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Selective determination of famotidine in human plasma by high performance liquid chromatography in alkaline media with solid phase extraction

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A new method is described for the determination of famotidine by solid phase extraction from alkalinized human plasma followed by reversed phase (RP) HPLC in acetonitrile/alkaline buffer with molsidomine as an internal standard. Different acetonitrile/aqueous buffer mobile phases as well as various RP columns were used. Alkaline medium allowed the limit of quantitation to be lowered to 5 ng/mL of plasma as the famotidine gives more intense absorption at about 286 nm (at pH values higher than 7). Moreover, work in alkaline media and at this wavelength is highly selective as peaks corresponding to impurities present in most samples are well separated. A method using a mildly alkaline mobile phase (acetonitrile/10 mM phosphate with 10 mM 1-heptanesulphonic acid, pH 7.5) was successfully used for determination of famotidine in human plasma in a pharmacokinetic study.

Key Words: Famotidine; Molsidomine; HPLC; Solid phase extraction; Ion pairing chromatography

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1 Introduction

Famotidine, (3-[2-(diaminomethyleneamino)-4-thiazolylmethylthio]-*N*-sulphamoylpropionamidine), is a highly selective and potent antagonist of histamine receptors of the H_2 type used widely in therapy of duodenal and gastric ulcers [1]. It is twenty times more efficient than cimetidine and seven times more potent than ranitidine in inhibiting the gastric acid secretion in man. As the efficacy of famotidine is high, the dosage may be rather low, which is reflected in relatively low levels of this drug in human plasma. Hence, any analytical method for determination of famotidine should be sufficiently sensitive.

The basicity of famotidine is reflected in its different ionization and spectral properties at acidic and at alkaline pH. Whereas the acidic form absorbs at 265 nm, in alkaline media famotidine exhibits maximal absorption at 286 nm [2]. The reversed phase HPLC methods described in the literature [3–10] are, however, based on the determination of famotidine at acidic pH when famotidine is protonized. This approach has thus led to experimental difficulties such as poor resolution or higher quantification limits, as is apparent from the chromatograms presented in the respective papers and from the fact that the lowest values of famotidine concentrations used in calibration curves usually started at 25 or 100 ng/mL.

The disadvantages of using acidic media may be summarized as follows: (i) The ionized, protonized form of famotidine is eluted together with other polar components from the biological matrix, which lowers the selectivity of the method, and (ii) the acidic form absorbs in the region where other, aromatic compounds also exhibit maximal absorption, which again contributes to lower selectivity of the determination. An alternative approach for improving the selectivity is the use of more sophisticated detection method. This was the route adopted by Campanero et al. [11] who used successfully the tandem MS detection.

The aim of this work was to take advantage of properties of famotidine in the alkaline form and to find the conditions conducive to reliable determination of this analyte in human blood plasma. For sample preparation, a solid phase extraction method with a C18 stationary phase was chosen as recommended for structurally related antiulcerotic agents, ranitidine, cimetidine, and nizatidine after equilibration of column with 25 mM phosphate (pH 9.0) [12]. Hence, all the procedures described here were performed in alkaline media. Three types of chromatographic columns with stationary phases claimed by the manufacturers to be stable at pH values higher than 7 were chosen for HPLC (XTerra® from Waters, Polyspher® RP and LiChrospher® RP C8 from Merck). The last one (LiChrospher) with a mobile phase for ion pairing chromatography (10 mM Na/phosphate with 10 mM 1-heptanesulfonic

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acid, pH 7.5/acetonitrile/water at ratio of 20:10:70) has been shown to be the most suitable for routine analyses of blood plasma samples. Molsidomine (having both chromatographic properties during SPE and spectral characteristics similar to those of famotidine) was selected as internal standard. The method has been shown to be suitable for determination of famotidine in plasma during the study of famotidine pharmacokinetics in man [13].

2 Experimental Methods

2.1 Reagents and Chemicals

Famotidine and molsidomine were obtained from PRO.-MED.CS Praha a.s. (Czech Republic) and were of highest purity available (Cz. and Eur. Pharmacopeia), 1-heptanesulphonic acid sodium salt was purchased from Fluka (Switzerland) and acetonitrile from Merck (Germany), both specified for HPLC. Water and methanol were HPLC grade, all other chemicals (Lachema, Czech Republic) were analytical reagent grade.

2.2 Sample preparation

To 2 mL of human plasma spiked with famotidine were added 75 μL of 0.2 M borate buffer (pH 10) together with 75 μL of 10^{-5} M molsidomine aqueous solution as an internal standard. The samples were vortexed for 10 s and applied onto a C-18 Supelclean SPE column (Supelco, USA) preconditioned with 1 mL of methanol and with 2 mL of 0.1 M borate buffer (pH 10). The SPE column was washed with 3 mL of citrate buffer (pH 5.5) and the analytes were then eluted by 3 mL of methanol. The solvent was evaporated at 45°C under a gentle stream of nitrogen. Dry sample was dissolved in 150 μL of mobile phase (see Section 2.4, Chromatography).

2.3 HPLC instrumentation

Chromatographic separation of famotidine and internal standard was carried out at ambient temperature on a chromatographic system (Thermo Separation Products, USA) consisting of a P-1500 binary pump, a UV 3000 detector, and an AS 3500 autosampler with a 100 μL loop. Data were collected and evaluated with ChromQuest analytical software (Thermo Separation Products, USA) using the analyte and internal standard peak area ratios with linear regression.

2.4 Chromatography

The HPLC analyses were performed on i) a polystyrene-based Polyspher® RP C18 (150 × 4.6 mm ID) column (Merck, Germany), ii) a pH-resistant hybrid silica-organosiloxane based RP C18 column XTerra® (Waters, USA), and iii) a modified silica column LiChrospher® 100 RP8 (250 × 4.6 mm ID) with particle size 5 μm (Merck, Germany). For XTerra® and Polyspher® columns the mobile phases consisted of acetonitrile and borate buffer (pH 10) (14.5:85.5, *v/v*), and for the LiChrospher® 100 RP8 column the mobile phase was 10 mM phosphoric acid containing 10 mM 1-heptanesulphonic acid (pH 7.5), acetonitrile, and water (20:10:70 *v/v/v*). The compounds were separated isocratically at a the flow rate of 1 mL min^{-1}. Eluted famotidine and molsidomine (internal standard) were detected at 285 nm.

3 Results and discussion

Three HPLC columns were used for determination of famotidine in alkaline media. As the p*K* of famotidine was reported to be 6.7 [14], under experimental conditions used here the molecule exhibits the spectral properties of the alkaline form with a spectral maximum of about 286 nm (287 nm at pH 10.0, 285 nm at pH 7.5, **Figure 1**). The first mobile phase tested was the acetonitrile/borate buffer (pH 10.0) (see Section 2.4) with the Polyspher® or XTerra® stationary phases. The results obtained with Polyspher® (of a C18-modified styrene polymer matrix) were not satisfactory as the resolution was poor (results not shown).

This was why another stationary phase expected to be suitable for alkaline media was chosen, namely XTerra® (modified silica-organosiloxane polymeric base). HPLC determination of famotidine with the XTerra® stationary phase yielded well-resolved responses with the retention time of famotidine being about 7.7 min and that of the internal standard 9.7 min, respectively (a typical chromatogram is shown in **Figure 2**). Here, the wavelength used for detection was red-shifted even more to 295 nm to achieve the clearest separation of the respective signals.

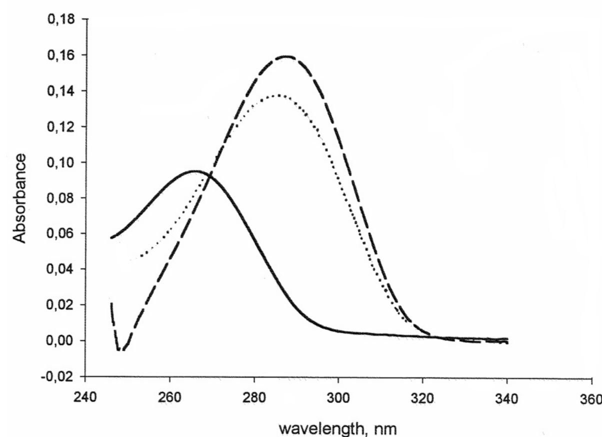

Figure 1. UV absorption spectra of famotidine at different pH. 10^{-5} M famotidine, pH: full line, pH 4, dotted, pH 7.5, dashed, pH 10.

Short Communication

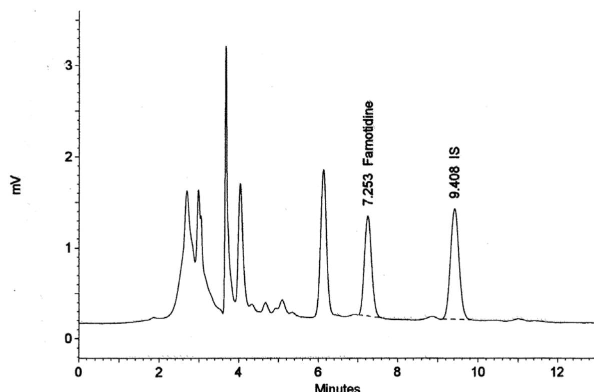

Figure 2. HPLC chromatogram (XTerra® column) of human plasma spiked with famotidine and molsidomine (internal standard). Concentrations of famotidine, 30 ng/mL, molsidomine, 90 ng/mL.

The results of the validation procedure can be summarized as follows:

Calibration, linearity: The calibration curve from spiked plasma was linear in the concentration range of 10.2 to 298.0 ng mL^{-1} (at six concentration levels with six parallel determinations each) giving the equation $y = 0.717295x$ (y, famotidine/internal standard peak area ratio; x, famotidine/internal standard concentration ratio), goodness of fit (r^2) 0.9906.

Precision, accuracy, LLOQ: The precision was expressed as coefficient of variation (%CV) and the accuracy was expressed as percentage of deviation (difference) from the theoretical value of famotidine concentration in spiked samples. The respective values are listed in **Table 1**. With the exception of the lowest concentration level, the precision was better than 13% and the accuracy of the determination was of similar quality, not exceeding 15% of the target value. The lower limit of quantification (LLOQ) was lower than 10 ng mL^{-1} (determined as the lowest concentration determined with the CV not exceeding 15%).

Table 1. Intraday precision and accuracy of famotidine determination using the XTerra® and LiChrospher® chromatographic columns.

Nominal conc. [ng mL^{-1}]	Found conc. [ng mL^{-1}]	Precision [%C.V.]	Accuracy [% difference]
XTerra®			
10.2	9.5	13.0	−7.3
50.6	47.2	6.0	−6.8
101.2	104.8	6.0	+3.6
151.8	173.4	11.1	+14.2
236.2	226.3	4.6	−4.2
298.0	299.5	3.6	+0.5
LiChrospher®			
8.4	7.9	13.4	−5.9
42.2	37.6	14.4	−10.8
84.4	90.7	9.8	+7.5
118.1	112.9	10.9	−4.4
160.3	157.8	12.7	−1.6
202.5	205.3	3.7	+1.4

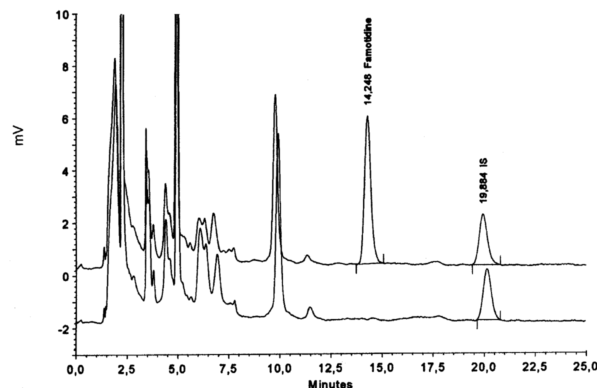

Figure 3. HPLC chromatogram (LiChrospher® column) of human plasma extracts. Lower trace, blank plasma spiked with molsidomine (internal standard), 90 ng/mL. Upper trace, sample taken 2 h after intake of 40 mg of famotidine. Famotidine level corresponds to 112 ng/mL, internal standard 90 ng/mL as above.

This method has been shown to give satisfactory results. On the other hand, the stationary phase started to deteriorate after 20 to 30 h (or, approximately, after 100 samples analysed) which could be seen from the appearance of less sharp peaks in the respective chromatogram. Hence this method cannot be recommended for a routine analytical work. It may, however, be suitable for analyses of small numbers of samples, e.g. in basic or applied research.

The third experiment described here was based on the use of the LiChrospher® C8 stationary phase under mildly alkaline conditions. A typical chromatogram is displayed in **Figure 3**, together with the trace obtained for blank plasma with an internal standard only. The retention time of famotidine was 14.2 min and that of molsidomine (internal standard) 19.9 min. The disadvantage of the relatively long retention times is well balanced by good separation and by high stability of the stationary phase. The optimal wavelength of the absorption maximum of the basic form of famotidine (286 nm) was chosen for detection. The validation procedure gave the following results:

Calibration, linearity: The linearity of famotidine determination was checked in spiked samples of human plasma in the concentration range of 8 ng mL^{-1} up to 200 ng mL^{-1}. The respective equation was $y = 0.703374x$, where the meaning of the symbols is the same as above. Goodness of fit was found to be (r^2) 0.9960.

Precision, accuracy, LLOQ: The parameters were expressed as given in the above method and the respec-

tive values are also listed in Table 1. The coefficient of variation (in %) found was no higher than 14%, and the accuracy expressed as difference from the theoretical concentration value was between 10.8 and 7.5%. The results presented here were again obtained with six samples at six levels of concentration in each case. The lower limit of quantification was 5 ng mL^{-1} of plasma when determined as the concentration for which CV did not exceed 15%. The mobile phase in this (third) method contained an ion-pairing agent, 1-heptanesulphonic acid. This approach helped to improve the separation significantly, either by possibly influencing the retention times of polar impurities or by blocking polar parts of the famotidine molecule. Ion-pairing chromatography has recently been applied to the determination of famotidine by Tahboub et al. [6]. However, those authors used an acidic medium and encountered the difficulties inherently associated with this approach (resolution far from ideal, detection limit higher than found here).

Before discussing the advantages of the method used, a more general remark on the determination of the lowest limit of quantification (LLOQ) is in place. In this work, the most rigorous definition of the LLOQ is used (see above), which is accordance with recent recommendations in the literature [15] as well as those of the regulatory authorities (www.fda.gov/cder/guidance). The earlier approaches based on signal-to-noise ratios 10:1, 5:1, or 4:1 gave lower LLOQ values which, however, do not fulfil the need for a precise and accurate measurement. This is mentioned to explain why the lowest concentration levels of famotidine in earlier literature were much greater than those claimed to be the LLOQ. From the data presented it follows that the method described here is applicable already from the concentration levels corresponding to the LLOQ. The advantages of the experimental approach used in this work can be summarized as follows:

First, the extraction procedure used here (C18 Supelclean column, binding of analyte at about pH 10, i.e. in a nonprotonated form, elution with methanol) gives relatively high recoveries (at least 90% as determined by absorption spectra with aqueous solutions, 80% with plasma samples). This method has been found to be superior to those described in the literature (SPE [6, 10], extraction to organic phase [7, 8] or deproteination [9]). Washing of the column by diluted acid leads to elution of the impurities. This is reflected in the fact that an impurity found in preliminary experiments to be eluted during the subsequent HPLC separation with a retention time very close to that of famotidine is then either absent from the sample or its level is low.

Secondly, detection of the alkaline form has the advantage of higher sensitivity due to higher values of the molar absorption coefficient (15000 L mol^{-1} cm^{-1} as against 10000 L mol^{-1} cm^{-1} for the acidic form) [2] and of high selectivity as the detection at longer wavelengths (280–295 nm) excludes the impurities which mostly absorb at wavelengths corresponding to aromatic structures (255–275 nm). This fact, together with the previously described SPE method, represents the greatest improvement in the determination of famotidine in comparison with earlier methods [3–10].

The third advantage of this method lies in its relative simplicity. The difficulties encountered in the determination of famotidine in human plasma in acid media resulted, for example, in a proposed HPLC method based on column switching [15] using subsequent chromatography on C4 and C8 stationary phases with mobile phases of different pH (6.8 and 3.5) with a gradient pump needed to achieve the optimal conditions of separation. The method described here is, in contrast, readily accomplished using one HPLC pump working under isocratic conditions. Also, the tandem MS detection as used by Campanero et al. [16], which seems to be the method of choice, is still not universally available.

The difficulties sourrounding the determination of this highly basic drug resulted in numerous earlier attempts in the literature. However, these papers have essentially been modifications of the original method of Vincek [3]. Various efforts to improve it by adopting more sophisticated approaches have been published in recent years [11, 16]. The new procedure outlined here (with a LiChrospher column) has been successfully used for determination of famotidine in human plasma in a bioequivalence study [13] with famotidine concentrations in plasma ranging from 5 to 175 ng mL^{-1}, thus documenting the applicability of this method in routine analysis.

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