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Short communication

Simple and robust high-performance liquid chromatographic method for the determination of ranitidine in microvolumes of human serum

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Abstract

A simple robust high-performance liquid chromatographic method is described for the determination of ranitidine in microvolumes of human serum. The drug of interest was isolated using liquid–liquid extraction with dichloromethane and back-extraction with 0.1% phosphoric acid and separation was obtained using a reversed-phase column under isocratic conditions, with ultraviolet detection at 313 nm. Intra-day and inter-day coefficients of variation ranged from 1 to 6% and 3 to 10%, respectively. Accuracy of the assay was less than 10% at all concentrations. The limit of detection and the limit of quantitation were 2 and 7 ng/ml, respectively. The linearity was assessed in the range 10–1000 ng/ml. It was shown that a group of common drugs co-administered with ranitidine did not interfere with its determination. The applicability of this method for the pharmacokinetic study of ranitidine following i.v. infusion in patients was demonstrated using only 100 μl of serum. The ruggedness of the assay was demonstrated over a three-year period.

Keywords: Ranitidine

1. Introduction

Ranitidine (N-[3-[[[5-(dimethylamine)methyl-2-furanyl]methyl]thio]ethyl]-N′-methyl-2-nitro-1,1-ethenediamine hydrochloride) is the H_2-receptor antagonist most frequently used in Intensive Care Units (ICUs) to prevent stress ulcer bleeding in the critically ill patient. The issue of optimal dosing for the prevention of stress ulcer bleeding remains to be elucidated. One of the hampering factors is the lack of knowledge about the pharmacokinetic–pharmacodynamic relationship and therapeutic concentrations for this clinical condition [1]. Pharmacokinetics play an important role in the outcome of therapy in this unstable heterogeneous population of critically ill patients. In order to monitor plasma levels of ranitidine in critical care patients and to carry out pharmacokinetic studies of the drug, a simple, sensitive and reproducible method for its determination is required. Quantitation of ranitidine in biological samples has been accomplished by high-performance liquid chromatography (HPLC) using liquid–liquid extraction [2–6] and automated solid-phase extraction (SPE) [7,8]. On the whole, these methods are time-consuming, require large volumes of serum or involve equipment not usually available in a clinical

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setting. A radioimmunoassay (RIA) for ranitidine in biological fluids, with a limit of detection of 2 ng/ml was reported [9]. Our paper describes a simple and useful HPLC method for ranitidine determination in microvolumes of human serum that does not require an evaporation step, and gives low limits of detection (LOD) and quantitation (LOQ).

2. Experimental

2.1. Equipment

The HPLC system consists of a Waters Instrument equipped with computer system for acquisition and integration of data (Maxima 820 chromatography data station), two M-45 pumps, a 484 variable-wavelength ultraviolet detector and a 715 Ultrawisp autosampler.

2.2. Reagents

Ranitidine hydrochloride and AH20480, N-[3-[5[[(dimethylamine) - methyl] - fenoxy]propyl]] - N′-methyl-2-nitro-1,1-ethenediamine, used as the internal standard, were received from Glaxo (Madrid, Spain). Water, methanol, acetonitrile and dichloromethane were provided by Prochem (Wesel, Germany). Potassium dihydrogen phosphate, sodium hydroxide and concentrated phosphoric acid were obtained from Merck (Darmstadt, Germany). Human blood and serum without drug were obtained from healthy volunteers. A 20% solution of Intralipid, Lipofundina MTC/LTC was received from B. Braun Medical (Barcelona, Spain). Bilirubin was provided by Fluka Chemika-BioChemika (Buchs, Switzerland). All chemicals used were of analytical-reagent grade.

2.3. Chromatographic conditions

The mobile phase was acetonitrile–10 m*M* phosphate buffer, pH 3.75 (15:85, v/v), filtered and degassed through a 0.45-μm Millipore filter and delivered at a flow-rate of 1.0 ml/min. Separation was accomplished at room temperature on a μBondapak C_{18} column (10 μm, 300×3.9 mm I.D.). The ultraviolet detector was set to 313 nm.

2.4. Extraction procedure

Venous blood samples were withdrawn to the Vacutainer tubes (Becton Dickinson Systems Europe, Meyland, France) and left at room temperature to coagulate. They were then centrifuged at 700 *g* for 10 min, and 100 μl of serum were added to a 1.5-ml polypropylene tube. The internal standard (100 μl) and 50 μl of 1 *M* NaOH were added. This mixture was carefully vortex-mixed. Extraction was performed by adding 1 ml of dichloromethane to the tubes and shaking for 1 min. After centrifugation at 700 *g* for 10 min, the whole organic layer was separated. To this, 100 μl of a 0.1% phosphoric acid solution were added. The mixture was vortex-mixed and left at room temperature for 5 min. The phosphoric layer was then separated and a 50-μl aliquot was injected into the HPLC system.

2.5. Drug standards

Working stock solutions for ranitidine and AH20480 were prepared in methanol at a concentration of 1 mg/ml and stored at 4°C. Internal standard, AH20480, was used as a 10 μg/ml solution in water. Serum standards from the aqueous solutions were prepared at concentrations of 10, 50, 100, 500 and 1000 ng/ml, using drug-free serum from healthy volunteers.

2.6. Quality control

Quality control (QC) samples were prepared at low, medium and high levels (40, 200 and 800 ng/ml) in the same way as serum standards and were analyzed on the day of preparation in sextuplicate (time 0), before starting pharmacokinetics studies, and again in duplicate on the same day as the patients' samples. Not all three levels were processed each day. The stability of the serum samples was monitored over the entire period that the samples were analyzed (around three years) from quality control samples [10].

2.7. Analytical variables

Absolute extraction recoveries of ranitidine and AH20480 from human serum were estimated using

standard samples at concentrations ranging from 10 to 1000 ng/ml of ranitidine ($n=6$) and a concentration of 10 μg/ml of the internal standard, by comparing the peak heights from processed serum standard samples with those from a calibration curve prepared from analytes in the mobile phase.The peak-height ratio of ranitidine to internal standard was plotted against the concentration of ranitidine. Serum standard samples (10, 50, 100, 500, 1000 ng/ml) were analyzed in sixtuplicate on one day during intra-assay validation. Inter-assay validation was assessed from the revalidation data made on days when patients' samples were analyzed. Linearity of standard curves, intra- and inter-assay precision (calculated as the coefficient of variation) and accuracy (calculated as relative error) were determined from validation and revalidation data. The limit of detection (LOD) and the limit of quantitation (LOQ) of ranitidine were determined from the peak and the standard deviation of the noise level, S_N. The LOD and LOQ were defined as the sample concentration of ranitidine resulting in a peak height of three and ten times S_N, respectively. The LOQ was also assessed by predefined accuracy and precision (coefficients of variation and relative error <20%).

2.8. Interference studies

A series of drugs normally used in the treatment of the critically ill patients were chosen to study their possible chromatographic interference, in aqueous solution, with the ranitidine peak. Some of these drugs, such as amikacin, metamizole, metronidazole and cefotaxime, are frequently used with ranitidine. The drugs whose retention times were similar to ranitidine or internal standard retention times were prepared and analyzed in serum. Hemolysis, lipemia and bilirubinemia effects were also studied.

2.9. Application

The assay was used in patients who were given 50 mg of ranitidine every 6 h in a short infusion of 15 min in pharmacokinetic studies. Samples from patients were taken during a dose interval prior to the dose ($t=0$ min), immediately after ($t=15$ min) and 5 min after the end of infusion ($t=20$ min), and at 4 and 6 h after administration of the dose. Comparison of peak-height ratios from the unknown samples with

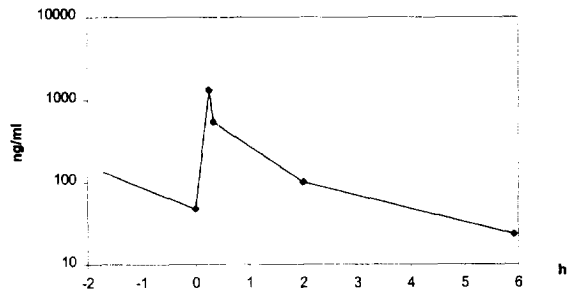

Fig. 1. Mean serum ranitidine concentration versus time profiles in a patient given a multiple infusion i.v. 50 mg dose.

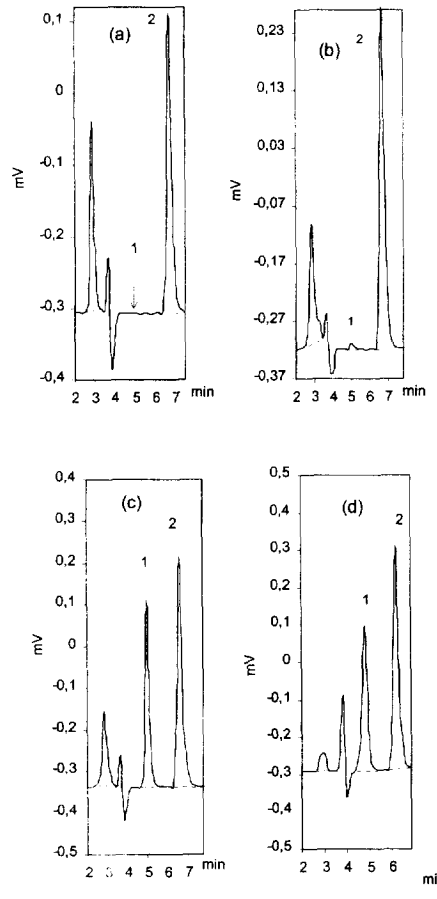

Fig. 2. (a) Chromatogram of blank pooled human serum. (b) Chromatogram of pooled human serum spiked with 10 ng/ml of ranitidine and 10 μg/ml of internal standard (peaks: 1=ranitidine; 2=AH20480, internal standard). (c) Chromatogram of pooled human serum spiked with 500 ng/ml of ranitidine and 10 μg/ml of internal standard (peaks: 1=ranitidine; 2=AH20480, internal standard). (d) Chromatogram of a serum sample from a patient at 20 min after dosing (peaks: 1=ranitidine; 2=AH20480, internal standard), the concentration of ranitidine was 539 ng/ml.

Table 1
Validation of the analytical method

Concentration added (ng/ml)	Concentration found (ng/ml)	C.V. (%)	Accuracy (%)
Intra-assay (n=6)			
10	11.1	5.7	10.9
50	48.9	1.3	−2.3
100	99.8	2.2	−0.2
500	495.7	2.7	−0.9
1000	999.6	0.8	−0.04
Inter-assay (n=13)			
10	10.6	9.1	6.3
50	50.4	6.7	0.8
100	100.7	2.7	0.7
500	497.1	2.2	−0.6
1000	995.7	2.7	−0.4

those from the calibration curve permitted quantitation of the assayed samples. Concentrations of ranitidine measured in serum samples obtained from a patient given a multiple infusion i.v. 50 mg dose of ranitidine are shown in Fig. 1.

3. Results and discussion

Fig. 2 illustrates representative chromatograms. Drug-free pooled human serum yielded clean chromatograms with no significant interfering peaks. Retention times of ranitidine and AH20480 were 4.9±0.2 and 6.5±0.3 min, respectively.

The extraction used in this method was simple and rapid to carry out. The method required small volumes of sample (100 μl) and organic solvent (1 ml), which is a clear advantage compared to the similar methods described [6]. Mean recoveries of extraction for six different concentrations of ranitidine in human serum (5, 10, 50, 100, 500 and 1000 ng/ml, $n=6$) were 66.4, 69.5, 64.8, 71.9, 67.0 and 81.2%, respectively, whereas the mean recovery for internal standard at a concentration of 10 μg/ml was 71.9%. The one-way ANOVA test demonstrated that there were no statistical differences between recoveries of different ranitidine concentrations ($p=0.3010$). Linearity of the standard curves used during the entire study was found in the range from 10 to 1000 ng/ml and was statistically confirmed (F test for lack of fit) [11]. The determination coefficient (r^2), the slope of the curve (b) and the y-intercept (a) for the straight lines were 0.9986±0.002, $1.67 \cdot 10^{-3} \pm 1.6 \cdot 10^{-4}$ and $5.25 \cdot 10^{-3} \pm 1.01 \cdot 10^{-2}$, respectively. The LOD of ranitidine was 2 ng/ml, comparable to the most sensitive method reported [5,6,9], whereas the LOQ was the lowest reported (7 ng/ml). The method demonstrated a good accuracy with values below ±15% of the nominal value [12]. Results of intra-assay validation and inter-assay validation are summarized in Table 1.

Neither of the drugs, nor the hemolysis, lipemia and bilirubinemia effects, tested for interferences, were found to interfere with the ranitidine and internal standard determination. The ruggedness of the method was studied from QC samples over time.

Table 2
Results of quality control

Concentration added (ng/ml)	Concentration found (ng/ml)	C.V. (%)	Accuracy (%)	n^a
40	41.1	9.6	2.6	31
200	202.8	11.5	1.4	46
800	741.7	6.9	−7.3	20

[a] Not all three levels were processed each day.

under different analysts, different lots of reagents and different columns [13,14]. The QC results show that ranitidine was stable in serum samples at −40°C for three years. Results of QC are shown in Table 2.

This analytical method has been applied to more than 500 clinical human samples in pharmacokinetic studies. Mean serum ranitidine concentrations, corresponding to an interval dose versus time profile in a patient, are illustrated in Fig. 1.

This paper describes a simple, sensitive, specific, rapid and robust reversed-phase HPLC method with UV detection for the measurement of ranitidine in human serum. It can also be successfully used with plasma samples. The method has proven suitable for use in clinical pharmacokinetic studies of ranitidine in humans.

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