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Short communication

Reliable method for the determination of ranitidine by liquid chromatography using a microvolume of plasma

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Abstract

The aim of the present study was to develop a simple method to measure ranitidine, using 100 μl of plasma, by high-performance liquid chromatography with a Symmetry C_{18} column and UV detection at 313 nm. Linearity was assessed in the range from 50 to 1500 ng ml^{-1} and had a correlation coefficient of 0.999. The inter- and intra-day coefficients of variation were less than 7%. The limits of detection and quantitation were 5 and 15 ng ml^{-1}, respectively. Drug levels were determined satisfactorily in three patients. A simple and reliable method was developed which uses a microvolume of plasma, particularly useful in low-weight children.

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1. Introduction

Ranitidine (*N*-[3-[[[5-(dimethylamine)methyl-2-furanyl]methyl]thio]ethyl]-*N′*-methyl-2-nitro-1,1-ethenediamine hydrochloride) is a histamine H_2-receptor antagonist most frequently used in intensive care units (ICUs) to prevent stress ulcer bleeding in the critically ill patient [1]. Gastro-oesophageal reflux (GOR) is a very common disease in children, caused by the return of gastric contents to the esophagus. The incidence of GOR in newborns is ~24%; these children can suffer later from low and very low body-weight. Ranitidine is used either for the oral treatment of peptic ulcer in children or to treat GOR in hospitalized pediatric patients, some of very low weight. It is thus been necessary to develop a reliable method to measure plasma levels of ranitidine in the pediatric population employing a minimum volume of plasma for quantitation.

Castañeda-Hernández et al. [2] employed 1 ml of plasma for bioequivalence studies. They reported a simple extraction technique, however, the mobile phase included several solvents, which complicated the technique. This is a disadvantage because determination requires greater resources and more time. Other authors, such as Schaiquevich et al. [3] and Segelman et al. [4], reported methods with some

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advantages, for instance, simplicity and sensitivity; however, they employed 100 μl or more of rat plasma. A small number of articles report ranitidine measurement in plasma of children, but only Hare et al. [5] have specifically determined this. They used 500 μl of plasma, which is still a disadvantage. The purpose of the present study was to develop a method for HPLC determination of ranitidine using a microvolume of plasma (100 μl), which can be especially useful to measure the drug in neonates with low or very low body-weight, where the sample volume must be as small as possible.

2. Experimental

2.1. Reagents

All solvents and chemicals were analytical grade. Ranitidine hydrochloride was commercially available from ICN Biomedicals (OH, USA) and nizatidine was available from Proquifa (Mexico). Acetonitrile and methanol were HPLC grade (Merck, Darmstadt, Germany), and deionized water was obtained from a Simplicity 185 water purification system (Millipore, Molsheim, France). Potassium dihydrogen phosphate, sodium hydroxide and dichloromethane were from Merck (Darmstadt, Germany).

2.2. Equipment

The chromatographic system consisted of a model 590 programmable pump, a model 717 automatic injector, and a model 486 variable wavelength UV–Vis detector. A Symmetry C_{18} 5 μm (150×3.9 mm I.D.) reversed-phase column was used. Chromatographic data were analysed by Millennium software version 32.0. All items were purchased from Waters (Milford, MA, USA).

2.3. Chromatographic conditions

The mobile phase consisted of 0.05 *M* potassium dihydrogenphosphate (pH 6.5)–acetonitrile (88:12, v/v). Following preparation, the mobile phase was filtered through a membrane filter and air was extracted by vacuum. The flow rate was kept constant at 1 ml min^{-1} at room temperature (25 °C). Absorbance was measured at 313 nm.

2.4. Extraction procedure

The method used here was a modification of methods reported by Castañeda et al. [2] and Wong et al. [6]. A reference solution of 2000 ng ml^{-1} ranitidine was prepared. Aliquots were diluted in plasma for each planned concentration to determine linearity and other parameters. For analysis, 100 μl of each concentration were added to 50 μl of a reference solution of 1000 ng ml^{-1} nizatidine (internal standard) and alkalinized with 50 μl of a 2.5 *M* sodium hydroxide solution, adding 3 ml of dichloromethane as extraction solvent. This mixture was carefully vortexed for 1 min and centrifuged at 800 *g* for 5 min. The organic layer was evaporated at 45 °C under a gentle nitrogen stream and the dry residue was dissolved in 200 μl of mobile phase. Then 100-μl aliquots were injected into the chromatographic system.

2.5. Quality control

Quality control samples were prepared at low, medium and high concentrations (150, 700 and 1300 ng ml^{-1}) using the same method as for plasma standards. The samples were used to determine the inter- and intra-day precision and accuracy of the method. To assay stability, plasma samples were stored at −10 °C for 0, 14 and 31 days. The assay for specificity consisted of the addition of certain clinically use drugs, such as amoxicillin, sulfamethoxazole plus trimethoprim, acetaminophen and naproxen, to ranitidine.

2.6. Analytical variables

Absolute extraction recovery of ranitidine and nizatidine from human plasma was estimated using standard samples at concentrations ranging from 50 to 1500 ng ml^{-1} of ranitidine ($n=5$) and 500 ng ml^{-1} of the internal standard. The reported value is the mean of the percent of recovered ranitidine obtained by comparing peak height data in plasma and in the solution at the concentrations marked on the curve. Standard plasma samples (150, 700 and

1300 ng ml^{-1}) were analyzed during 1 day (n=5) for intra-day validation. Inter-day validation was assessed from the revalidation data obtained on different days. Linearity of standard curves, inter- and intra-day precision and accuracy were determined from validation and revalidation data. The levels of ranitidine were measured in three patients of low body-weight, 2.0 h post-dose.

3. Results

Calibration curves were derived from injections of six concentrations of ranitidine (50, 100, 250, 500, 1000 and 1500 ng ml^{-1}) plus 500 ng ml^{-1} nizatidine.

The following regression equation was obtained:

$$y = 8.795x + (-197.56) \quad r = 0.999 \text{ and } r^2 = 0.998$$

Mean recovery was 100.9% (C.V. 10.5%) for ranitidine. The limits of detection (LOD) and quantitation (LOQ) were 5 and 15 ng ml^{-1}, respectively. LOD was calculated on the basis of 3σ and LOQ on the basis of 10σ, according to Miller and Miller [7].

The coefficient of variation was less than 7%. Results of the parameters to test the validation of our method and the quality control determined by precision and accuracy of the analytical method are summarized in Table 1. Fig. 1 shows: (a) specificity results where none of the four added drugs was detected, (b) retention times of the compounds; as can be seen, the retention times were 2.8 and 4.2 min for ranitidine and nizatidine, respectively, (c) chromatogram obtained from a patient sample; 770.3 ng ml^{-1} of ranitidine was measured in this case. The levels of the other two patients were 1010.1 and 1098.5 ng ml^{-1}. Patients were given 150 mg of ranitidine every 12 h. Comparison of peak-height ratios from unknown samples with those from the calibration curve allowed measurement of the assayed samples of patients. As regards stability on days 0, 14 and 31 of storage, the coefficients of variation were 6.3, 14.7 and 33.3%, respectively. The samples remained stable for up to 14 days; the 15% coefficient of variation was not surpassed.

4. Discussion

To obtain 100 μl of plasma, ~200 μl or a maximum of 250 μl of blood were needed. From the results and based on the criteria for acceptance of the tested validation parameters (such as coefficient of variation and correlation between response of detection and added amount), we concluded that the precision, accuracy and specificity of the developed method is good. Besides, one chromatographic separation takes ~6 min. This is one of the advantages of our method, because previous reports required much longer [2,5]. In general, a separation lasts ~8–15 min mainly due to ranitidine retention time. This retention time ranges from 5.9 to 7.7 min, while the retention time in our method is 2.8±0.15 min. Ranitidine concentration in patients was found to be within the linearity range of the method and we decided to draw samples at 2 h post-dose due to the value of C_{max} (reported between 2 and 3 h). One of the characteristics of methods for therapeutic drug monitoring is that they must be fast, because results are often urgently required. This is why we consider our method optimal for therapeutic drug monitoring,

Table 1
Precision and accuracy of the HPLC method for ranitidine determination

Concentration of ranitidine added (ng/ml)	Inter-assay reproducibility(n=9)		Intra-assay reproducibility(n=5)		Accuracy (%)[b]
	Concentration found after extraction (mean±SD)[a] (ng/ml)	C.V. (%)	Concentration found after extraction (mean±SD)[a] (ng/ml)	C.V. (%)	
150	154±8	5	152±6	4	+1.33
700	711±28	4	718±18	3	+2.57
1300	1409±44	3	1363±45	3	+4.85

[a] Calculated from linear regression equation.
[b] Accuracy=(concentration found−concentration given)/concentration given×100.

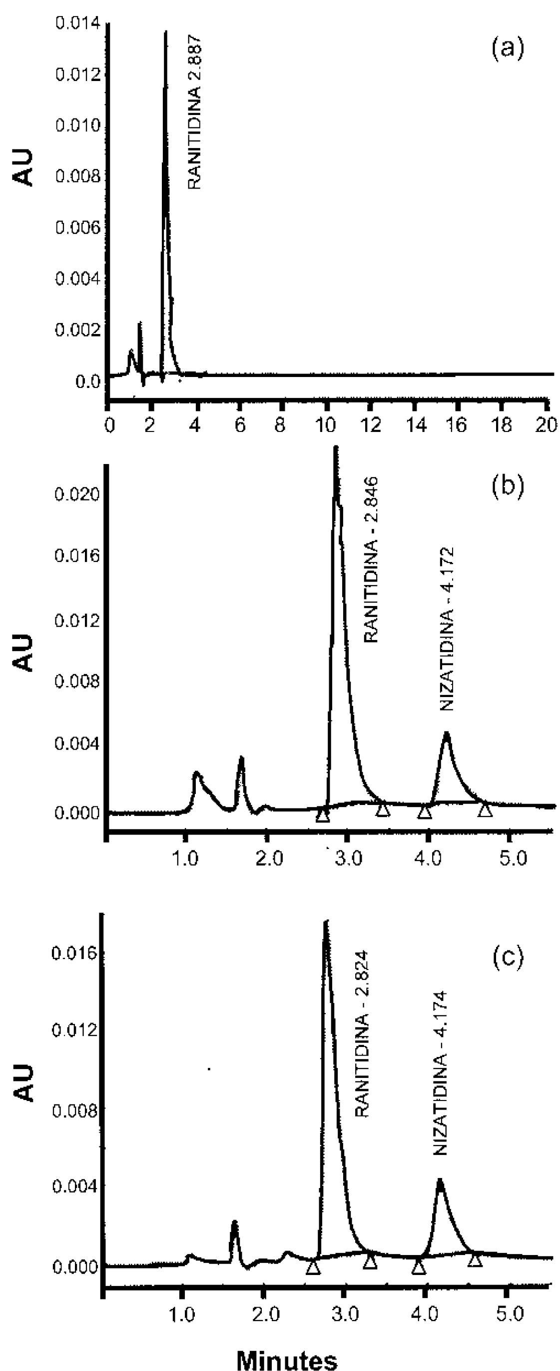

Fig. 1. Determination of ranitidine: (a) specificity test, (b) retention times of compounds and (c) in patient sample.

especially in children of low and very low body-weight who are unfortunately very common in our hospital. However, the method may also be applied to children of normal body-weight. Besides, the method proposed here can be applied to future studies in drug pharmacokinetics, bioavailability and bioequivalence, which are greatly needed in our country. After applying this method successfully to measure the plasma levels of three patients in the newborn intensive care unit, where most patients are of low or very low body-weight, we can state that the developed method is simple, fast and precise.

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