

AN OVERVIEW OF DETERMINATION OF FAMOTIDINE BY DIFFERENT ANALYTICAL METHODS

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Abstract: This review article summarized the analytical methods for the quantitative determinations of H₂ receptor antagonist famotidine by spectral methods, electrochemical procedures (potentiometric titration, polarography, differential pulse voltammetry) as well as separation procedures such as high-performance liquid chromatography (HPLC), thin layer chromatography (HPTLC) and capillary electrophoresis. The clinical and pharmaceutical analysis of drug of interest required effective analytical procedures for quality control and therapeutic drug monitoring. An extensive survey of the literature published in various analytical and pharmaceutical chemistry-related journals has been presented in this review. The application of described methods for determination of famotidine in bulk, various pharmaceutical formulations and biological samples has also been discussed.

Introduction

Famotidine (FMT) was chemically 3-([2-(diaminomethyleneamino) thiazol-4-yl] methylthio)-N-sulfamoylpropanimidamide. It is commonly used in the treatment of peptic ulcer disease and gastro esophageal reflux disease. Famotidine is histamine H₂-receptor antagonist which blocks the action of histamine on stomach cells and reduces acid production. Famotidine is useful in promoting the healing of stomach and duodenal ulcers and reducing ulcer pain [1].

New methods, enabling determination with maximum precision, selectivity, specificity and accuracy should ensure simultaneous determination of individual components in multicomponent preparations and in biological media. Development and validation of analytical methods were of basic importance to optimize the analysis of drugs in the pharmaceutical industry and to guarantee quality of the commercialized product [2]. The present review comprised analytical methods that have been used for the determination of famotidine in individual dosage form or in combination with other drugs as well as in biological fluids. The drug is official in both the British Pharmacopoeia (BP) [3] and the United States Pharmacopoeia (USP)

[4]. The BP recommended a potentiometric non aqueous method for the determination of FMT using perchloric acid as the titrant, while the USP proposed a similar approach for the determination of FMT in its bulk form, and an HPLC method using a mixture of acetate buffer of pH 6: acetonitrile (93:7) as a mobile phase with UV detection at 275 nm. According to the European Pharmacopoeia [5] and USP, the drug was determined in pharmaceutical preparations by potentiometric titration and HPLC methods. Thin layer chromatographic method has been reported for the determination of the related substances of FMT.

UV-VIS spectrophotometry, spectrofluorimetry and flow injection analysis

Visible spectrophotometry was still considered to be a very convenient and economical technique because of its simplicity and speed, the inexpensive equipment needed and accuracy of results. Visible spectrophotometric methods based on different reactions have been proposed for the assay of FMT in pharmaceutical dosage forms.

- Three rapid and simple spectrophotometric methods were described for the determina-

tion of famotidine [6]. The methods were based on charge transfer complexation of the drug with following reagents: chloranil, dichloro dicyano benzoquinone and dichloro-nitrophenol in methanol to give intensely coloured products. The absorbance was measured at the wavelengths of maximum absorption 458, 460 and 425 nm respectively. The effect of several variables on colour development was studied. The mole ratio of the reactants in each case has been established. The proposed methods have been applied successfully for the analysis of famotidine in raw materials and also in tablets.

- Basavaiah et al. have found that amino groups in FMT reduced iron (III) to iron (II) [7]. The iron (II) reacted with ferricyanide to form specific coloured product named Prussian blue with absorption maximum at 760 nm. The application of the method to the analysis of tablets yielded a recovery of 98.64-102.34%.
- A simple spectrophotometric method for determination of famotidine was described from Reddy et al. [8]. The method was based on bromination of the drug with excess brominating mixture in acidic medium. The yellow colour produced was measured at 350 nm against distilled water blank. Beer's law was obeyed in the range of 40-200 $\mu\text{g/ml}$.
- A kinetic method for the accurate and sensitive determination of famotidine has been described [9]. The determination was based on the alkaline oxidation of the drug with potassium permanganate at a fixed time of 10 min. The absorbance of formed manganate ion was measured at 610 nm. The concentration of famotidine was calculated using the calibration equation for the fixed time method. Beer's law was obeyed in the range of 1-10 $\mu\text{g/ml}$ and the RSD ($n = 10$) was 0.47%. The method has been applied successfully to commercial tablet dosage form.
- Nafisur and co-authors have developed a simple and fast spectrophotometric procedure for the determination of famotidine [10]. The method was based on the interaction of ninhydrin with primary amino group present in the famotidine. The proposed method has been applied to quality control of tablets with satisfactory results.
- Three simple, accurate, sensitive and selective spectrophotometric methods (A, B and C) for the determination of famotidine in bulk sample, in dosage forms and in the presence of its oxidative metabolites have elaborated by Amin et al. [11]. The first method A was based on oxidation of the drug by N-bromosuccinimide (NBS) and determination of the unreacted NBS by measuring the decrease in absorbance of Amaranth dye (AM) at a suitable λ_{max} (521 nm). The methods B and C involved addition of excess cerium sulphate and determination of the unreacted Ce(IV) by decrease the red colour of chromotrope 2R (C2R) at λ_{max} 528 nm for method B or decrease the orange pink colour of rhodamine 6G (Rh6G) at λ_{max} 526 nm for method C.
- A simple, accurate and sensitive spectrophotometric method for analysis of FMT has been developed [12]. The method was based on the reaction of drug with N-bromosuccinimide and subsequent reaction of the remaining NBS with fluorescein (FLC) to give a pink colored product that was measured at 518 nm. Different variables affecting the reaction conditions were carefully studied and optimized. Under the optimum conditions, Beer's law was obeyed in the drugs concentration range of 0.5-35 g/ml. The assay limits of detection and quantitation were 0.13-1.32, and 0.44-4.42 g/ml, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2%. The reaction stoichiometry and mechanism were studied. The proposed method was successfully applied to the analysis of the investigated drug in pure and pharmaceutical dosage forms without interference from the common excipients.
- A simple, accurate and sensitive spectrophotometric method for determination of FMT has been developed from Hussein et al. [13]. Method was based on the reaction of the drug with NBS and subsequent measurement of the excess N-bromosuccinimide by its reaction with p-aminophenol to give a violet colored product (λ_{max} at 552 nm). Decrease in the absorption intensity (ΔA) of the colored product, due to the presence of the drug, was correlated with its concentration in the sample solution. The proposed method was successfully applied to the analysis of drug

from interest in bulk substance and in pharmaceutical dosage forms.

- Spectrophotometric and spectrofluorimetric methods were adopted for the analysis of famotidine and ranitidine depending on their reaction with 1,4-benzoquinone reagent at pH 5.2 and 5.6, respectively [14]. The absorbances of the resulting condensation products were measured at 502 and 508 nm for famotidine and ranitidine, respectively. Concentrations adhering to Beer's law were from 40-160 $\mu\text{g/ml}$ for famotidine and from 20-100 $\mu\text{g/ml}$ for ranitidine. Furthermore the resulting condensation products exhibited fluorescence at 665 nm when excited at 290 nm and the calibration graphs were linear from 0.4-1.4 $\mu\text{g/ml}$ for famotidine and from 0.21 $\mu\text{g/ml}$ for ranitidine. These methods were applied to the pharmaceutical preparations and the results were satisfactory. The spectrofluorimetric method was a hundred times more sensitive than the spectrophotometric procedure.
- Koricanac et al. has found that famotidine and palladium (II) ions formed a complex, Pd(II): FMT = 1:1, which has an absorption maximum at 345 nm [15]. The formation of the complex between famotidine and palladium (II) chloride in Britton-Robinson buffer solution in the pH range 2.23-8.50 was studied. The conditional stability constant of the complex at the optimum pH 2.62 and ionic strength 0.5M was found to be $\log K' = 3.742 \pm 0.025$. The proposed method was found to be suitable for accurate and sensitive analysis of famotidine both as the substance and its dosage forms.
- A simple, sensitive and specific method was developed for the determination of FMT in pharmaceutical preparations and biological fluids [16]. The proposed method was based on ternary complex formation of famotidine with EDTA and terbium chloride in acetate buffer of pH=4. Alternatively, the complex was formed via the reaction with hexamine and either lanthanum chloride LaCl₃, or cerium chloride CeCl₃ in borate buffer of pH=6.2 and 7.2 respectively. In all cases, the relative fluorescence intensity of the formed complexes was measured at 580 nm after excitation at 290 nm. The fluorescence intensity - concentration plots were linear over

the concentration range of 10-100, 5-70, and 5-60 ng/ml, with minimum quantification limits (LOQ) of 2.4, 2.2, and 5.2 ng/ml, and minimum limits of detection (LOD) of 0.79, 0.74, and 1.7 ng/ml upon using TbCl₃, LaCl₃, and CeCl₃ respectively. The proposed method was applied successfully for the analysis of famotidine in dosage forms and in human plasma. The kinetics of both alkaline and oxidative induced degradation of the drug was studied using the proposed method. The apparent first order rate constant and half life time were calculated.

- Four new extraction-free spectrophotometric methods have been established for the quantitation of famotidine [17]. The methods are based on the formation of yellow ion-pair complexes between the drug of interest and four sulphonphthalein dyes viz., bromothymol blue (method A), bromophenol blue (method B), bromocresol purple (method C) and bromocresol green (method D) in dioxane or acetone medium. The proposed methods were applied successfully to the determination of famotidine in tablets with good accuracy and precision and without interferences from common excipients.
- Two simple, rapid and inexpensive methods, a titrimetric one and spectrophotometric one, have been developed for the determination of famotidine in pure form and in its dosage forms involving the use of chloramine-T as the oxidimetric reagent [18]. In the titrimetric method, 3-15 mg sample was titrated directly with chloramine-T in hydrochloric acid medium to methyl orange end point. The spectrophotometric procedure was based on the oxidation of the drug with chloramine-T in excess followed by the estimation of the unreacted oxidant with reaction that yielded a characteristic red colour with an absorption maximum at 520 nm. The consumed amount of chloramine-T corresponded to the drug content. The analytical conditions of both methods were investigated and optimised. The molar ratio drug: oxidant of the titration reaction was found to be 1:2 and the probable reaction scheme was suggested in conformity with the stoichiometry. Spectrophotometry was valid within the concentration range of 0.0-40.00 $\mu\text{g/ml}$ with an apparent molar absorptivity $2.78 \times 10^3 \text{ l.mol}^{-1}/\text{cm}$ and

Sandell sensitivity of $121.20 \text{ ng}\cdot\text{cm}^{-2}$. The methods were reproducible and accurate and have been applied to the assay of famotidine in tablets and injections.

- Quantitative determination of famotidine in its dosage forms was carried out spectrophotometrically, analysing the coloured complex resulting from a charge-transfer interaction between the drug as an electron-donor and chloranilic acid as an electron-acceptor [19]. Famotidine-Chloranilic acid complex displayed λ -max at 525 nm. Conditions and time for complex formation have been optimized. The stoichiometry of the complex is 3:2 donor to acceptor mole ratio. Excellent recovery values (99.01-100.71%) was obtained for the drug in both its pure and pharmaceutical dosage forms.
- A simple, accurate and sensitive spectrophotometric method has been developed and validated for determination of H₂-receptor antagonists: cimetidine, famotidine, nizatidine, and ranitidine hydrochloride [20]. The method was based on the oxidation of these drugs with cerium (IV) in presence of perchloric acid and subsequent measurement of the excess Ce (IV) by its reaction with p-dimethylaminocinnamaldehyde to give a red colored product (λ max at 464 nm). The value of ΔA of the colored product, due to the presence of the drug was correlated with its concentration in the sample solution. Different variables affecting the reaction were carefully studied and optimized. Under the optimum conditions, linear relationships with good correlation coefficients (0.9985-0.9994) were found between ΔA values and the concentrations of the drugs in a concentration range of 1-16 $\mu\text{g}/\text{ml}$. The assay limits of detection and quantitation were 0.12-0.44 and 0.37-1.33 $\mu\text{g}/\text{ml}$, respectively. The method was validated, in terms of accuracy, precision, ruggedness, and robustness; the results were satisfactory. The proposed method was successfully applied to the analysis of the investigated drugs in their pure and pharmaceutical dosage forms (recovery was 98.8-102.5 \pm 0.79-1.72%) without interference from the common excipients.
- A spectrophotometric stability—indicating method have been presented for the deter-

mination of famotidine in the presence of its degradation products [21]. Method was based on measuring the peak height of the second—derivative maximum at 304 nm. The proposed method was used for the analysis of famotidine in its pharmaceutical dosage forms. The results obtained were precise and accurate.

- Three new, simple, cost effective, sensitive and accurate UV spectrophotometric methods have been carried out for the determination of FMT in bulk drug and in tablets [22]. The methods were based on measurement of absorbance of FMT in 0.1 M NaOH solution at 286 nm (method A), 0.1 M HCl solution at 265 nm (method B) and in 0.1 M H₂SO₄ solution at 265 nm (method C). Beer's law is obeyed over the concentration ranges 0.63 - 15.0 $\mu\text{g}/\text{ml}$ for method A and 1.25 - 30.0 $\mu\text{g}/\text{ml}$ for methods B and C, respectively. The molar absorptivity values are calculated to be $2.14 \times 10^4 \text{ l}/\text{mol}/\text{cm}$ (method A), $1.18 \times 10^4 \text{ l}/\text{mol}/\text{cm}$ (method B) and $1.22 \times 10^4 \text{ l}/\text{mol}/\text{cm}$ (method C) and the corresponding Sandell sensitivity values are 0.016, 0.029 and 0.028 $\mu\text{g}/\text{cm}^2$.

In conclusion, most of the above visible spectrophotometric methods suffered from one or other disadvantage such as poor sensitivity, poor selectivity, use of expensive reagent or heating step, narrow linear range, strict pH control.

Flow injection analysis proved to be a suitable technique for on line analysis because of its low reagent and sample consumption and to its simplicity, high sampling frequency and the repeatability of its results.

- A flow injection determination of famotidine has been described by Kamath et al. [23]. The method was based on the reaction of the drug with cupric acetate to form a blue complex which shows absorption maxima at 314 nm and 630 nm. Samples could be analysed at rates up to 60 per hour with a relative standard deviation less than 1.4%. The method was evaluated by analysis of the pure drug and pharmaceutical formulations.
- A flow injection kinetic spectrophotometric method has been developed for the determination of famotidine in pharmaceutical preparations by Helali et al. [24]. The method was based on a kinetic investigation of the oxydation reaction of the drug in alkaline potassium

permanganate. The absorbance of the produced green coloured manganate species was monitored at 610 nm. Flow injection variable parameters such as reagent concentration, injected volume, reactor length and flow rate were carefully investigated and optimised. The flow injection method can be satisfactorily applied to the determination of famotidine in pharmaceutical preparations with a sampling frequency of 60 samples per hour.

Electrochemical methods

- Two new potentiometric methods for determination of famotidine in pure form and in its pharmaceutical tablet form have been presented [25]. In the first method, the construction of poly(vinyl chloride) (PVC) matrix-type famotidine ion-selective membrane electrode and its use in the potentiometric determination of famotidine in pharmaceutical preparations has been described. It is based on the use of the ion-associate species, formed by famotidine cation and tetraphenyl borate (TPB) counterion. The electrode exhibited a linear response for 1×10^{-3} – 1×10^{-5} M of famotidine solutions over the pH range 1–5 with an average recovery of 99.26% and mean standard deviation of 1.12%. Common organic and inorganic cations showed negligible interference. In the second method, the conditions for the oxidimetric titration of famotidine have been studied. The method depended on using lead (IV) acetate for oxidation of the thioether contained in famotidine. The titration takes place in presence of catalytic quantities of potassium bromide. Direct potentiometric determination of 1.75×10^{-2} M famotidine solution showed an average recovery of 100.51% with a mean standard deviation of 1.26%. The two methods have been applied successfully to commercial tablet.
- A differential pulse voltammetric method for the determination of famotidine in pharmaceutical preparations has been elaborated [26]. The method was based on electrochemical oxidation of famotidine at a glassy carbon or platinum electrode. The proposed method showed good reproducibility, and sample preparation was simple.
- Wallash et al. described the development of a sensitive, rapid polarographic method

for the determination of famotidine in pure form and in certain dosage forms [27]. The proposed method depends upon studying the polarographic activity of Nickel (II)-famotidine complex in Britton Robinson buffer over the pH range 4–8 and its usefulness in the analysis of famotidine using direct current, differential pulse, and alternating current polarography. The different experimental parameters affecting the cathodic waves were carefully investigated and optimized. Moreover, in order to check the validity of the proposed method, the standard addition method was applied by adding famotidine to the previously analyzed tablets. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of commercial tablets and the recovery study suggested that there is no interference from any excipients, which are present in tablets. Statistical comparison of the results was performed with regard to accuracy and precision using student's t-test and F-ratio at 95% confidence level.

- Two titrimetric and two spectrophotometric methods have been described for the assay of famotidine in tablets using N-bromosuccinimide [28]. Method A was direct in which FMT was titrated with NBS in HCl medium using methyl orange as indicator. The remaining three methods are indirect in which the unreacted NBS was determined after the complete reaction between FMT and NBS by iodometric back titration (method B) or by reacting with a fixed amount of either indigo carmine (method C) or neutral red (method D). The method A and method B were applicable over the range of 2–9 mg and 1–7 mg, respectively. In spectrophotometric methods, Beer's law was obeyed over the concentration ranges of 0.75–6.0 $\mu\text{g/ml}$ (method C) and 0.3–3.0 $\mu\text{g/ml}$ (method D). The applicability of the developed methods was demonstrated by the determination of FMT in pure drug as well as in tablets.

Separation techniques

- A simple and rapid capillary zone electrophoresis (CZE) method with UV detection has been developed for the determination of famotidine and its potential impurities in

pharmaceutical formulations [29]. The electrophoretic separation of these compounds was performed using a fused silica capillary and 37.5 mmol/l phosphate buffer pH 3.5 as the electrolyte. Under the optimised conditions, six impurities could be resolved from the famotidine peak in less than 7 min. The calibration curves obtained for the seven compounds were linear over the concentration range investigated (from 1.5 to 78.5 µg/ml). The intra- and inter-day relative standard deviations for the migration times and corrected peak areas were less than 2% and 5%, respectively. The detection limits were found to be 0.09 µg/l for famotidine, and from 0.1 to 0.62 µg/l depending on the impurities. The method has been successfully applied to the determination of famotidine in commercial dosage forms.

- A simple and sensitive capillary electrophoresis method using UV detection has been developed for the direct determination of ranitidine and famotidine in serum, urine and pharmaceutical formulations [30]. A buffer consisting of 60 mM phosphate buffer adjusted to pH 6.5 was found to provide a very efficient and stable electrophoretic system for the analysis of both drugs. The detection limits obtained were 0.08 µg/ml for ranitidine and 0.16 µg/ml for famotidine.
- A quantitative method using silica gel high performance thin layer chromatography plates with fluorescent indicator, automated sample application, and UV absorption vid-eodensitometry was carried out for the determination of famotidine tablets [31]. Three pharmaceutical tablet products containing famotidine as the active ingredient were analyzed to test the applicability of the new method. Precision was evaluated by replicate analyses of the samples and accuracy by analysis of a sample, spiked with standard, and reanalysis (standard addition). The percent famotidine in the tablets ranged from 92.5% to 140% compared to label values, precision from 1.25% to 2.55% relative standard deviation, and the error in the standard addition analysis was 1.76% compared to the fortification level.
- A high-performance thin-layer chromatographic method has been described for in-process control and content uniformity testing of pharmaceutical formulations containing ranitidine hydrochloride and famotidine [32]. HPTLC separation was performed on silica precoated plates using the mobile phase toluene–methanol–diethylamine (9:1:1, v/v) for both drugs. The samples were applied on a HPTLC plate automatically. Quantification was done by densitometry at in situ UV absorption maxima of ranitidine hydrochloride and famotidine at 320 nm and 276 nm, respectively. The method was validated in terms of selectivity (related compounds and placebo effect), system suitability, range (30 to 230 ng for ranitidine hydrochloride and 80 to 580 ng for famotidine), accuracy, precision, ruggedness and analyte stability. A large number of analyses were performed simultaneously with a low solvent consumption. It was found that the method was fast, accurate and cost-effective.
- A simple, sensitive, and rapid reversed-phase high-performance liquid chromatographic method has been developed for determination of famotidine and its impurities in pharmaceutical formulations [33]. Separations were performed on a Supelcosil LC18 column with an isocratic mobile phase—13:87 v/v acetonitrile–0.1 M dihydrogen phosphate buffer containing 0.2% triethylamine (pH 3.0). The mobile phase flow rate was 1 ml/min and the detection wavelength was 265 nm. Response was linearly dependent on concentration between 1 and 80 µg/ml. RSD from determination of method repeatability (intraday) and reproducibility (interday) were <2% (n=6). Lowest detectable concentrations ranged from 0.08 to 0.14 µg/ml.
- A HPLC method has been developed for the determination of famotidine and related compounds in drug raw materials and formulations [34]. The minimum detectable amount of the available related compounds is less than 0.02% and the minimum quantifiable amount is less than 0.1%. Famotidine impurity levels were found to be between 0.5 and 2.5% in raw materials, 0.44% in one tablet sample and about 3% in an injection solution.
- A method for the determination of famotidine in tablets and vials has been described [35]. The procedure was based on the use of the reversed-phase high-performance liquid

chromatography, and of the second-derivative ultraviolet spectra, by utilizing the linear relationship between drug concentration and derivative peak amplitude. The minimum concentration detectable by derivative spectrophotometry was 0.5 µg/ml, and by HPLC 0.1 µg/ml. The relative standard deviations observed were approx. 1.5% for derivative spectrophotometry, and 1.2% for HPLC. The proposed methods, which give thoroughly comparable data, were simple and rapid, and allow one to obtain precise and accurate results.

- A HPLC method has been developed and validated for the simultaneous determination of ibuprofen and famotidine in combined pharmaceutical formulation [36]. Separation was achieved with a C8 (250 mm x 4.6 mm, 5 µm) column, ambient temperature with isocratic mode with mobile phase containing acetonitrile and 0.5 M potassium dihydrogen phosphate buffer pH 2.2 adjusted with ortho-phosphoric acid (25:75). UV detection was performed at 280 nm. The flow rate was 1.2 ml/min. The retention times of ibuprofen and famotidine were found to be 3.19 min and 8.37 min, respectively. The responses were linear ($R^2 > 0.9999$) in the range of 20 – 160 µg/ml for ibuprofen and 0.68 – 5.4 µg/ml for famotidine. The % recovery for ibuprofen and famotidine was 99.75 and 99.24, respectively. No chromatographic interference from the tablet excipients was found. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which can be applied for the routine analysis of ibuprofen and famotidine in tablet dosage forms.
- A simple, specific, precise and accurate (RP-LC) method has been proposed for the simultaneous determination of ketoprofen and famotidine in laboratory mixtures [37]. The chromatographic separation was performed on a LiChrosorb C18, 125 mm x 4.6 mm, 5 µm column at a detector wavelength of 230 nm and a flow rate of 1.5 ml/min. The mobile phase was composed of phosphate buffer (pH adjusted to 7.4 with ortho-phosphoric acid) and acetonitrile (20:80 v/v). The retention times of ketoprofen and famotidine were found to be 3.05 and 6.96 min, respectively.

The method was validated for the parameters like specificity, linearity, precision, accuracy, ruggedness, limit of quantitation and limit of detection. The calibration curves were linear in the concentration range of 25.00-200.0 µg/ml for ketoprofen and 5.00-40.00 µg/ml for famotidine. The % recovery for both drugs was in the range between 98.33% and 99.85% with RSD values not greater than 2.55.

- An improved, rapid and specific high-performance liquid chromatographic assay has been described for the determination of famotidine in human plasma and urine [38]. Plasma samples were alkalinized and the analyte and internal standard (cimetidine) extracted with water-saturated ethyl acetate. The extracts were reconstituted in mobile phase, and injected onto a C18 reversed-phase column; UV detection was set at 267 nm. Urine samples were diluted with nine volumes of a mobile phase-internal standard mixture prior to injection. The lower limits of quantification in plasma and urine were 75 ng/ml and 1.0 µg/ml, respectively; intra- and inter-day coefficients of variation were $\leq 10.5\%$. This method is currently being used to support renal function studies assessing the use of intravenously administered famotidine to characterize cationic tubular secretion in man.
- 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 [39]. The procedure employed ranitidine as an internal standard. 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.

- A rapid, sensitive and robust reverse-phase high performance liquid chromatographic method with column switching and an internal standard for the quantitative determination of famotidine in human plasma has been developed [40]. Famotidine and the internal standard were isolated from plasma samples by cation exchange solid phase extraction with SCX cartridges. The chromatographic separation was accomplished by an Inertsil C4 column with a mobile phase of acetonitrile/phosphate aqueous solution, connected by a switching valve to a BDS Hypersil C8 column with a mobile phase of acetonitrile/sodium dodecyl sulfate and phosphate aqueous solution. UV detection was set at 267 nm. The standard curve was linear in the concentration range of 1–100 ng/ml. The intraday coefficients of variation at all concentration levels were less than 10%. The interday consistency was assessed by running QC samples during each daily run. The limit of quantification for famotidine in human plasma was 1 ng/ml. The method has been utilized to support clinical pharmacokinetic studies in healthy volunteers who received famotidine 10 mg orally.
- A new method has been reported by Anzenbacherová et al. [41] for the determination of famotidine by solid phase extraction from alkalized human plasma followed by reversed phase HPLC in acetonitrile/alkaline buffer with molsidomine as an internal standard. Different acetonitrile/aqueous buffer mobile phases as well as various 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.
- A sensitive high-performance liquid chromatographic method for the quantitation of famotidine in human plasma has been developed [42]. Clopamide was used as the internal standard. Plasma samples were extracted with diethyl ether to eliminate endogenous interferences. Plasma samples were then extracted at alkaline pH with ethyl acetate. Famotidine and the internal standard were readily extracted into the organic solvent. After evaporation of ethyl acetate, the residue was analysed by HPLC. The chromatographic separation was accomplished with an isocratic mobile phase consisting of acetonitrile—water (12:88, v/v) containing 20 mM disodium hydrogenphosphate and 50 mM sodium dodecyl sulphate, adjusted to pH 3. The HPLC microbore column was packed with 5 μ m ODS Hypersil. Using ultraviolet detection at 267 nm, the detection limit for plasma famotidine was 5 ng/ml. The calibration curve was linear over the concentration range 5–500 ng/ml. The inter- and intra-assay coefficients of variation were found to be less than 10%. Applicability of the method was demonstrated by a bioavailability/pharmacokinetic study in normal volunteers who received 80 mg famotidine orally.
- A rapid, specific and sensitive high-performance liquid chromatographic method for the determination of famotidine in human plasma has been reported [43]. Famotidine and the internal standard were chromatographically separated from plasma components using a Lichrocart Lichrospher 60 RP select B cartridge for solid-phase separation with a mobile phase composed of 0.1 % (v/v) triethylamine in water (pH 3) and acetonitrile (92:8, v/v). UV detection was set at 270 nm. The calibration curve was linear in the concentration range of 10.0 – 350.0 ng/ml. The method was implemented to monitor the famotidine levels in patient samples.
- A stability-indicating HPLC analytical method has been developed for the determination of famotidine in the presence of its degradation products [44]. The method utilizes reversed phase chromatography with UV detection and internal calibration techniques. The mobile phase was comprised of 84% ammonium acetate buffer (pH 2.9) and 16% acetonitrile and pumped at a flow rate of 1.5 ml/min. Quantitation was performed by measuring the peak height ratio of drug to internal standard (salicylic acid). The lim-

- it of famotidine detection was determined to be 10 ng (0.4 µg/ml) with a signal to noise ratio of 3:1. Within day coefficient of variation of the method was 2.22% (2.5 µg/ml) and 0.82% (10 µg/ml). Between day coefficient of variation based on the slopes of daily prepared standard curves was 4.70%. The developed method was used to determine the drug content of famotidine tablets. Further, it was used to investigate the kinetics of degradation of the drug in an acidic solution.
- A HPLC method has been reported for the quantitation of famotidine in tablet formulation using a mobile phase consisting of 0.1M phosphate buffer (84%), acetonitrile (11%) and methanol (5%) at a pH of 6.5 [45]. The detection wavelength was set at 285 nm. The method is precise and adaptable for quality control purposes as well as in tablet dissolution investigations.
 - A rapid and sensitive HPLC method using a monolithic column has been developed for quantification of famotidine in plasma [46]. The assay enables the measurement of famotidine for therapeutic drug monitoring with a minimum detectable limit of 5 ng/ml. The method involves simple, one-step extraction procedure and analytical recovery was complete. The separation was carried out in reversed-phase conditions using a Chromolith Performance (RP-18e, 100 mm × 4.6 mm) column with an isocratic mobile phase consisting of 0.03 M disodium hydrogen phosphate buffer–acetonitrile (93:7, v/v) adjusted to pH 6.5. The wavelength was set at 267 nm. The calibration curve was linear over the concentration range 20–400 ng/ml. The coefficients of variation for inter-day and intra-day assay were found to be less than 8%.
 - An analytical method has been reported for the determination of famotidine, potential degradates, and preservatives in several pharmaceutical formulations [47]. The method employed reversed-phase chromatography on a silica column with a methanol–phosphate solution as the mobile phase. The influence of the concentration of phosphate and organic modifier are discussed. Accuracy and precision for this method along with assay data from different formulations of famotidine were presented.
 - A HPLC method for the determination of famotidine in human plasma, a basic polar drug with poor solubility in organic solvents, has been presented [48]. In order to optimize the mass detection of famotidine, several parameters such as ionization mode, fragmentor voltage, m/z ratios of ions monitored, type of organic modifier and eluent additive, were investigated. Each analysis required 5 min. The calibration curve of famotidine in the range 1–200 ng/ml was linear with a correlation coefficient of 0.9992 (n=6), and a detection limit a signal-to-noise ratio of 3 was 0.2 ng/ml. The within- and between-day variations in the famotidine analysis were 5.2 (n=6) and 6.7% (n=18), respectively. The applicability of this method was also demonstrated for the analysis of plasma samples in a Phase-I human pharmacokinetic study.
 - A sensitive and rapid HPLC method for assay of famotidine in both plasma and urine samples has been reported [49]. Ranitidine and cimetidine were used as internal standards for urine and plasma measurements. For plasma, the mobile phase was acetonitrile-water (15: 85 v/v), 45 mM sodium dodecyl sulphate (SDS) and 20 mM disodium hydrogen phosphate adjusted to pH 3. A mixture of methanol-phosphate buffer (20: 80 v/v) adjusted to pH 6.3 was used as the mobile phase for the determination of the compound in urine. The separation was performed on an analytical 150 - 3.9 mm i.d. reversed-phase Novapack C18 (4 µm particle size) column using UV detector (267 nm for plasma and 282 nm for urine). The detection limits for famotidine in plasma and urine were 7.5 ng/ml and 160 ng/ml, respectively. The inter- and intra-assay coefficients of variation were found to be less than 10%.
 - A rapid, sensitive and robust assay procedure using liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) for the determination of famotidine in human plasma and urine has been described [50]. Famotidine and the internal standard were isolated from plasma samples by cation-exchange solid-phase extraction with benzenesulfonic acid (SCX) cartridges. The urine assay used direct injection of a diluted urine sample. The chromatographic separation was accomplished by using a BDS Hy-

persil silica column with a mobile phase of acetonitrile–water containing trifluoroacetic acid. The MS/MS detection of the analytes was set in the positive ionization mode using electrospray ionization for sample introduction. The analyte and internal standard precursor–product ion combinations were monitored in the multiple-reaction monitoring mode. Assay calibration curves were linear in the concentration range 0.5–500 ng/ml and 0.05–50 µg/ml in plasma and urine, respectively. For the plasma assay, a 100 µl sample aliquot was subjected to extraction. To perform the urine assay, a 50 µl sample aliquot was used. The intra-day relative standard deviations at all concentration levels were <10%. The inter-day consistency was assessed by running quality control samples during each daily run. The limit of quantification was 0.5 ng/ml in plasma and 0.05 µg/ml in urine. The methods were utilized to support clinical pharmacokinetic studies in infants aged 0–12 months.

- A simple and rapid high-performance liquid chromatographic method has been developed for the separation and determination of small amounts of process impurities viz., thiourea, diaminomethyleneamino(chloromethyl)thiazole and diaminomethyleneamino(1-amino-1'-iminomethylene)thiomethylthiazole in famotidine [51]. The separation was achieved on a reversed-phase C8 column using acetonitrile-0.01M aqueous potassium dihydrogenphosphate (25:75, v/v; pH 3.15) as eluent. The method was used not only for quality assurance but also for monitoring the reactions involved in process development of famotidine. The mean recovery of famotidine from authentic samples was 99.48±1.87% and the limit of detection was 5·10⁻⁹ g.
- An efficient reversed phase high performance liquid chromatographic method has been reported for quantitation of famotidine and its process impurities which may coexist in bulk drugs and in solid pharmaceutical dosage forms [52]. The separation was achieved on a C18 column (250 mm x 4.6 mm) using a mobile phase of acetonitrile, methanol and 1-hexane sodium sulfonate. Flow rate was 1.5 ml/min. The photo diode array detector was operated at 266 nm. The method was

validated for specificity, linearity, precision, accuracy and limit of quantification. The degree of linearity of the calibration curves, the percent recoveries of famotidine and impurities, the limit of detection and quantitation, for the HPLC method were determined. The method was found to be simple, specific, precise, accurate and reproducible. The method was applied for the quality control of commercial famotidine tablets to quantify the drug and its related substances and to check the formulation content uniformity.

- A rapid and specific high-performance liquid chromatographic method has been developed for quantitative analysis of famotidine in pharmaceutical dosage forms [53]. The method was applied to medicines on the Turkish market. A LiChrospher RP-18 column, HP-1050 UV-detector and methanol-0.1 M aqueous ammonium acetate (30:70) as the solvent system (flow rate 1.0 ml/min) were used. Quantitative determination of famotidine was carried out at 254 nm wavelength. The method was found to be rapid - under 5 min per assay-and was selective enough to allow the determination of famotidine in the presence of certain excipients.

Conclusion

In this paper various analytical methods for the determination of H₂-antagonist famotidine in bulk material, pharmaceutical formulations and biological specimens were reviewed. Spectrophotometric techniques provided practical (less-time-consuming, simple, and more convenient) and significant economic advantages over other methods; therefore, they are a frequent choice for pharmaceutical analyses. HPLC methods generally required complex and expensive equipment, provision for use and disposal of solvents, tedious sample preparation procedure, and personal skills in chromatographic techniques. In addition, most of the HPLC methods summarized have the potential application to clinical research of drug combination, multidrug pharmacokinetics, and interactions.

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