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Development of an HPLC method for the determination of ranitidine and cimetidine in human plasma following SPE

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Abstract

A selective, sensitive and accurate high-performance liquid chromatographic method has been developed, validated and applied for the determination of ranitidine and cimetidine in plasma samples. The effects of mobile phase composition, buffer concentration, mobile phase pH and concentration of organic modifiers on retention of investigated drugs were investigated. Sample preparation was carried out by adding an internal standard, famotidine, and the clean-up procedure was accomplished using solid-phase extraction (SPE). This method uses ultraviolet detection, the separation used a Lichrocart Lichrospher 60 RP-select B column and the mobile phase consisted of 0.2% triethylamine (TEA), 0.04 mol l^{-1} KH_2PO_4 at pH 6.8 and 14% acetonitrile. The recovery, selectivity, linearity, precision and accuracy of the method were evaluated from spiked human plasma. The method has been implemented to monitor ranitidine levels in clinical samples.

Keywords: Ranitidine; Cimetidine; High-performance liquid chromatography

1. Introduction

Ranitidine, cimetidine and famotidine are histamine H_2-receptor antagonists. The most prominent effects of H_2-receptor antagonists are on basal acid secretion; less profound but still significant is suppression of stimulated (feeding, gastrin, hypoglycemia, or vagal stimulation) acid production [1].

There are several investigations concerning the determination of ranitidine and/or cimetidine in pharmaceutical preparations by high performance liquid chromatography (HPLC) [2–5].

A number of assay methods for determination of histamine H_2-receptor antagonists in biological fluids have been reported [6–23]. Maurer [7] reviewed chromatographic procedures for the identification and quantification of 60 histamine H_1- and 10 H_2-receptor blockers in biosamples. Vinas et al. [8] reported on a liquid chromato-

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graphic method for the determination of ranitidine and its metabolites in biological fluids using post-column fluorescence derivatization. Another method for the determination of ranitidine entailed direct injection of the plasma samples after deproteination using perchloric acid has been proposed by Ahmadiani and Amini [9]. Many authors have performed the determination of ranitidine [10–14] or cimetidine [15] in plasma, serum or urine after liquid–liquid extraction using dichloromethane [10–12], a mixture of acetonitrile–ethyl acetate [13], acetonitrile [14] or ethyl acetate [15]. However, the disadvantage of these methods employing liquid–liquid extraction from biological fluids is that they involve several steps yielding poor separation from the plasma, serum or urine endogenous interferences and gave highly variable and relatively low recoveries.

On the other hand, Xu et al. [16] proposed a method for quantitative analysis of cimetidine in human plasma using liquid chromatography coupled with atmospheric pressure chemical ionization and selected reaction monitoring mass spectrometry. In the published methods, a fully automated solid-phase extraction (SPE) assay for HPLC determination of ranitidine in serum and plasma [17] and in urine, plasma and peritoneal dialysate [18] have been reported. Farthing et al. [19] proposed a HPLC method for the determination of ranitidine in human plasma using SPE and midbore chromatography. Many investigators have performed the separation of cimetidine from plasma or serum with SPE on different cartridges such as phenyl [20] and C_{18}-bonded silica [21–23]. However, SPE performed on C18 cartridge, gave the poor separation of investigated drugs due to interferences from the plasma.

In this paper, a new method for the determination of ranitidine and cimetidine in plasma samples using SPE technique on a RP-select B cartridges is proposed. Namely, developed method allowed determination of each agent without the need for development of separate and distinct methods for each analyte. Also, this method can be use for analysis of patient samples after oral administration of ranitidine or cimetidine using the same mobile and stationary phase. The method was first developed for the separation and determination of ranitidine and cimetidine concentrations using an internal standard method by optimizing the experimental parameters and determining linearity for the investigated drugs. Then, the method for the determination of ranitidine and cimetidine concentrations was validated by evaluating recovery, selectivity, linearity, precision and accuracy. Finally, the method was used for the determination of ranitidine in plasma samples obtained from healthy volunteers.

2. Experimental

2.1. Chemicals

Ranitidine working standard was purchased from Mileage Products International B.V. (Netherlands). Cimetidine and famotidine were kindly provided by Sigma (Deutschland). All chemicals were analytical-grade: acetonitrile and methanol from Across Organics (Belgium), triethylamine (TEA), *o*-phosphoric acid, potassium dihydrogen phosphate and sodium carbonate from Merck (Germany). Cartridges for SPE were supplied by Merck (Germany).

2.2. Apparatus

Chromatography was performed on a Perkin–Elmer liquid chromatography system (USA) consisting of a pump PE LC series 200, autosampler PE LC ISS Series 200, diode array detector PE LC 235 C and column oven PE model 101. The chromatographic system is controlled by the software package Turbochrom Version 4.1. plus and UV-spectrometric data were produced by the program TurboScan Version 2.0.

2.3. Chromatography

In order to develop a convenient and easy-to-use method for the simultaneous determination of ranitidine and cimetidine in human plasma, and with respect to the location and shape of the peaks of investigated drugs and internal standard (famotidine) in the corresponding chromatograms, a set of column packings including C8, C18 and RP-

select B with different lengths and particle sizes were tested. When the separations were performed using C8 and C18 HPLC columns the investigated drugs showed peak tailing. The peak tailing may be attributed to the secondary interaction between the silanol groups on the column packing material and the amino groups on the investigated drugs. The final choice of the stationary phase that provided satisfactory peak shape, resolution and run time was a Lichrocart Lichrospher 60 RP-select B, 250×4 mm I.D. (5 μm, particle size), protected by a guard column Lichrospher RP-select B 4×4 mm (5 μm). All experiments were carried out at ambient temperature, approximately 25 °C, and the flow-rate of the mobile phase was 1 ml min^{-1}. The effluent was monitored over the dative UV region and the wavelength of 230 nm exhibited the highest response.

2.4. Preparation of standards

Stock solutions of ranitidine, cimetidine and famotidine were prepared at a concentration of 1000 μg ml^{-1} by the dissolving appropriate amounts in methanol. Stock solutions, after storage at 4 °C over the period of 1 month, were analyzed and no change in stability was observed. The working solutions were prepared by diluting appropriate portions of these solutions with distilled water (HPLC grade).

2.5. Sample preparation and extraction

Human plasma was prepared from heparinized whole blood samples. Blood samples were collected from healthy volunteers who later participated in a bioequivalence study for ranitidine and the blood was stored at −20 °C. After thawing, blood samples were spike with the working standard solutions of ranitidine, cimetidine and famotidine.

A SPE vacuum manifold (Merck) was used for sample preparation. Satisfactory values for recovery of ranitidine and cimetidine were obtained with a single extraction using the RP select B solid-phase cartridge (200 mg) for the isolation of the drugs and the internal standard (famotidine) from plasma samples. Before the analysis, the plasma samples (0.5 ml) were spiked with 100 μl of internal standard that was buffered with 0.5 ml of 0.1 mol l^{-1} sodium carbonate solution. The cartridge was conditioned sequentially by 2 ml of methanol and 2 ml water. The buffered plasma sample was introduced into the cartridge under vacuum at 5 psi. Water (3 ml) was used to rinse the cartridge. The analytes were eluted with 2 ml methanol. The eluate was evaporated to dryness under N_2 for about 20 min at 40 °C. After reconstitution of the residue with 200 μl of mobile phase, the sample was filtered using a filter with pore size of 0.45 μm (Milipore) and 100 μl volume was injected into the HPLC system.

2.6. Calibration standards

Five calibrators each of ranitidine and cimetidine were prepared by making serial dilutions from stock solutions and spiking them into drug-free human plasma. The calibration range was 50.0–1000.0 ng ranitidine and 50.0–2000.0 ng of cimetidine per ml of plasma. The standard samples were prepared according to the procedure as unknown samples. The calibration curves were obtained by plotting the peak area ratio of target (ranitidine or cimetidine) to internal standard versus concentration of target in ng ml^{-1}. The regression equations were calculated by the least-squares method. Quality control (QC) samples were prepared at low-, medium- and high levels in the same way as plasma samples for the calibration.

3. Results and discussion

3.1. Method development

When 14% acetonitrile was used as the mobile phase ranitidine and cimetidine were not overlapped and the best resolution was obtained. On the other hand, the amount of organic modifier present in the mobile phase will influence on analytes that are retained predominantly by adsorption onto the stationary phase. Fig. 1 shows the results that were obtained over an acetonitrile range of 10–20% in the mobile phase composed of

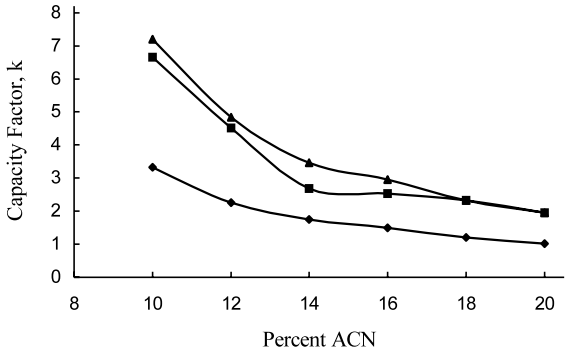

Fig. 1. The effect of the organic modifier concentration on analytes retention (■, ranitidine; ▲, cimetidine; ◆, famotidine).

0.2% (v/v) TEA, 0.04 mol l^{-1} KH_2PO_4 at pH 6.8. This data was used to determine an optimal amount of organic modifier that should be used for the separation of ranitidine, cimetidine and internal standard, famotidine. As observed in Fig. 1, the retention time of the investigated drugs decreased with increasing acetonitrile. The best resolution between two peaks of ranitidine and cimetidine can be obtained when the percentage of acetonitrile in the mobile phase is 14%.

Further, the effect of TEA percentages from 0.0 to 1.0% in mobile phase was investigated on the separation of ranitidine, cimetidine and famotidine. Fig. 2 shows the results that were obtained with mobile phase consisted of 0.04 mol l^{-1} KH_2PO_4 at pH 6.8 and 14% acetonitrile. As can be seen from Fig. 2, before the addition of TEA, ranitidine and cimetidine overlapped. When TEA was added to the mobile phase ranitidine and cimetidine were resolved. Their resolution did not improve at TEA percentages higher than 0.2% and this percentage was chosen as optimal for the chromatographic separation.

Also, the effect of the buffer concentration from 0.0 to 0.08 mol l^{-1} in mobile phase was investigated for the analytes retention and separation (Fig. 3). The retention of ranitidine, cimetidine and famotidine decreased with increasing buffer concentration, but the resolution was much better using mobile phase that consisted of 0.2% TEA, 0.04 mol l^{-1} KH_2PO_4 at pH 6.8 and 14% acetonitrile.

An additional study was done to determine the effect that the mobile phase pH would have on the analytes retention and resolution, and the results are shown in Fig. 4. As can be seen, the retention of ranitidine, cimetidine and famotidine decreased with decreasing mobile phase pH. The best separation of these drugs was obtained when the mobile phase pH was higher than 6.5. Additionally, a mobile phase pH of 6.8 was also chosen since it provides good retention and resolution between the three peaks, and also shorter time of analysis. The use of mobile phase with higher pH values may cause partially dissolution of the silica bond stationary phase.

From these data it was determined that a mobile phase that consisted of 0.2% TEA, 0.04 mol l^{-1} KH_2PO_4 at pH 6.8 and 14% acetonitrile, would provide good retention and resolution for ranitidine, cimetidine and famotidine as well as an acceptable runtime of less than 11 min.

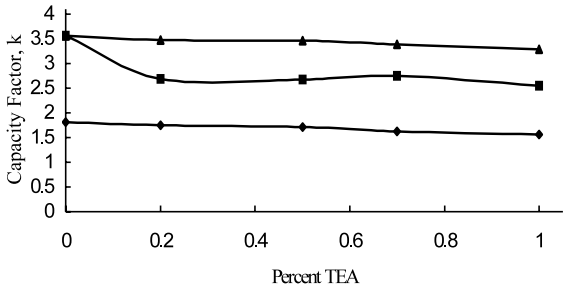

Fig. 2. The effect of the TEA concentration on analytes retention (■, ranitidine; ▲, cimetidine; ◆, famotidine).

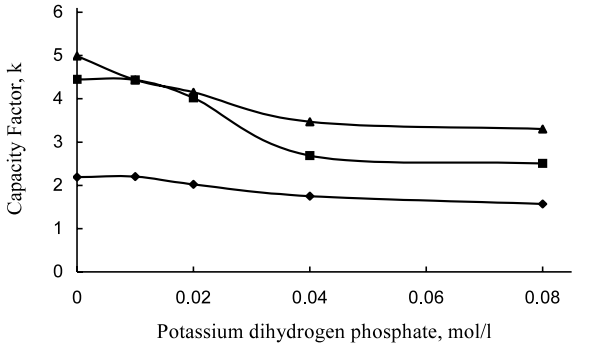

Fig. 3. The effect of the buffer concentration on analytes retention (■, ranitidine; ▲, cimetidine; ◆, famotidine).

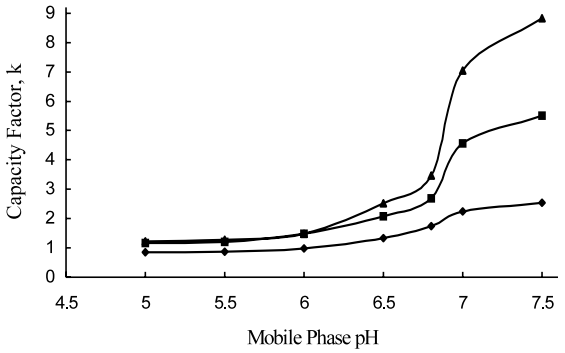

Fig. 4. The effect of the mobile phase pH on analytes retention (■, ranitidine; ▲, cimetidine; ◆, famotidine).

A typical chromatogram of standard solutions of ranitidine, cimetidine and famotidine produced by the developed HPLC method is shown in Fig. 5(a). The retention times for famotidine, ranitidine and cimetidine are 5.2, 7.8 and 8.9 min, respectively.

In addition, different cartridges for the SPE (C18 and RP-select B) were tested in order to obtain satisfactory recoveries of cimetidine and ranitidine. The extraction recoveries were calculated by comparing the peak height of ranitidine, cimetidine and internal standard (famotidine) obtained for low-, medium- and high level quality control samples ($n = 3$ for each level for ranitidine and cimetidine, $n = 9$ for internal standard) and those resulting from the direct injection ($n = 3$, working solutions) of the theoretical amount of either ranitidine, cimetidine or internal standard (= 100% recovery).

Results from this investigation show those acceptable recoveries of ranitidine, cimetidine and famotidine were obtained when plasma samples were extracted with both types of solid-phase cartridges. When the RP-select B cartridge was used for sample preparation values for recovery for all three drugs were ranged from 96.7 to 101.1%. When SPE was performed using the C18

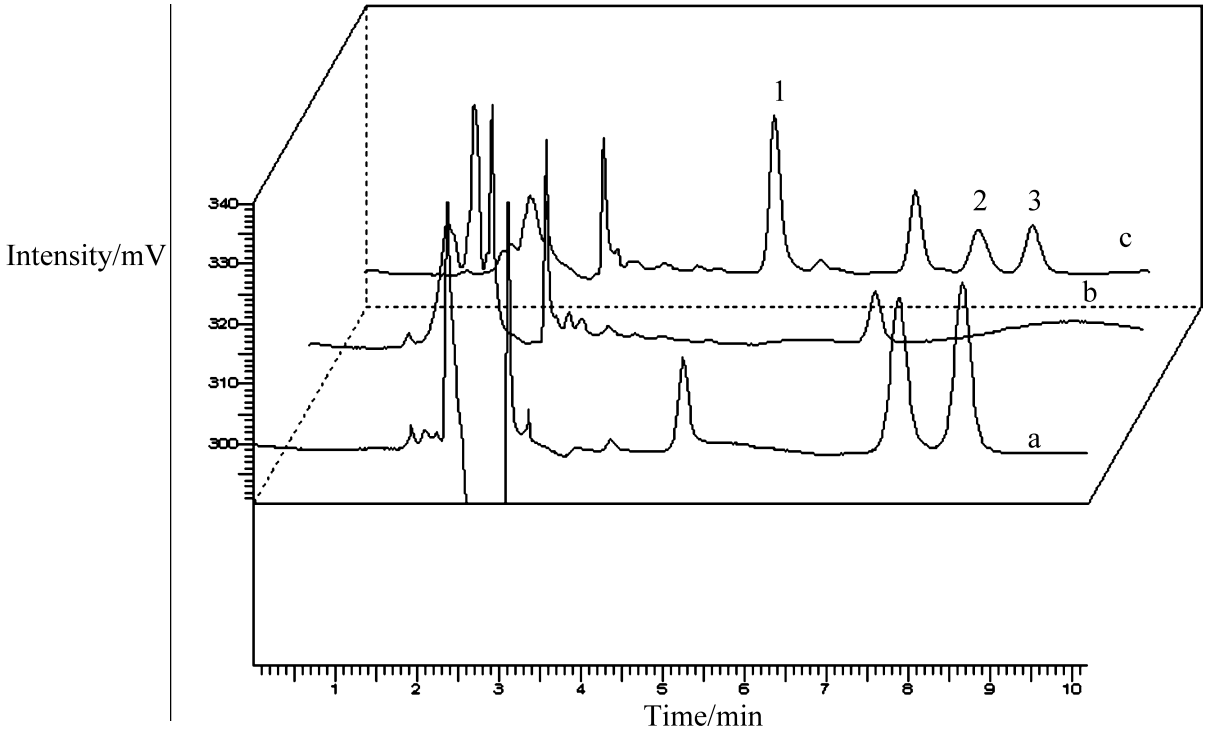

Fig. 5. Chromatograms of standard solutions (a) of famotidine (1.0 μg ml^{-1}), ranitidine and cimetidine (1.5 μg ml^{-1}); 1–famotidine, 2–ranitidine, 3–cimetidine, blank (b) and spiked plasma (c) samples containing 0.25 μg ml^{-1} of ranitidine, 0.30 μg ml^{-1} of cimetidine and 0.80 μg ml^{-1} of famotidine.

cartridges, recoveries ranged from 94.3 to 99.5% for ranitidine and cimetidine and from 85.6 to 92.3% for famotidine. Also, when the SPE used the C18 cartridge, the separation of investigated drugs was poor due to interferences from the plasma.

Under the chromatographic conditions described, famotidine, ranitidine and cimetidine peaks were well resolved. Endogenous plasma components did not show any interfering peaks. Typical chromatograms of blank plasma in comparison to spiked sample are shown in Fig. 5(b and c).

The developed HPLC method that was developed was used for analysis of plasma samples from healthy volunteers after oral administration of ranitidine. Typical chromatograms of plasma samples of patients before and after the administration of 150 mg ranitidine are shown in Fig. 6(a and b). Also, we can conclude that this method is selective and specific because there are no peaks from metabolites in the chromatogram obtained from plasma samples from healthy volunteers after oral administration of ranitidine (Fig. 6(b)).

3.2. Method validation

The linearity of the method was tested over 2 days at five different concentration that ranged from 50.0 to 1000.0 ng ml^{-1} of ranitidine and 50.0 to 2000.0 ng ml^{-1} of cimetidine in plasma samples. Plasma samples were spiked with the internal standard (famotidine) at a concentration of 800.0 ng ml^{-1}. Respective regression equations were: $y = 0.0018\gamma + 0.0086$ for ranitidine and $y = 0.0019\gamma - 0.0299$ for cimetidine. The correlation coefficients were 0.9998 and 0.9997, respectively.

Spiked samples from each concentration used for construction of calibration curves were prepared in triplicate and analyzed by the proposed HPLC method in two different days. Then, the corresponding coefficients of variation were calculated. The intra- and inter-day variations of the

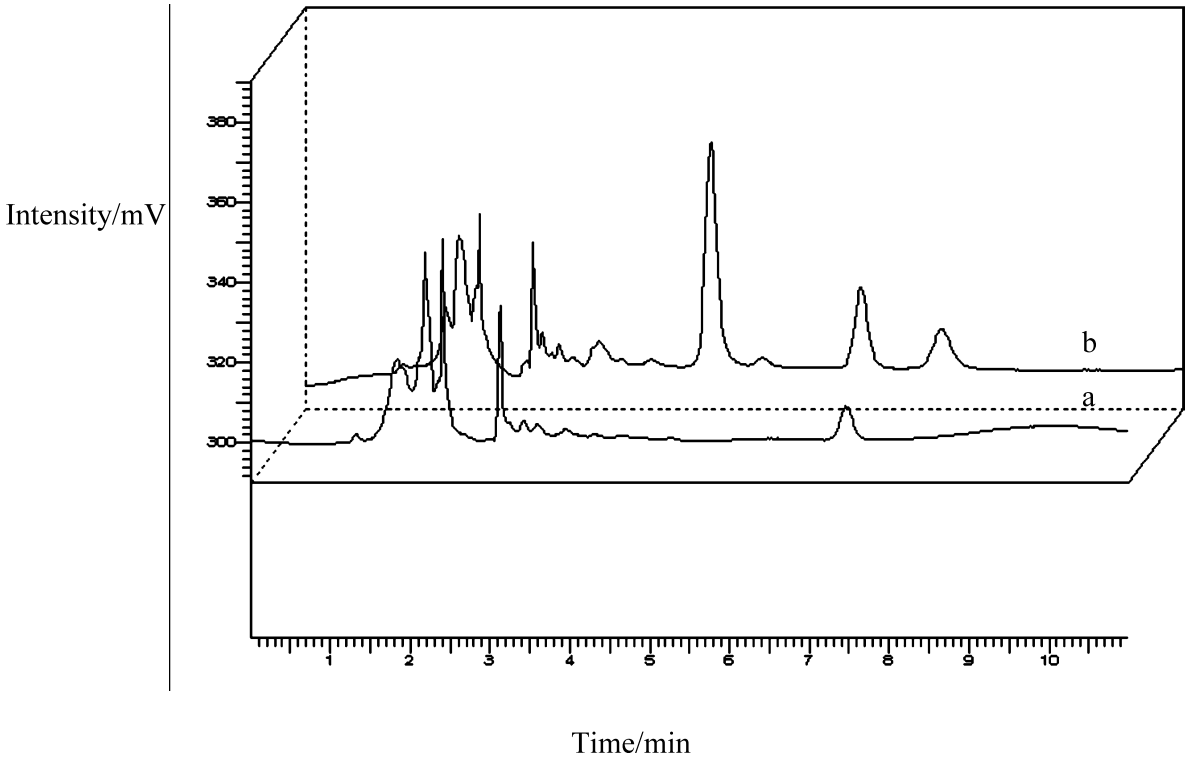

Fig. 6. Chromatograms of plasma samples from healthy volunteers before (a) and after (b) administration of 150 mg ranitidine.

method throughout the linear range of concentrations are shown in Table 1. These data indicate a considerable degree of precision and reproducibility for the method.

Intra- and inter-day accuracy was determined by measuring plasma quality control samples at low-, middle- and high concentration levels of ranitidine and cimetidine. An indication of accuracy was based on the calculation of the relative error of the mean observed concentration as compared to the nominal concentration. Accuracy data are presented in Table 2. Relative errors at all three concentrations studied for ranitidine and cimetidine are less than 2.88% and it is obvious that the method is accurate which ensures reliable results.

The limit of quantification was defined as the lowest amount detectable with a precision of less than 15% ($n = 5$) and an accuracy of $\pm 15\%$ ($n = 5$). The limits of quantification were found to be 50.0 ng ml^{-1} for ranitidine and cimetidine. The limit of detection was defined as signal to noise ratio 1:3 and they were found to be 10.0 ng ml^{-1} for ranitidine and cimetidine.

Ruggedness was tested on the second HPLC column of the same type by determining linearity, precision and accuracy. Linearity was performed at five concentration points for ranitidine and cimetidine in plasma in concentration range from 50.0 to 1000.0 and 50.0 to 2000.0 ng ml^{-1}, respectively. The regression equations were: for ranitidine $y = 0.0018\gamma - 0.0033$ and for cimetidine $y = 0.0017\gamma - 0.0003$. The correlation coefficients were 0.9991 and 0.9999, respectively. Intra-day precision and accuracy were determined by measuring two series of plasma quality control samples. Relative standard deviations at all three concentrations studied for ranitidine and cimetidine were less than 2.3%. The relative errors ranged from 0.07 to 2.11% of the nominal concentrations of investigated drugs. As can be seen, the results of this assessment are very similar to those obtained by previous investigation on the first HPLC column. That means that this HPLC method for simultaneously determination of ranitidine and cimetidine in spiked human plasma samples is rugged.

3.3. Stability of ranitidine and cimetidine in plasma samples

Stability of ranitidine and cimetidine in plasma was investigated using spiked samples at two different concentration levels prepared in duplicate. Spiked samples were analysed after different storage conditions: immediately, after being on the autosampler for 2, 12 and 24 h, after one and two freeze/thaw cycles and after 1 month stored at −20 °C. The results from this investigation show

Table 1
Intra- and inter-day precision data

Nominal concentration (ng ml^{-1})	Intra-day		Inter-day	
	Mean ($n = 3$) observed concentration (ng ml^{-1})	Relative standard deviation (%)	Mean ($n = 6$) observed concentration (ng ml^{-1})	Relative standard deviation (%)
Ranitidine				
50.0	51.5	1.47	52.1	1.22
100.0	103.4	2.66	102.8	2.17
250.0	255.0	1.23	255.1	2.26
500.0	495.6	0.77	493.2	3.08
1000.0	1000.4	2.23	1001.6	2.07
Cimetidine				
50.0	52.9	1.24	52.9	0.97
100.0	100.9	3.60	100.8	2.50
300.0	292.8	1.65	295.4	2.60
800.0	778.4	0.51	774.8	0.54
2000.0	2009.2	2.50	2010.1	1.66

Table 2
Intra- and inter-day accuracy data

Nominal concentration (ng ml^{-1})	Intra-day		Inter-day	
	Mean ($n = 3$) observed concentration (ng ml^{-1})	Relative error (%)	Mean ($n = 6$) observed concentration (ng ml^{-1})	Relative error (%)
Ranitidine				
80.0	78.8	−1.50	79.4	−0.75
350.0	344.0	−1.71	346.7	−0.94
600.0	611.1	1.85	604.7	0.78
Cimetidine				
80.0	77.7	−2.88	78.4	−2.0
500.0	496.9	−0.62	500.5	0.1
1000.0	995.4	−0.46	1003.2	0.32

that relative errors at two different concentrations studied are less than 2.7% for ranitidine and 4.7% for cimetidine and it is obvious that ranitidine and cimetidine added to plasma are stable at the different storage conditions.

4. Conclusion

The proposed HPLC method employing SPE for sample preparation is simple and convenient for the determination of ranitidine and cimetidine in plasma samples. Ranitidine, cimetidine and the internal standard, famotidine have been successfully separated. The method has been developed and validated for the quantitative determination of ranitidine and cimetidine in human plasma. The method reported here provides an efficient clean up of the complex biological matrix and high recovery of investigated drugs. The validation data demonstrates good precision and accuracy, which proves the reliability of the proposed method. Finally, the method has been implemented to monitor ranitidine levels in clinical samples.

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