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A validated HPLC method for the determination of ranitidine in human plasma: Application to bioavailability studies

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ABSTRACT

A simple and precise reversed-phase high performance liquid chromatography (HPLC) method for the determination of ranitidine in human plasma was developed and validated. Using 4-aminoantipyrene as an internal standard (IS), separation was achieved on Symmetry Shield RP-18 column. The mobile phase, 0.02 M potassium phosphate (dibasic), acetonitrile, and methanol (80:10:10, v/v) was delivered at a flow rate 1.0 ml/min. The eluent was monitored spectrophotometric at 317 nm. Plasma samples were prepared using centrifree filters. No interference in blank plasma or of commonly used drugs was observed. The relationship between the concentration of ranitidine in plasma and peak area ratio of ranitidine to the IS was linear over the range of 0.03–2.0 µg/ml. The intra-day and inter-day coefficients of variation were ≤ 8.0% and ≤ 11.0%, and corresponding biases were ≤ 13.4% and ≤ 8.3%, respectively. Mean extraction recovery of ranitidine and IS from plasma samples was 99.4% and 87%, respectively. The method was used to determine ranitidine level in plasma samples obtained from healthy subject and to assess stability of ranitidine in plasma under various conditions encountered in the clinical laboratory.

KEYWORDS

Ranitidine;
4-Aminoantipyrene;
Human plasma;
HPLC.

INTRODUCTION

Ranitidine hydrochloride, (CAS: 66357-59-3), (*N*-(2-[(5-[(dimethylamino) methyl] furan-2-yl) methylthio]ethyl)-*N'*-methyl-2-nitroethene-1,1-diamine), is an H2-receptor antagonists that inhibits stomach acid production. It is mainly used in the treatment of gastrointestinal ulcers and hyper acidity[1]. Its oral bioavailability is about 50%, with a mean peak plasma concentration of 0.44 - 0.54 µg/ml at about 2- 3 hours after ingestion of one 150 mg[2].

Several analytical methods have been reported for the determination of ranitidine in plasma including high performance liquid chromatography[3-9], capillary electrophoresis[10-11], radioimmunoassay[12], and liquid chromatography mass spectrometry[13], using either liquid-liquid extraction[14-17] or solid-phase extraction[18-21] for sample preparation. Most of the reported methods involve multiple extraction steps associated with increase complexity and time of sample preparation. Further, some of this reported methods[10-12] are not economically feasible for routine use in pharmacokinetic studies where numerous samples have to be analyzed.

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Campanero et. al[22] reported a simple and sensitive method for the determination of ranitidine in human plasma using a single step solvent extraction. Although the method is simple, requires at least one milliliter of plasma.

This paper describes a simple and precise assay that requires only 0.5 ml human plasma and does not involve extraction. The method was validated and used to determine ranitidine level in plasma samples from a healthy subject. Further, it was used for determination of stability of ranitidine under various laboratory conditions.

EXPERIMENTAL

Apparatus

Chromatography was performed on HPLC Waters Alliance e2695 Separation Module, consisting of quaternary pump, auto sampler, column thermostat, and 2998 photodiode array (PDA) detector. A reversed-phase Symmetry Shield RP-18 column (150 x 4.6 mm, 5μm) and (Symmetry C_{18}, 5-μm 4.6 x 10 mm) guard column (Waters Associates Inc, Milford, MA, USA) were used for separation. Data were collected with a Pentium IV computer using Empower Chromatography Software.

Chemical and material

All reagents were of analytical-reagent grade unless stated otherwise. Ranitidine hydrochloride (USP) was purchased from USP, Rockville, MD, USA, and 4-Aminoantipyrene from Sigma, St. Louis MD, USA. Acetonitrile, methanol (HPLC grade), phosphoric acid, and potassium phosphate (dibasic) were purchased from Fisher Scientific, Fairlawn, NJ, USA. Water for HPLC analysis was generated by "reverse-osmosis" and further purified by passing through a Synergy Purification System (Millipore Co., Bedford, MA, USA). Drug-free human plasma was obtained from the blood bank of King Faisal Specialist Hospital & Research Centre (KFSHRC) Riyadh, Saudi Arabia. Samples from volunteers were collected after obtaining approval from the Research Ethical Committee of KFSHRC.

Chromatographic conditions

The mobile phase was composed of 0.02 M dibasic potassium phosphate (pH=6.5 adjusted with phosphoric acid) acetonitrile, and methanol (80:10:10, v/v). Before delivering into the system, the mobile phase was filtered through 0.45 μm polyetersulfone membrane and sonicated under vacuum for 5 minutes. The analysis was carried out under isocratic conditions using a flow rate of 1.0 ml/min at 23°C and a run time of 10 minutes. Chromatograms were recorded at 317 nm using a PDA detector.

Preparation of standard and quality control samples

Stock solution of ranitidine hydrochloride (0.1 mg/ml) in methanol and 4-aminoantipyrene (0.1 mg/ml) in water were prepared and diluted with blank human plasma and water, respectively to produce working solutions of 2.0 μg/ml and 10.0 μg/ml. Nine calibration standards in the range of 0.03-2.0 μg/ml were prepared in human plasma. Five quality control (QC) samples were prepared as follows: 0.03 μg/ml, 0.09 μg/ml, 0.45 μg/ml 1.0 μg/ml, and 1.8 μg/ml were prepared in human plasma, and stored at -20 ºC until used.

Sample preparation

Aliquots of 0.5 ml of calibration curve, quality control, or volunteer samples were allowed to equilibrate to room temperature. 150 μl IS working solution (10 μg/ ml in water) were added and vertexed for 30 seconds. Samples were filtered using centrifree (Millipore, Waters USA). 75 μL, of the filtrate was injected in HPLC system.

Stability studies

Three QC samples were used for ranitidine stability studies: 0.03, 0.45, and 1.8 μg/ml: Five aliquots of each QC sample were extracted and immediately analyzed (baseline). Five aliquots of two QC samples (0.03 or 1.8 μg/ml) were allowed to stand on the bench-top for 24 hours at room temperature before being processed and analyzed (counter stability, 24 hours at room temperature) five aliquots were stored at –20 °C for 1-27 weeks before being processed and analyzed (long term freezer storage stability), and ten aliquots were processed, reconstituted, and stored at room temperature for 24 hours or 48 hours at –20 C before analysis (autosampler stability). Fifteen aliquots of two QC (0.45 and 1.8 μg/ml) were stored at –20 °C for

24 hours. They were then left to completely thaw unassisted at room temperature. Five aliquots of each sample were extracted and analyzed and the rest returned to –20 °C for another 24 hours. The cycle was repeated three times (freeze-thaw) stability.

Method validation

The method was validated according to standard procedures described in the US Food and Drug Administration (FDA) bioanalytical method validation guidance[23]. The validation parameter included: specificity, linearity, accuracy, precision, recovery, and stability.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Under the optimal experimental conditions consisting of a mobile phase of 0.02 M dibasic potassium phosphate (pH=6.5 adjusted with phosphoric,) acetonitrile, and methanol (80:10:10, v/v) delivered at flow rate 1.0 ml/min, ranitidine, IS and plasma components exhibited a well defined chromatographic separation within a 10 minute run. The retention times of ranitidine and IS were around 3.8 and 9.6 respectively.

Specificity

Specificity is defined the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. Potential interfering substances in plasma samples include endogenous components, metabolites, and decomposition products. We screened six batches of blank human plasma and eight frequently used medications (aspirin, acetaminophen, omeprazole, nicotinic acid, ascorbic acid, caffeine, ibuprofen, and diclofenac) for potential interference. No interference was found in plasma and none of the drugs co-eluted with ranitidine or the IS. Figure 1 depicts a representative chromatogram of drug free human plasma used in the preparation of standard and quality control samples.

Limits of quantification, detection and linearity

The lower limit of quantification of ranitidine in human plasma defined as the lowest concentration on the calibration curve that can be determined with acceptable precision and accuracy (i.e., coefficient of variation and relative error ≤ 20%) was 0.03 µg/ml, whereas the lowest detection limit was 0.015 µg/ml. Linearity of ranitidine was evaluated by analyzing ten curves of nine standard concentrations over the range (0.03-2.0 µg/ml) prepared in human plasma. Figure 2 represents an overlay of chromatograms of extracts of 0.5 ml human plasma spiked with the IS and one of nine concentrations of ranitidine. The peak area ratios were subjected to regression analysis. Figure 3 depicts the ten calibration curves that were used over the period of method validation. The mean regression equation was $y = 1.9174 x – 0.0200$. The suitability of the calibration curves was confirmed by back-calculating the concentration of ranitidine from the calibration curves (TABLE 1).

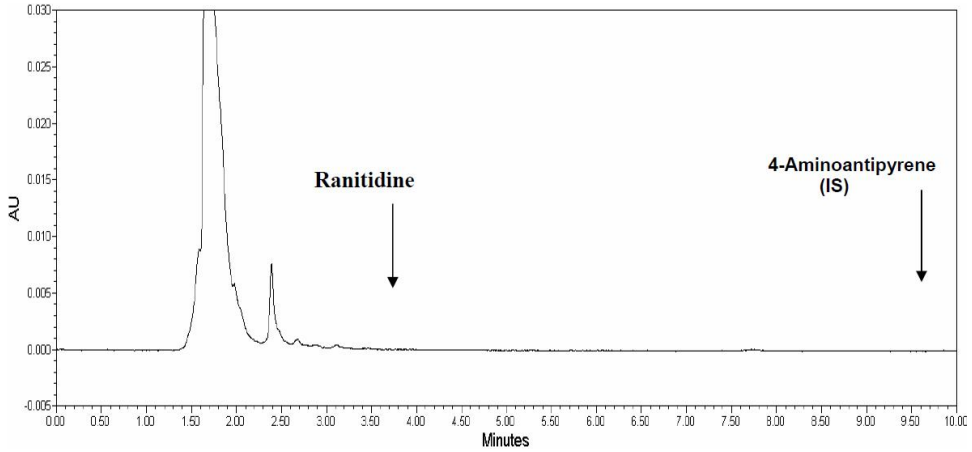

Figure 1 : Representative chromatogram of a drug-free human plasma. The arrows indicate the retention times of ranitidine (3.8 min) and the internal standard, 4-aminoantipyrene (9.8min)

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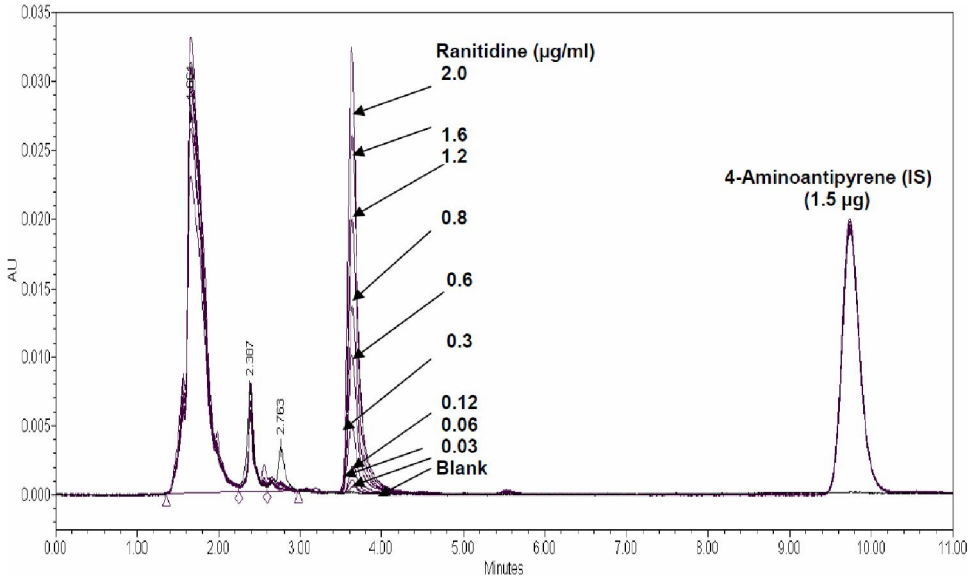

Figure 2 : Overlay of chromatograms of extracts of 0.5 ml human plasma spiked with the internal standard (IS) and one of ten concentrations of ranitidine, 0.00, 0.03, 0.06, 0.8, 0.12, 0.30, 0.60, 1.20, 1.60, and 2.00 ìg/ml.

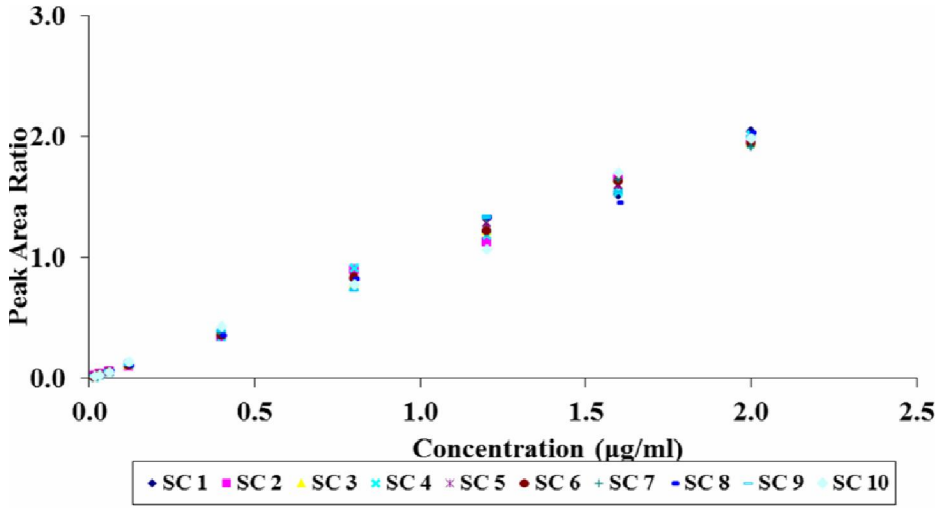

Figure 3 : Ten calibration curves used during method validation.

Accuracy and precision

According to standard procedure[23], accuracy and precision were determined for four QC concentrations (0.03, 0.09, 1.0, and 1.8 µg/ml). The inter-day precision and accuracy (bias %) of the assay were determined over three different days. The intra-day (n=10) and inter-day (n=20) coefficients of variation were ≤ 8.0% and ≤ 11.0%, and biases were ≤ 13.4% and ≤ 8.3%, respectively. The results are summarized in TABLE 2. The results indicate that the method was reliable within the studied concentration range.

Recovery

The absolute recovery of ranitidine was assessed by direct comparison of absolute peak areas from plasma vs. mobile phase samples, using five replicates for each of the four QC concentrations (0.03, 0.06, 1.0 and 1.8 µg/ml). Similarly, the recovery of the IS was determined by comparing the peak areas of the IS in 5 aliquots of human plasma spiked with 15 µg/ml IS with the peak areas of equivalent samples prepared in mobile phase. The results are presented in TABLE 3**.** Mean recovery of ranitidine and IS was 99% and 87%, respectively.

Stability

The stability of the ranitidine in processed and

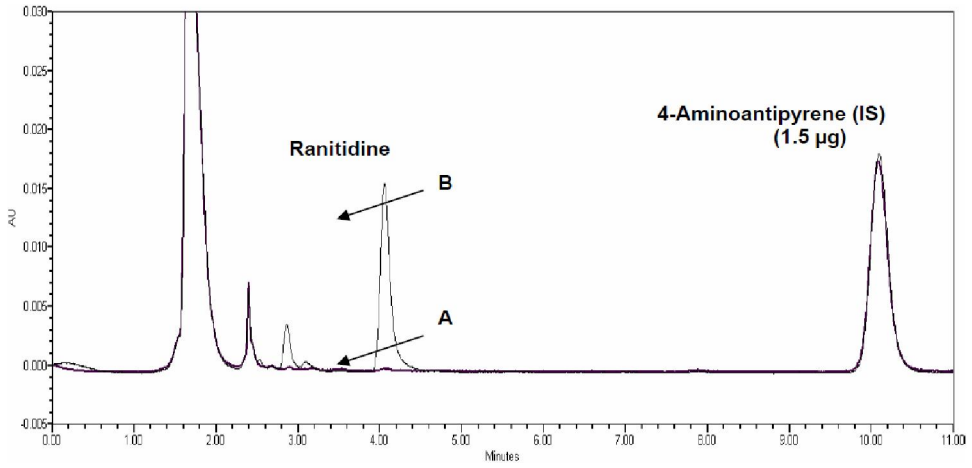

Figure 4 : An overlay of chromatograms of plasma samples obtained from a healthy volunteer before (A) and 2.5 h after (B) ingestion 150 mg ranitidine hydrochloride.

TABLE 1: Back-calculated ranitidine concentrations from ten calibration curves

Nominal Level (µg/ml)	Calculated Level (µg/ml)		CV (%)	Bias (%)
	Mean	SD		
0.03	0.0333	0.0026	7.8	11.0
0.06	0.0590	0.0049	8.3	-1.7
0.12	0.1201	0.0034	2.8	0.1
0.30	0.2862	0.0129	4.5	-4.6
0.60	0.5833	0.0148	2.5	-2.8
0.80	0.7816	0.0136	1.7	-2.3
1.20	1.1789	0.0280	2.4	-1.8
1.60	1.5894	0.0232	1.5	-0.7
2.00	2.0104	0.0181	0.9	0.5

SD, standard deviation; CV, standard deviation divided by mean measured concentration x100 Bias, measured level - nominal level divided by nominal level x 100.

TABLE 2: Intra - and inter-day bias and precision of ranitidine assay

Nominal Level (µg/ml)	Measured Level (µg/ml)		CV (%)	Bias (%)
	Mean	SD		
Intra-day (n=10)				
0.03	0.0326	0.0026	8.0	8.7
0.09	0.0964	0.0040	4.1	7.1
1.0	1.0849	0.0431	4.0	8.5
1.8	2.0411	0.1305	6.4	13.4
Inter-day (n=20)				
0.03	0.0317	0.0035	11.0	5.7
0.09	0.0930	0.0060	6.5	3.3
1.0	1.0430	0.0540	5.2	4.3
1.8	1.9496	0.1354	6.9	8.3

SD, standard deviation; CV, standard deviation divided by mean measured concentration x100 Bias, measured level - nominal level divided by nominal level x 100.

TABLE 3 : Recovery of ranitidine and the internal standard from 0.5 ml of human plasma

Concentration (µg/ml)	Human Plasma*	Mobile Phase*	Recovery (%)
Ranitidine 0.03	4101 (386)	3943 (96)	100
0.09	13677 (503)	13390 (219)	100
1.0	161318 (1472)	172722 (1548)	93
1.8	306674 (2116)	313223 (3763)	98
Internal Standard 10	271274 (5723)	312545 (1404)	87

*** Mean peak area (SD), n = 5.**

TABLE 4 : Stability of ranitidine under various clinical laboratory conditions

Stability (%)							
Plasma samples							
Nominal	Unprocessed		Processed		Freeze-Thaw		
Level (µg/ml)	24 hrs RT	27 wks -20 °C	24 hrs RT	48 hrs -20 °C	Cycle 1	2	3
0.03	100	100	97	98	-	-	-
0.45	-	-	-	-	100	100	100
1.8	100	100	100	100	97	100	89

Stability (%) = mean measured concentration (n=5) at the indicated time divided by mean measured concentration (n=5) at baseline x 100. *Spiked plasma samples were processed and analyzed immediately (baseline, data not shown), after 24 hours at room temperature (24 hrs RT), after freezing at –20 ºC for 27 weeks (27 wks. –20 ºC), or processed and then analyzed after storing for 24 hours at room temperature (24 hrs RT) or 48 hours at –20 ºC (48 hrs -20 ºC).

unprocessed plasma samples was investigated. No significant decrease in the measured concentration or change in chromatographic behavior of the ranitidine or the IS was observed. Processed samples containing ranitidine (0.03 and 1.8 µg/ml) found to be stable 24

hours at room temperature and 48 hours at —20°C (97% and 98%), respectively. Plasma samples containing ranitidine (0.03 and 1.8 μg/ml) stored at –20 °C were found to be stable (98% and 100%) for at least 27 weeks and plasma samples containing ranitidine (0.45 and 1.8 μg/ml) stable at least after three freeze-thaw cycles (100% and 89%). TABLE 4 summarizes the results of stability studies of ranitidine. Similarly, ranitidine stock solution (100 μg/ml, methanol) found to be stable for 24 hours at room temperature and 27 weeks at -20°C (100% and 94%) respectively.

Application to a volunteer sample

Figure 4 depicts an overlay chromatogram of samples collected from a volunteer before and after 2.5 hours ingestion of a single dose of 150 mg ranitidine. The measured levels of ranitidine were zero and 0.52 μg/ml, respectively.

CONCLUSION

The described HPLC assay is simple, precise, and accurate for measurement of ranitidine in human plasma. The assay requires only 0.5 ml plasma and utilizes a simple filtration procedure for sample preparation. The assay was applied to monitor stability of ranitidine under various conditions generally encountered in the clinical laboratories. Further, the assay was successfully applied to determine levels of ranitidine in samples obtained from a healthy volunteer.

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