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Validated HPLC method for the determination of ranitidine in plasma

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A validated HPLC method for the determination of ranitidine in human plasma is presented. Sulfanilamide as internal standard (IS) was used. Plasma samples were purified by solid phase extraction (SPE) using a copolymeric [poly(divinyl-benzene-co-*N*-vinylpyrrolidone)] column ("Oasis Waters"). Mobile phase consisting of dibasic potasium phosphate 0.08 M/acetonitrile/methanol/triethylamine 0.05% (89.5 : 3 : 7 : 0.05) pH5 was used at a flow rate of 0.9 ml/min on a C18 column (Nova-Pack, 3,9 × 300 mm, Waters). The eluate was monitored using an UV/Vis detector set at 300 nm. Ratio of peak area of ranitidine to sulfanilamide was used for the quantitation of plasma samples. FDA criteria for bioanalytical validation was used to validate the method. Linearity was assessed between 100–1600 ng/ml, the limit of quantitation was 100 ng/ml and recovery was greater than 94%. Accuracy, precision and selectivity met the current recommendations for bioanalytical method validation. The method was successfully used in a bioavailability study of a ranitidine tablet in healthy volunteers.

1. Introduction

A validated method for quantitation of drugs in biological fluids is essential in bioavailability and bioequivalence studies. Such method has to be sensitive enough to properly define the plasma concentration profiles of the drug. It is of general acceptance that the last concentration in the terminal phase has to be 1/10 to 1/5 of the maximum in order to establish at least 80% of the total area under the curve of plasma concentration versus time through experimental data. Solid phase extraction (SPE) is an efficient way to purify and concentrate biological samples. Among different fillers, a copolymer [poly(divinyl-benzene-co-*N*-vinylpyrrolidone)] ("Oasis Waters") has demonstrated a good performance to extract basic, acidic and neutral compounds [1–3].

H_2 receptor antagonists like ranitidine competitively inhibit the interaction of histamine with H_2 receptors in a highly selective way. Ranitidine is 50–60% orally absorbed presenting a concentration peak about 1.5 h after oral administration; a second maximum could be observed at 4 h due to enterohepatic recycling. Its pharmacokinetics are characterized by a half life of 2.5 h, a volume of distribution of 1–2 l/kg and a clearance of 10 ml/min/kg. Plasma protein binding is 15% and presystemic clearance has been reported [4, 5].

The aim of this work was to develop and validate an analytical method to quantify ranitidine in human plasma, appropriate for application in a bioavailability study of a 300 mg ranitidine tablet, produced by a local manufacturer. According to the dose, plasma concentrations ranging from 120 to 1200 ng/ml were expected.

2. Investigations, results and discussion

A typical chromatogram of a plasma sample is shown in Fig. 1. No endogenous interfering peaks were observed in 6 plasma blanks at the retention times of ranitidine and sulfanilamide, confirming the selectivity of the method. Both analyte and IS were well separated with retention times of 11.6 and 4.2 min, respectively.

Calibration curves were linear in the proposed range; r^2 for individual curves ranged from 0.99 to 0.997.

The LOQ was found to be 100 ng/ml, base in a RSD < 20% and accuracy of 80 to 120%. Inter-day and intra-assay precision fulfilled the FDA criteria: CV not higher than 20% for lower concentrations and not exceeding 15% for the other levels. Accuracy, measured as mean relative error, was also within the limits of 80–120% for lower and 85–115% for higher concentrations (Tables 1 and 2) [6].

Recovery was 94.2% for low concentration (100 ng/ml), 95.1% for medium concentration (600 ng/ml) and 97.3% for high concentration (1600 ng/ml).

Standard solutions were stable for 2 weeks under refrigerated conditions and protected from light; plasma samples were stable for 2 months at −20 °C and 3 freeze and thaw cycles.

Plasma concentrations of ranitidine in 24 human volunteers following the administration of 300 mg dose are shown in Fig. 2. Few samples exceed 1600 ng/ml, and in this situation half of plasma volume was cleaned up; inversely, for those samples with concentrations lower than 100 ng/ml, 1 ml of plasma sample was purified. In both situations, corrections were made to calculate the real sample concentration.

In conclusion, the analytical method considering SPE through the copolymer [poly(divinyl-benzene-co-*N*-vinyl-

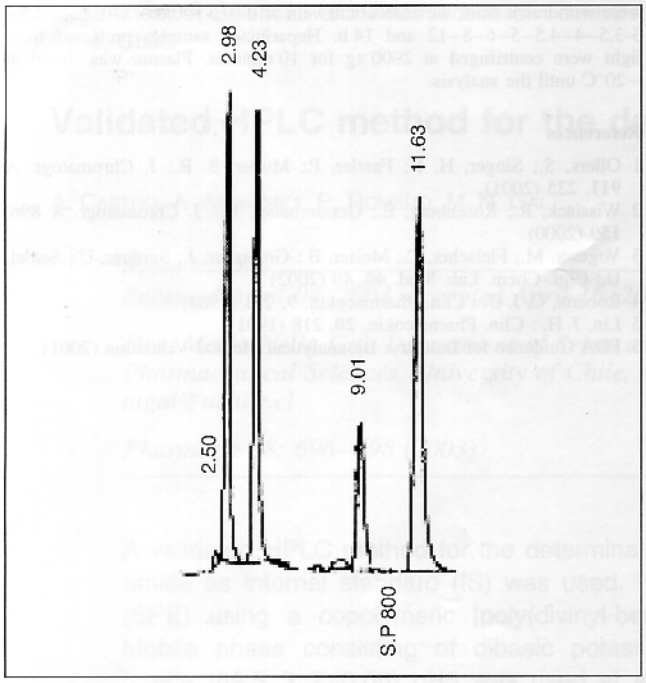

Fig. 1: Typical chromatogram obtained from a volunteer shows IS and ranitidine at 4.2 and 11.6 min, respectively

pyrrolidone)] and HPLC reversed phase chromatographic conditions resulted in a simple and efficient method for purification and quantitation of ranitidine in plasma samples, with suitable sensitivity, selectivity, accuracy and precision. Hence the method was appropriate for conducting a bioavailability study.

3. Experimental

3.1. Chemicals and reagents

Ranitidine and sulfanilamide pure samples were gifted by Laboratorios Chile. Methanol (Fisher Scientific) and acetonitrile (Fisher Scientific) were of HPLC grade. Triethylamine and dibasic potassium phosphate were p.a. from Merck. Double distilled water was used during HPLC process. Solid phase extraction was performed using an Oasis (Waters) column filled with 60 mg of a copolymer [poly(divinyl-benzene-co-N-vinylpyrrolidone)].

3.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a HPLC pump model L-6000 A (Merck Hitachi), fitted with a 200 ul loop. Separation was achieved on a reversed phase column Novapack RP-18 300 × 3.9 mm (Waters). Mobile phase consisting of dibasic potassium phosphate 0.08 M/acetonitrile/methanol/triethylamine 0,05% (89.5 : 3 : 7 : 0.05) pH5, was used at a flow rate of 0.9 ml/min. The eluate was monitored at 300 nm using an UV/Vis detector model L-4200 UV-VIS (Merck Hitachi).

3.3. Standard solutions

A primary stock solution of 0.2 mg/ml of ranitidine was prepared in the mobile phase; the same concentration was used for sulfanilamide, dissolved in methanol not exceeding 5% of the total volume and water to reach the final volume. Appropriate dilutions of ranitidine were made in the mobile phase to produce working stock solutions of 1, 4 and 8 mg/ml and 0.02 mg/ml of sulfanilamide. Calibration samples were prepared by spiking 500 µl of blank plasma with an appropriate amount of drug and 125 µl of the sulfanilamide 0.02 mg/ml solution. All the solutions were protected from light.

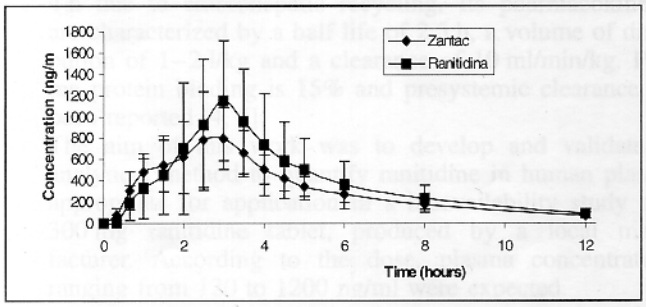

Fig. 2: Mean plasma concentrations (± SD) of ranitidine after the administration of the reference and study products in 24 healthy volunteers

Table 1: Interday precision obtained from 6 calibration curves with 3 levels of spiked control samples

	Concentration level		
	100 ng/ml	600 ng/ml	1600 ng/ml
Curve 1	146.44	593.11	1459.78
Curve 2	125.90	588.90	1558.90
Curve 3	99.00	626.27	1477.18
Curve 4	118.64	617.73	1558.64
Curve 5	116.80	591.8	1449.80
Curve 6	96.22	609.56	1630.67
Mean	117.17	604.56	1522.49
SD	18.46	15.55	71.56
C.V (%)	15.76	2.57	4.70
Mean relative error (%)	17.17	0.76	4.84

Table 2: Intraday precision obtained from a calibration curve with 6 sets of spiked control samples

	Concentration level		
	100 ng/ml	600 ng/ml	1600 ng/ml
	97.19	506.01	1206.41
	102.20	480.96	1359.72
	109.22	535.07	1388.78
	93.19	532.06	1502.00
	106.21	513.03	1520.04
	101.20	559.12	1431.86
Mean	101.54	521.04	1401.47
SD	5.83	27.07	114.05
C.V (%)	5.74	5.20	8.14
Mean relative error (%)	1.54	13.16	12.41

3.4. Extraction procedure

125 µl of internal standard solution (0.02 µg/ml) were added to 500 µl of blank plasma using tubes protected from light. SPE column was activated with 1 ml of methanol and washed with 1 ml of water. Sample passed slowly through the column, then was washed with 1 ml of methanol/water 95/5 and was eluted with 1 ml of methanol; the eluate was received in a light protected conic tube and evaporated to dryness under nitrogen stream at 40 °C. The residue was reconstituted with 300 µl of mobile phase and vortex for 30 s. The solution was filtered through a 0.22 µm GVWP Millipore filter. A 200 µl aliquot was injected into the HPLC system.

3.5. Selectivity

To demonstrate the selectivity of the method, blank plasma samples from 6 different subjects were used.

3.6. Linearity and limit of quantitation

Calibration curves were prepared in the range 100–1600 ng/ml by spiking 500 µl of plasma with appropriate amounts of drug and internal standard on the day of analysis. The limit of quantitation (LOQ) was defined as the lowest concentration at which the relative standard deviation and deviation from the nominal concentration were less than 20%.

3.7. Precision and accuracy

The repeatability (intra-assay precision), the intermediate (inter-day) precision and the accuracy were calculated from data obtained during 6-day validation. Standard curve between 100 and 1600 ng/ml and quality control samples from high, medium and low range of the standard curve were prepared. Precision was expressed as the coefficient of variation and accuracy was expressed as the mean relative error of the interpolated concentrations.

3.8. Recovery

Recovery was determined at three concentrations (low, medium and high), comparing extracted samples with unextracted standards that represent 100% recovery

3.9. Stability

Stability of refrigerated stock solutions, frozen samples (−20 °C) and samples after three freeze and thaw cycles were determined.

3.10. Application to bioavailability study

The assay method was used in a bioavailability study in 24 healthy subjects. Doses of 300 mg of ranitidine were administered after an overnight fast. Zantac® 150 mg (2 tablets) was used as reference. Blood samples were withdrawn from the antecubital vein at 0–0.33–0.67–1–1.5–2–2.5–3-3.5–4–4.5–5–6–8–12 and 14 h. Heparinized samples protected from light were centrifuged at 2000 xg for 10 minutes. Plasma was stored at −20°C until the analysis.

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