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Validated HPLC method for the determination of ranitidine in human serum and its application in a clinical pharmacokinetic study

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Ranitidine is a histamine (H_2) receptor antagonist. It is widely accepted as effective in the treatment of gastric and duodenal ulcer [1, 2]. The oral bioavailability of ranitidine is $52 \pm 11\%$. Ranitidine is hardly protein bound ($15 \pm 3\%$), clearance is 10.4 ± 1.1 ml/min · kg and has volume of distribution of 1.3 ± 0.4 L/kg in humans [3]. Metabolic studies of ranitidine have revealed that it is mainly metabolized by oxidation to give N-oxide XIII, S-oxide XV, and desmethyl ranitidine XIV[4, 5]. Ranitidine is a highly water soluble drug with a log P value of 0.2 and pKa values of 8.2 and 2.7 [5].

Various analytical methods using HPLC [6–8] and radioimmunoassay [9] have been developed for the determination of ranitidine in biological samples. Each HPLC technique has its own advantages and disadvantages. The radioimmunoassay technique was found to be more sensitive, with a limit of 2 ng/ml, and requires only 0.1 ml of plasma sample. But it is more expensive than the HPLC methods.

This paper describes a simple HPLC method which enables the determination of ranitidine with great accuracy, even at low ranitidine drug concentrations in serum. Here, the possibility of using ion-pair HPLC method of ranitidine in serum was investigated and an HPLC method using sodium lauryl sulphate as a counter ion has been developed. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

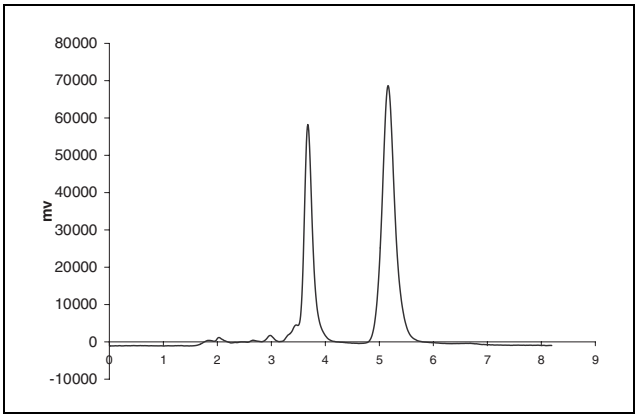

Fig.: Typical HPLC Chromatogram of ranitidine 2 h after dosing: serum sample from a subject collected 2 h after dosing. The respective concentration was 485.2 ng/ml of ranitidine. IS – Internal standard

A chromatogram of serum sample obtained 2 h after oral administration of ranitidine and blank serum sample of one of the volunteer is shown in the Fig. No endogenous interfering peaks were visible in individual blank serum at the retention times of ranitidine and metoclopramide (internal standard I.S.), thereby confirming the specificity of the analytical method. Both the analyte and I.S. were well separated with retention times of 3.6 and 5.2 min, respectively (Fig.). System suitability parameters for the method were as follows: theoretical plates for ranitidine and I.S. were 2722 and 2465 respectively; tailing factor was less than 1.5 for both ranitidine and I.S.

The ratio of peak area of ranitidine to that of I.S. was used for the quantification of ranitidine in serum samples. The equation of the calibration curve obtained from 8 points was $y = 0.0004x + 0.0043$; ($r^2 = 0.9977$).

The limit of quantification (LOQ), established by determining the concentration of four spiked calibration standards having a reproducibility with a relative standard deviation (RSD) less than 20% and an accuracy of 80 to 120% was found to be 10 ng/ml. Using this method, it is possible to further increase sensitivity, by increasing the serum/injection volume. The intra-day RSD ranged from 0.79–3.66, 2.42–4.75, 0.64–1.27 and 0.61–1.08 for 10, 100, 500 and 1000 ng/ml respectively. These values were within the limits (<15%) specified for inter and intra-day precision [5, 10]. The recovery of ranitidine from serum was estimated to be 10, 100, 500 and 1000 ng/ml concentrations. The absolute recoveries ranged from 70–80%.

Table: Intra and inter-day precision of ranitidine determination in serum

Concentration (ng/ml)	Day	Mean concentrations (ng/ml)		
		Mean	S.D.	R.S.D
Intra day variation (n = 3)				
10	0	10.03	0.08	0.79
	1	9.99	0.12	1.20
	5	10.09	0.37	3.66
	10	9.96	0.15	1.5
100	0	97.61	2.37	2.42
	1	100.57	4.78	4.75
	5	97.37	2.57	2.63
	10	101.47	3.25	3.2
500	0	495.27	6.33	1.27
	1	501.7	4.76	0.95
	5	502.17	3.48	0.69
	10	498.53	3.22	0.64
1000	0	995.23	5.35	0.54
	1	996.57	10.77	1.08
	5	1000.23	10.01	1.0
	10	997.0	6.1	0.61
Inter day variation (n = 12)				
10		10.03	0.08	0.8
100		98.27	2.84	2.89
500		495.03	5.05	1.02
1000		990.03	10.05	1.01

Mean pharmacokinetic parameters of ranitidine in 8 human volunteers following oral administration of 150 mg ranitidine were as follows: peak concentration of 402.34 ± 184.34 ng/ml (C_{max}, mean $\pm$ SD) for ranitidine reached at 2.6 ± 1.1 h (t_{max}, mean $\pm$ SD). The half-life was found to be 4.4 ± 1.3 h. Systemic exposure, area under the serum concentration ($AUC_{(0-\square)}$) was found to be 2154 ± 834 ng · h/ml. These parameters were comparable with those reported earlier [3, 11, 12].

These experiments confirm that the present method for determination of ranitidine in human serum is simple, sensitive, specific, precise and accurate and requires relatively small volumes of serum (500 µl). The calibration curve was linear in the concentration range from 10 to 1000 ng/ml and hence, the method is suitable for conducting pharmacokinetic studies.

Experimental

1. Materials

Ranitidine and metoclopramide pure samples were gifted by Glaxo India Ltd, India and Astra-IDL, India, respectively. Acetonitrile (HPLC grade) was obtained from Qualigens Chemicals, Mumbai, India. Ammonium acetate (AR grade), sodium lauryl sulfate (AR grade) and Dichloromethane (AR grade) were purchased from S.D. fine chemicals, Mumbai, India, Loba chemie Pvt. Ltd., Mumbai, India, respectively. Ranitidine 150 mg tablet (Zenetac 150®) were obtained from Glaxo India Ltd., Mumbai, India. Double distilled water was used during the entire HPLC procedure.

2. Extraction procedure

To 500 µl of serum, 10 µl of metoclopramide solution (1 mg/ml) and 600 µl of 2M NaOH were added and mixed well for 2 min. To this approximately 500 mg of NaCl and 8 ml of dichloromethane were added and vortexed for 5 min followed by centrifugation at 5000 rpm for 30 min. Organic phase was separated and evaporated under nitrogen gas at 45 °C. The residue was reconstituted in 100 µl of mobile phase and 20 µl of this solution was spiked on to the HPLC column.

3. Chromatographic conditions

The HPLC system (Shimadzu, Japan) consisted of a LC-10AT solvent delivery module, a SPD-10A, UV-visible spectrophotometric detector with LC10 software. The column used was Tracer analytica, Nucleosil, C8 (stainless steel column of length 25 cm and internal diameter of 0.4 cm packed with porous silica spheres of 5 µ diameter). A mobile phase consisting of 50 mM ammonium acetate containing 25 mM sodium lauryl sulfate (pH 3.8, adjusted with glacial acetic acid), acetonitrile and methanol mixture (40 : 40 : 20, v/v) was used at a flow rate of 1.0 ml/min. The eluate was monitored at 330 nm. The sensitivity was set at 0.005 AUFS.

4. Application to a clinical pharmacokinetic study

The assay method was used to determine ranitidine concentrations in serum following oral administration ofa ranitidine 150 mg tablet to 8 healthy male human volunteers after an overnight fast. Blood samples (5 ml) were withdrawn from the ante cubital vein at the intervals of 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h following drug administration. The samples were allowed to clot and centrifuged at 3500 rpm for 15 min. The serum was separated and stored at −20 °C until the commencement of analysis.

Pharmacokinetic parameters like peak serum concentrations (C_{max}), time to reach peak concentrations (T_{max}), area under the curve (AUC), elimination half-life ($t_{1/2}$), volume of distribution (Vd/f) and total clearance (CL/f) for ranitidine were obtained for each subject using a computer program KINETICA (Inna phase corporation, 1999) meant for calculation of model independent parameters.

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