residue was reconstituted in 100 μl of distilled water. Injection volumes of 20 μl were used in a concentration range of 0.1—10 μg/ml. Volumes of 50 μl were injected in the range below 0.1 μg/ml.

The spiked urine was diluted 1:50 with water, vortexed for a few seconds and then centrifuged for 5 min at 1200 *g*. The supernatant (1 ml) was extracted following the procedure described for plasma.

Red blood cell partition studies

Red blood cells were supplied by Civitan Blood Bank. The erythrocytes were gently suspended in an equal volume of isotonic Hank's solution [9] and centrifuged for 10 min at 1200 *g*. The washing procedure was repeated three times. The packed red blood cells (2 ml) were spiked with different amounts of ranitidine by adding the calculated volume of the drug's isotonic stock solution. After addition of the internal standard, Hank's solution was added to obtain a total volume of 4 ml of red blood cell suspensions. For the calibration

curves the same samples were prepared with 2 ml of Hank's solution instead of the red blood cells. Total ranitidine concentrations were in the range 0.01—10 μg/ml. The suspensions were allowed to equilibrate for 30 min. During this time the hematocrit was determined using a microcentrifuge with capillary tubes. After centrifugation for 10 min at 1200 *g*, 1.8 ml of the supernatant plasma water were removed and extracted to be analysed by HPLC.

The procedure was repeated with samples taken 15, 60 and 180 min after addition of the drug in order to investigate a time dependency of the red blood cell partitioning.

RESULTS AND DISCUSSION

Chromatography

Under the described conditions the chromatographic peaks of ranitidine and internal standard were well resolved and did not interfere with endogenous plasma or urine constituents. Typical chromatograms obtained following extraction of human plasma and urine are shown in Fig. 2.

The retention times of ranitidine and internal standard were 4.2 and 5.8 min at a flow-rate of 1.7 ml/min and 3.1 and 4.2 min at a flow-rate of 2.0 ml/min, respectively.

The limit of sensitivity was 1 ng/ml in plasma and with care 0.5 ng/ml could still be detected.

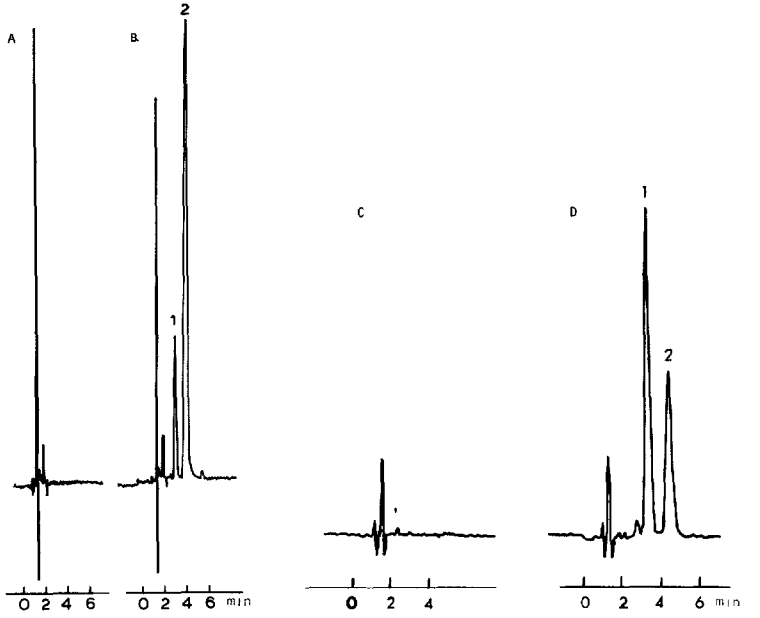

Fig. 2. Chromatograms corresponding to extracts of ranitidine from plasma and urine. (A) A 2-ml sample of blank human plasma; (B) a 2-ml sample of human plasma spiked with 40 ng/ml ranitidine (1) and internal standard (2) (0.01 a.u.f.s.); (C) 1 ml of blank urine diluted 1:50; (D) 1 ml of urine collected 4—8 h after oral administration of a 150-mg ranitidine dose, spiked with the internal standard (2) and diluted 1:50. Attenuation of integrator: 2. Peak 1 corresponds to a ranitidine concentration of 220 μg/ml in urine.

Precision and accuracy

The precision and accuracy of the method were evaluated by repetitive analysis of spiked human plasma samples at different drug levels. Results obtained from within-day assays are shown in Table I. The method yields an average coefficient of variation of 3.6%. Table II demonstrates the inter-assay precision with coefficients of variation of less than 9%. Plasma samples were analysed at intervals of two days.

TABLE I

ACCURACY AND PRECISION OF THE METHOD

Actual concentration (ng/ml)	Assayed concentration (mean ± S.D., $n = 4$) (ng/ml)	Coefficient of variation (%)
20	21.0 ± 0.50	2.4
40	39.6 ± 1.2	3.0
200	200.3 ± 15.0	7.5
500	483.5 ± 5.0	1.0
1000	1014.7 ± 42.1	4.2

TABLE II

DAY-TO-DAY REPRODUCIBILITY

Actual concentration (ng/ml)	Assayed concentration (mean ± S.D.) (ng/ml)	n	Coefficient of variation (%)
40	40.7 ± 3.62	4	8.9
80	78.5 ± 6.2	5	7.9
200	204.0 ± 12.0	6	5.9
300	295.3 ± 12.1	4	4.1

Calibration

Calibration curves in plasma were linear over the range 0.001—5 μg/ml. For urine calibration curves a linear relationship with correlation coefficients of 0.999 and higher was obtained in the range 5—500 μg/ml.

Application

Fig. 3 shows the plasma concentration–time curve of ranitidine after administration of a single oral 150-mg dose to one healthy volunteer. The appearance of two peak levels after oral administration has been observed previously [10, 11]. A representative plot of the cumulative urinary excretion (Fig. 4) following oral administration of 150 mg ranitidine also demonstrates the applicability of the assay. The urinary recovery of 28% of the dose is in good agreement with literature values [10].

Red blood cell—plasma water partitioning

The red blood cell partition coefficient (D) of a drug is defined [12] as the

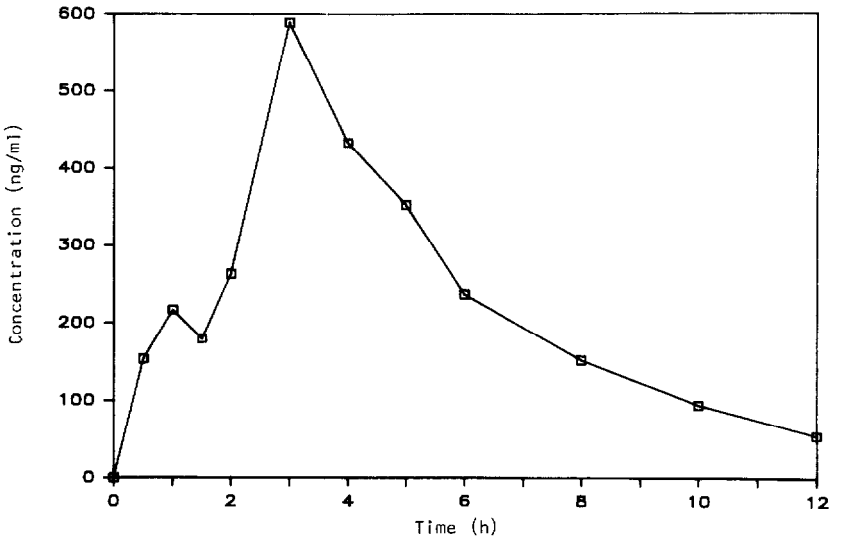

Fig. 3. Plasma concentration—time curve following oral administration of 150 mg ranitidine to one healthy volunteer.

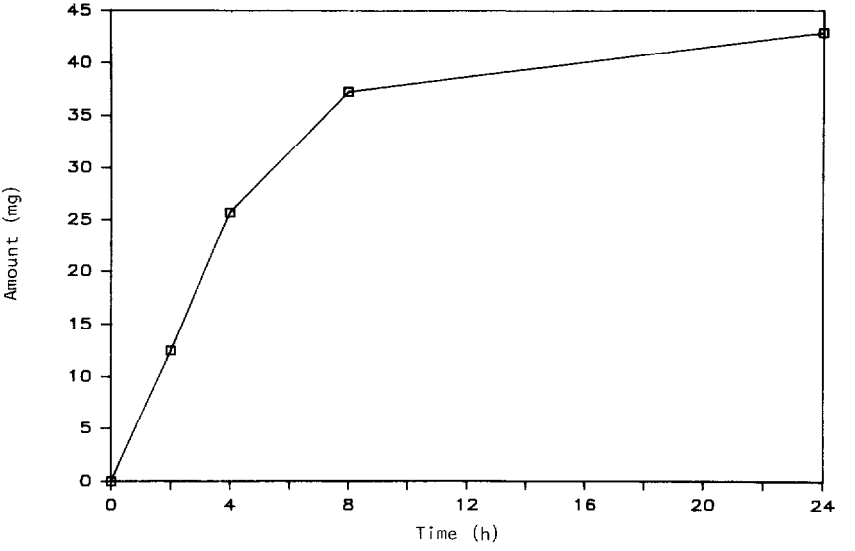

Fig. 4. Plot of ranitidine excretion in urine from one healthy volunteer 2, 4, 8 and 24 h after a single oral dose of 150 mg of the drug.

ratio of drug concentration in the erythrocyte, C_{RBC}, and in the surrounding plasma water, C_{PW} ($D = C_{RBC}/C_{PW}$).

The partition coefficient of ranitidine was measured to be 1.08 (± 0.13) for various concentrations and equilibrium times (Table III). No concentration dependence for partitioning in the concentration range up to 10 μg/ml was observed. Samples assayed after 30 min were not significantly different from those that were allowed to equilibrate for 15, 60 or 180 min. According to this, partitioning had been completed after 15 min and was not time-dependent within the studied range.

TABLE III

RED BLOOD CELL–PLASMA WATER PARTITION COEFFICIENTS (D) OF RANITIDINE DETERMINED FOR DIFFERENT RANITIDINE CONCENTRATIONS (C_{PW}) AND DIFFERENT INCUBATION TIMES

15 min		30 min		60 min		180 min	
C_{PW} (μg/ml)	D	C_{PW} (μg/ml)	D	C_{PW} (μg/ml)	D	C_{PW} (μg/ml)	D
0.025	1.00	0.010	1.04	0.026	0.90	0.497	1.01
0.039	1.08	0.019	1.18	0.045	1.30	0.662	1.18
0.095	1.10	0.033	1.18	0.090	1.28	0.860	1.14
0.206	0.92	0.452	1.26	0.373	1.19	1.097	1.00
0.304	0.97	0.076	0.97	0.696	1.20	1.305	0.99
0.683	1.26	0.088	1.06	0.911	1.26	1.494	1.00
		0.098	1.07			1.535	1.15
		0.155	0.92			1.960	1.02
		0.202	0.98			2.453	1.02
		0.275	1.19				
		0.411	0.94				
		0.469	1.17				
		0.683	1.26				
		0.918	1.24				
		5.541	0.80				
		6.179	0.94				
		11.025	0.81				
Mean	1.06		1.06		1.19		1.06
S.D.	0.11		0.15		0.13		0.07

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