METHOD VALIDATION AND QUANTITATIVE DETERMINATION OF FAMOTIDINE IN HUMAN PLASMA

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Abstract. A simple, selective and accurate reversed-phase high pressure liquid chromatographic method with UV detection at 274 nm has been developed for the determination of famotidine in human plasma. The procedure employed ranitidine as an internal standard (IS). A good chromatographic separation between famotidine, internal standard and interfering endogenous peaks was achieved using a C18 column and mixture of 0.1% triethylamine:phosphate buffer pH 6.8:acetonitrile (70:15:15% v/v/v) as mobile phase at a flow rate of 1.0 ml/min. The extraction procedure for RP-HPLC quantitation of famotidine was performed. The method involved reproducible liquid-liquid extraction of drug from biological matrix using a mixture of isopropanol:hexane (1:1). The method was validated with respect of specificity, accuracy and precision over a linear range of 4 – 40 µg/ml for famotidine. The LOQs and LODs were 1.0 and 0.2 µg/ml, respectively.

Key words: famotidine, liquid chromatography, validation, plasma, liquid-liquid extraction

Introduction
Famotidine, is a histamine H₂-receptor antagonist which competitively inhibits the action of histamine on the H₂-receptors of parietal cells and thereby reduces the gastric acid secretion under daytime and nocturnal basal conditions as well as secretion stimulated by food and pentagastrin. It is used for the short term treatment of duodenal ulcer and treatment of pathologic hypersecretory conditions like Zollinger-Ellison syndrome [1]. The assay of this drug in biological samples is required in therapeutic monitoring, pharmacokinetic and bioavailability studies.

Several techniques including HPLC [2-16], capillary electrophoresis [17] and spectrofluorometry [18] have been employed for determining famotidine in pharmaceutical dosage forms as well as different types of biological fluids.

The aim of present study was to develop a liquid-liquid extraction method for the elimination of plasma endogenous interferences on the determination of famotidine in human plasma.

EXPERIMENTAL SECTION

Materials and methods
Reagents and chemicals
HPLC acetonitrile was used to prepare the mobile phase. All other reagents were of analytical grade. Famotidine (99.84 %) and ranitidine reference substances were supplied from Sigma-Aldrich (Germany). Blank human blood was collected from healthy drug-free volunteers by local blood centre. Plasma was obtained by centrifugation of blood treated with EDTA as anticoagulant. Pooled plasma was prepared and then stored at -20°C until needed.

Equipment
The HPLC system consisted of a Shimadzu LC-10A system equipped with a LC-10 AS pump, SPD-10A variable wavelength detector, SIL-10A autosampler and SCL-10A system controller. Chromatographic column LiChrosorb RP-18, 250 mm x 4.6 mm, 5 µm (Merck, Germany), equipped with additional guard column (20 x 4 mm) was used.

Chromatographic conditions
The mobile phase of 0.1% triethylamine:phosphate buffer pH 6.8:acetonitrile (70:15:15% v/v/v) was delivered at a flow rate of 1 ml/min. The eluate was monitored using UV detector with wavelength at 274 nm at flow rate of 1 ml/min.

Extraction procedure
To 0.2 ml of plasma 2.0 ml mixture of isopropanol-hexane (1:1) containing 20 µg/ml ranitidine as internal standard was added. After shaking for 2
min on a Vortex mixer, the mixture was centrifuged at 3000 rpm for 3 min. A 1.0 ml aliquot of the clear organic solvent was removed and then evaporated to dryness at 65-70°C. The residue was dissolved in 1.0 ml of mobile phase and 20 μl aliquots were injected into the chromatographic system.

**Method validation**

**Linearity**
Calibration curves were constructed for famotidine in both plasma and mobile phase. Stock solutions of famotidine and internal standard were prepared in mobile phase. After thawing the plasma was spiked with appropriate amounts of stock solution to yield final concentration of famotidine in the range 4 to 40 µg/ml.

**Accuracy**
The accuracy of the method was verified by analysis of model mixtures obtained by adding known amounts of drug of interest to blank plasma. The concentrations of the mixtures were 10, 20 and 30 µg/ml. Five replicates were performed for each concentration studied. The extraction recovery of famotidine from biological matrix was calculated by comparing concentrations measured in the plasma with the concentrations added.

**Precision**
The precision of the method was assessed by repeated analysis of plasma specimens containing known concentration of the compound (20 µg/ml).

**Stability studies**
The stability of famotidine in plasma was evaluated with three studies: a short term stability study, a long term stability study and a freeze thaw study. Quality control (QC) samples were prepared as follows: blank plasma was spiked with famotidine at concentration 8 µg/ml, 24 µg/ml and 32 µg/ml and each concentration was carried out for five times. Plasma samples were extracted and subsequent HPLC analysis was carried out as described previously. Short-term stability test was performed at ambient temperature. Plasma samples spiked with famotidine were kept at room temperature for 12 hr, extracted and then analyzed. The long term stability study was performed with plasma blank samples spiked with famotidine, which were stored -20° and they were analyzed periodically 1 months against a standard curve prepared on the analysis day. For freeze thaw stability spiked samples were analyzed immediately after preparation and on a daily basis after repeated freeze thaw cycles at -20°C on three consecutive days.

**Results and discussions**
Under the described chromatographic conditions peaks of protein components (tr = 3.02 min), internal standard (tr = 5.46 min) and famotidine (tr = 9.07 min) were well resolved.

No interfering peaks were observed in chromatogram of blank plasma at the retention times of investigated compounds, which verify the specificity of analytical method.

**Validation data**
Peak area ratios of famotidine to internal standard showed a linear relationship to plasma concentration within the range 4 to 40 µg/ml. The quantification and detection limits for famotidine were 50 ng and 10 ng, respectively. The good linearity of the calibration curves was confirmed by the high value of correlation coefficients (r²=0.9998). Data concerning validation procedure were shown in Table 1 and Table 2. The average extraction recovery of examined ester from biological matrix was 97.54 %. The relative standard deviation (RSD) obtained in study of precision was below 5 %.

**Stability of famotidine**
Famotidine was stable for at least three freeze-thaw cycles. No significant decrease of drug concen-
Method validation and quantitative determination of famotidine in plasma was detected after exposing samples to three freeze/thaw cycles and % RSD was found to be 2.15 at 8 µg/ml, 3.24 at 16 µg/ml and 1.66 at 32 µg/ml. The short term stability test performed at room temperature showed that three QC samples were stable for 8 hr (% RSD 3.12, 2.25 and 2.83 at 8 µg/ml, 24 µg/ml and 32 µg/ml, respectively). While the long term stability indicated that famotidine samples were stable during 1 month, with a % RSD 2.27 at 8 µg/ml, 2.58 at 16 µg/ml and 1.89 at 32 µg/ml, respectively.

**Conclusion**

The proposed HPLC method can be regarded as selective, accurate, precise, and valid for determination of famotidine with a total run time of 12 min. The results showed that developed analytical procedure can be successfully applied for the quantitative determination of famotidine in human plasma.

### Table 1. Precision of the method

<table>
<thead>
<tr>
<th>Actual concentration, µg/ml</th>
<th>Measured concentration, µg/ml</th>
<th>RSD, %</th>
</tr>
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<tr>
<td>20.00</td>
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<td>3.33</td>
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<tr>
<td>9.84</td>
<td>9.57</td>
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<td>9.57</td>
<td>9.22</td>
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<td>9.22</td>
<td>9.39</td>
<td>2.08</td>
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<tr>
<td>Average</td>
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<tr>
<td>Sd</td>
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### Table 2. Recovery of famotidine from plasma

<table>
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<th>Taken amount, µg/ml</th>
<th>Found amount, µg/ml</th>
<th>Recovery, %</th>
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</thead>
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<td>93.6</td>
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<tr>
<td>Mean</td>
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<td>97.54</td>
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<td>Sd</td>
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<tr>
<td>RSD, %</td>
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</tbody>
</table>

**References**

11. **Anzenbacherová** E, Filipová K, Nobilis M, Anzenbacher P. Selective determination of famotidine in human plasma by high perfor-


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