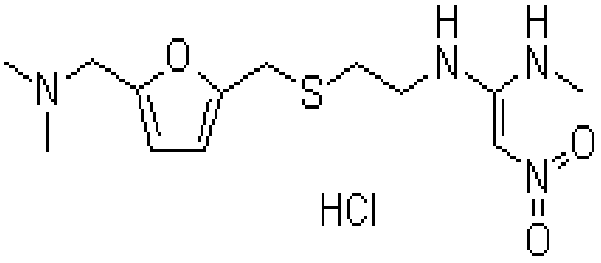
Introduction

No doubt that, it is critically vital to determine the purity and concentration of any therapeutic drug in high accuracy and precision. Since most of them are toxic in high concentration, detection of the dose is very essential. One of the most important procedures is the spectrophotometric method. In this study, a spectrophotometric method has been developed and validated for determination of H_2-receptor antagonists: cimetidine, ranitidine and famotidine in pure and pharmaceutical formulations. The applied method is characterized by its simplicity, selectivity and high sensitivity. In this chapter, short notes about the physical and chemical characters, mode of action and use are given. Also, a historical survey on some previous works concerned the determination of the drugs under investigation is shown briefly.

1- Literature survey for the determination of ranitidine hydrochloride

Systemic (IUPAC) name of ranitidine hydrochloride is N,N-dimethyl-5-[2-(1-methylamino-2-nitrovinyl)-ethylthiomethyl]furfurylamine monohydrochloride. Its structure is:



Molecular formula: $C_{13}H_{22}N_4O_3S.HCl$, molecular weight = 350.86

Ranitidine is a histamine H_2-receptor antagonist that inhibits stomach acid production. It is currently marketed under the trade name Zantac

by GlaxoSmithKline in prescription form and by Pfizer in over-the-counter form.

Ranitidine was found to have a far-improved tolerability profile (i.e. fewer adverse drug reactions), longer-lasting action, and ten times the activity of cimetidine. Ranitidine has 10% the affinity that cimetidine has to CYP450 so it causes fewer side effects, but other H_2 blockers famotidine and nizatidine have no CYP450 significant interactions.

Uses: ranitidine is used to treat ulcers; gastro esophageal reflux disease (GERD), a condition in which backward flow of acid from the stomach causes heartburn and injury of the food pipe (esophagus); and conditions where the stomach produces too much acid, such as Zollinger-Ellison syndrome. Over-the-counter ranitidine is used to prevent and treat symptoms of heartburn associated with acid indigestion and sour stomach. Ranitidine is in a class of medications called H_2 blockers. It decreases the amount of acid made in the stomach.

Ranitidine is also used sometimes to treat upper gastrointestinal bleeding and to prevent stress ulcers, stomach damage from use of nonsteroidal anti-inflammatory drugs (NSAIDs), and aspiration of stomach acid during anesthesia.

Ranitidine comes as a tablet, an effervescent tablet, effervescent granules, and a syrup to take by mouth. It is usually taken once a day at bedtime or two to four times a day. To prevent symptoms, it is taken 30 to 60 minutes before eating or drinking foods that cause heartburn.

Three simple, accurate, economical and reproducible UV spectrophotometric methods for simultaneous estimation of two

component drug mixture of ranitidine hydrochloride and ondansetron hydrochloride from combined tablet dosage form had been developed[1]. First developed method involved formation and solving of simultaneous equations at 267.2 nm and 314.4 nm. Second method was developed making use of first order derivative spectroscopy using 340.8 nm and 276.0 nm as zero crossing points for estimation of ranitidine hydrochloride and ondansetron hydrochloride respectively. Third method was based on two-wavelength calculation. Wavelengths selected for estimation of ranitidine hydrochloride were 266.1 nm and 301.8 nm and for ondansetron hydrochloride 305.7 nm and 319.2 nm. The results of analysis have been validated statistically and by recovery studies.

Four new methods were described[2] for the determination of ranitidine hydrochloride (RNH) in bulk drug and in formulations employing titrimetric and spectrophotometric techniques using potassium dichromate as the oxidimetric reagent. In titrimetry (method A), RNH was treated with a measured excess of dichromate in acid medium, and the unreacted oxidant was back titrated with iron(II) ammonium sulfate. The three spectrophotometric methods were also based on the oxidation of RNH by a known excess of dichromate under acidic conditions followed by the determination of surplus oxidant by three different reaction schemes. In one procedure (method B), the residual dichromate is treated with diphenylcarbazide and the absorbance measured at 540 nm. Calculated amount of iron(II) was added to residual dichromate and the resulting iron(III) was complexed with thiocyanate and measured at 470 nm (method C). Method D involved reduction of unreacted dichromate by a calculated amount of iron(II) and estimation of residual iron(II) as its orthophenanthroline complex after raising the pH, and measuring the absorbance at 510 nm.

In all the methods, the amount of dichromate reacted corresponds to the drug content. The experimental conditions were optimized. The titrimetric procedure was applicable over 5-10 mg range. In spectrophotometric methods, Beer's law was obeyed in the ranges 5-50, 5-80, and 10-100 µg ml^{-1} for method B, method C, and method D, respectively. The methods were validated for accuracy, precision and recovery. The proposed methods were applied to the analysis of RNH in the tablet and the injection forms, and the results were in agreement with those obtained by the reference method.

Two simple, rapid and cost-effective methods based on titrimetric and spectrophotometric techniques were described[3] for the assay of ranitidine hydrochloride (RNH) in bulk drug and in dosage forms using silver nitrate, mercury(II) thiocyanate and iron(II) nitrate as reagents. In titrimetry, an aqueous solution of RNH is treated with measured excess of silver nitrate in HNO_3 medium followed by determination of unreacted silver nitrate by Volhard's method using iron(III) alum indicator. Spectrophotometric method involved the addition of a known excess of mercury(II) thiocyanate and iron(II) nitrate to RNH, followed by the measurement of the absorbance of iron(III) thiocyanate complex at 470 nm. Titrimetric method was applicaple over 4-30 mg range and the reaction stoichiometry was found to be 1:1 (RNH:$AgNO_3$). In the spectrophotometric method, the absorbance was found to increase linearly with concentration of RNH which was corroborated by the correlation coefficient of 0.9959. The system obeyed Beer's law for 5-70 µg/mL. The apparent molar absorptivity and Sandell sensitivity values were found to be $3.27x10^3$ L $mol^{-1}cm^{-1}$and 0.107 µgcm^{-2}, respectively. The limits of detection and quantification were also reported for the spectrophotometric method. Intra-day and inter-day precision and accuracy of the method were evaluated as per ICH guidelines. The method were successfully applied to the assay of RNH in formulations

and the results were compared with those of a reference method by applying Student's t and F-tests. No interference was observed from common pharmaceutical excipients. The accuracy of the methods was further ascertained by performing recovery tests by standard addition method.

A novel method for the determination of Ranitidine in flow injection systems was developed[4]. The effects of various parameters on the sensitivity of the method were investigated. The conditions producing optimal performance were a pH value of 2, a scan rate value of 100 V/s, accumulation potential of (− 100) mV, and accumulation time of 0.4 s. Some of the advantages of the proposed method were as follows: the removal of oxygen from the test solution was not required any more, the detection limit of the method was sub-nanomolar and finally, the method was fast enough for determination of compounds in a wide variety of chromatographic methods. A special computer based numerical method was introduced for calculation of the analyte signal and noise reduction. After subtracting the background current from noise, the electrode response was calculated, based on partial and total charge exchanges at the electrode surface. The integration range of currents was set for all the potential scan ranges, including oxidation and reduction of the Au surface electrode, to obtain a sensitive determination. The waveform potential was continuously applied on an Au disk microelectrode (12.5 μm in radius). The detection limit of the method for Ranitidine was found to be 25 pg/ml. For 8 runs, the relative standard deviation of the method at 1.1×10^{-8} M was 2.1%. The method was successfully applied for fast determination of Ranitidine in its pharmaceutical formulations. Being very simple, precise, accurate, time saving and economical this method has many advantages compared to all previously reported methods.

A simple, fast and precise multicomponent mode analysis method had been developed for simultaneous estimation of ranitidine and domperidone in tablet formulation[5]. The sampling wavelengths selected for both the drugs were 229 nm, 245 nm, 270 nm, 285 nm, 294 nm on trial-and-error basis using methanol as solvent. The linearity for both the drugs at all the selected wavelengths lied between 3.0 and 50 µg/ml for ranitidine and 0.2 and 3.5 µg/ml for domperidone. The concentrations of both the drugs were evaluated in laboratory mixture and marketed formulation. The recovery study was carried out by standard addition method.

Three simple, rapid, reliable and cost-effective methods based on titrimetry and spectrophotmetry in non-aqueous medium were described for the determination of ranitidine in pharmaceuticals [6]. In titrimetry, the drug was dissolved in glacial acetic acid and titrated with acetous perchloric acid with visual and potentiometric end point detection, crystal violet being used as indicator for visual titration. Spectrophotometry involved adding different amounts of the drug to a fixed amount of perchloric acid-crystal violet mixture followed by measurement of absorbance at 570 nm. The absorbance was found to increase linearly with the concentration of the drug and formed the basis for quantification. The titrimetric methods were applicable over 1-15 mg range of ranitidine, and in spectrophotometry, calibration graph was linear from 10 to 70 µg /mL. The apparent molar absorptivity was 2.2×10^3 L mol^{-1} cm^{-1} and the calculated Sandell sensitivity was 161.7 ng cm^{-2}. The limits of detection and quantification were found to be 1.07 and 3.58 µg/mL, respectively. The procedures were used to determine ranitidine in pharmaceutical products and the results were found to be in good agreement with those obtained by the reference method. Associated pharmaceutical materials did not interfere. The accuracy and reliability of the methods were further ascertained by recovery

studies via standard-addition technique with percent recoveries in the range 96.3 to 102.5 %.

New ranitidine hydrochloride (RaCl)-selective electrodes of the conventional polymer membrane type were described[7]. They were based on incorporation of ranitidine-tetraphenylborate (Ra-TPB) ion-pair or ranitidine-phosphotungstate (Ra-PT) ion-associate in a poly(vinyl chloride) (PVC) membrane plasticized with dioctylphthalate (DOP) or dibutylphthalate (DBP). The electrodes were fully characterized in terms of the membrane composition, solution temperature, and pH. The sensors showed fast and stable responses. Nernstian response was found over the concentration range of 2.0×10^{-5} M to 1.0×10^{-2} M and 1.0×10^{-5} M to 1.0×10^{-2} M in the case of Ra-TPB electrode and over the range of 1.03×10^{-5} M to 1.0×10^{-2} M and 1.0×10^{-5} M to 1.0×10^{-2} M in the case of Ra-PT electrode for batch and FIA systems, respectively. The electrodes exhibited good selectivity for RaCl with respect to a large number of common ions, sugars, amino acids, and components other than ranitidine hydrochloride of the investigated mixed drugs. The electrodes were applied to the potentiometric determination of RaCl in pure solutions and in pharmaceutical preparations under batch and flow injection conditions with a lower detection limit of 1.26×10^{-5} M and 5.62×10^{-6} M at 25 ± 1°C. An average recovery of 100.91% and 100.42% with a relative standard deviation of 0.72% and 0.23% had been achieved.

Sodium tetraphenylborate and phosphotungstic acid were used as titrants for the conductimetric determination of phenylpropanolamine HCl (PPA.Cl), ranitidine HCl (Ra.Cl), hyoscyamine HBr (Hy.Br) and betaine HCl (Be.Cl) through ion-associate complex formation[8]. The molar combining ratio and the solubility products of the formed ion-associates were studied and calculated. The suggested method had

been applied to the determination of the mentioned drugs in their pure state and pharmaceutical preparations with mean recovery values of 97.71–102.97% and relative standard deviations 0.25–0.85%. The accuracy of the method was indicated by excellent recovery and low standard deviation. The results were compared with the pharmacopoeial or the official methods.

Basavaiah and Nagegowda[9] described four methods using titrimetry and spectrophotometry for the determination of ranitidine hydrochloride (RNH) with potassium bromate as the oxidimetric reagent and acid dyes, methyl orange, indigo carmine and metanil yellow. In direct titrimetry (method A), the drug was titrated directly with bromate in acid medium and in the presence of excess of bromide using methyl orange indicator. In back titrimetry (method B), the drug was treated with a measured excess of bromate in the presence of bromide and acid, and the unreacted bromine was determined iodometrically. Both spectrophotometric methods were based on the oxidation of RNH by a known excess of bromate in acid medium and in the presence of excess of bromide followed by estimation of surplus oxidant by reacting with either indigo carmine (method C) or metanil yellow (method D), and measuring the absorbance at 610 or 530 nm. In methods B, C and D, reacted oxidant corresponds to the drug content. The experimental conditions were optimized. Titrimetric procedures were applicable over the ranges 1–10 mg (A) and 1–17 mg (B), and the reaction stoichiometry is found to be 1:1 (BrO^{-}_{3}: RNH). In spectrophotometric methods, the absorbance was found to increase linearly with increasing concentration of RNH, which is corroborated by the calculated correlation coefficient (*r*) of 0.9984 (C) and 0.9976 (D). The systems obey Beer's law for 2–12 and 1–7 μg ml^{-1}, for methods C and D, respectively. Method D with a molar absorptivity of 9.82×10^4 l mol^{-l} cm^{-1} was found to be more sensitive than method C ($\varepsilon = 2.06 \times l0^4$ l

mol^{-1} cm^{-1}). The limits of detection and quantification were reported for both the spectrophotometric methods. The proposed methods were applied successfully to the determination of RNH in tablets and injections. The reliability of the assay was established by parallel determination by the official method and by recovery studies.

Two Spectrophotometric procedures were presented for the determination of two commonly used H_2-receptor antagonists, nizatidine (I) and ranitidine hydrochloride (II)[10]. The methods were based mainly on charge transfer complexation reaction of these drugs with either p-chloranilic acid (p-CA) or 2, 3 dichloro-5, 6-dicyanoquinone (DDQ). The produced colored products were quantified spectrophotometrically at 515 and 467 nm in chloranilic acid and DDQ methods, respectively. The molar ratios for the reaction products and the optimum assay conditions were studied. The methods determined the cited drugs in concentration ranges of 20-200 and 20-160 µg/mL for nizatidine and ranges of 20-240 and 20-140 µg/mL for ranitidine with chloranilic acid and DDQ methods, respectively. A more detailed investigation of the complexes formed was made with respect to their composition, association constant, molar absorptivity and free energy change. The proposed procedures were successfully utilized in the determination of the drugs in pharmaceutical preparations. The standard addition method was applied by adding nizatidine and ranitidine to the previously analyzed tablets or capsules. The recovery of each 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 (standard addition method) of the cited drugs suggested that there is no interference from any excipients, which are present in tablets or capsules.

Two methods for determining ranitidine hydrochloride (RNH) in pure drug and in formulations using potassium iodate and dichlorofuorescein were described[11]. Titrimetry involved the oxidation of RNH by a known excess of potassium iodate in acidic conditions followed by iodometric determination of surplus iodate. In spectrophotometry also, the drug was oxidized by a large excess of iodate and the iodine released was oxidized to ICl_2^- in the presence of chloride ions, and is used to iodinate 2,7-dichlorofluorescein dye and the amount of iodinated dye was measured. Reaction conditions of both methods was optimized. In titrimetry, the reaction stoichiometry was established and the reaction scheme of the spectrophotometric method was given. Titrimetry was applicable over 1-16 mg range. In spectrophotometry, the system obeyed Beer's law for 5-50 µg mL^{-1}. The molar absorptivity and Sandell sensitivity were calculated to be 3.88×10^3 L mol^{-1} cm^{-1} and 5.72 ng cm^{-2}, respectively. The calculated limits of detection and quantification were 2.14 and 7.15 µg mL^{-1}, respectively. The proposed methods were applied successfully to the determination of RNH in pharmaceutical preparations with recoveries in the range of 98.28 ± 0.88 to 103 ± 1.96% (titrimetry) and 99.46 ± 1.88 to 102.58 ± 0.73% (spectrophotometry). The reliability of the assay was established by parallel determination, by an established procedure and by recovery studies using standard addition technique.

Three simple, accurate and sensitive colorimetric methods (A, B and C) for the determination of ranitidine HCl (RHCl) in bulk sample, in dosage forms and in the presence of its oxidative degradates were described[12]. The first method A was based on the oxidation of the drug by N-bromosuccinimide (NBS) and determination of the unreacted NBS by measurement of the decrease in absorbance of amaranth dye (AM) at λ_{max} = 520 nm. The methods B and C involve the addition of excess Ce^{4+} and determination of the unreacted oxidant by decrease

the red color of chromotrope 2R (C2R) at λ_{max} = 528 nm for method B or decrease the orange pink color of rhodamine 6G (Rh6G) at a suitable λ_{max} = 526 nm for method C. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 0.2-3.6, 0.1-2.8 and 0.1-2.6 µg /mL for methods A, B and C, respectively. The apparent molar absorptivity, Sandell sensitivity, detection and quantitation limits were calculated. For more accurate results, Ringbom optimum concentration ranges were 0.3-3.4, 0.2-2.6 and 0.2-2.4 µg/mL for methods A, B and C, respectively . Analyzing pure and dosage forms containing RHCl tested the validity of the proposed methods. The relative standard deviations were less than or equal to1.38 with recoveries 98.9-101.0%.

The voltammetric behavior of ranitidine was studied in aqueous media with a mercury coated platinum ultramicroelectrode (Hg-UME) and by HMDE[13]. The LSV curves for the electroreduction of ranitidine showed that the compound presents two reduction waves in pH < 4.0 and only one in pH > 4.0, the observed waves being attributed to the reduction of the nitro group to hydroxylamine. A linear relation between the current peak or limiting current and the ranitidine concentration using HMDE or Hg-UME was observed, demonstrating that these ultramicroelectrodes can be used in the analytical determination of ranitidine. An alternative and more sensitive methodology for the analytical determination of ranitidine by SWV was also developed, with a detection limit of 3.5×10^{-8} mol L^{-1} (or 11 µg L^{-1}). The apparent recovery (AR) studies proved the accuracy and precision of the assay developed. The excipients found in commercial Antak tablets (Glaxo Wellcome) and the generic from EMS did not interfere in the determination.

The spectrophotometric determination of trace amounts of ranitidine was carried out by liquid–liquid extraction using bromothymol blue with a flow system [14]. The determination of ranitidine in the range of 1×10^{-5}–1×10^{-4} mol l^{-1} was possible with a sampling frequency of 40 samples h^{-1}. The method was satisfactorily applied to the determination of ranitidine in pharmaceutical preparations and the recovery was quantitative and no interferences from excipients were observed.

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[15]. The absorbance 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 $\mu g.ml^{-1}$ for famotidine and from 20-100 $\mu g.ml^{-1}$ for ranitidine. Furthermore the resulting condensation products exhibited fluorescence at 665 nm when excited at 290 nm and the calibration graphs were rectilinear from 0.4-1.4 $\mu g.ml^{-1}$ for famotidine and from 0.21 $\mu g.ml^{-1}$ for ranitidine.
Different parameters affecting these reactions were thoroughly studied. Also these methods were applied to the pharmaceutical preparations and the results were satisfactory. The validities of the methods were ascertained by the standard addition technique revealing fine results in consideration to the mean recovery percent and standard deviation. The spectrofluorimetric method was a hundred times more sensitive than the spectrophotometric method. The proposed methods were sensitive, accurate, and precise as statistically compared with the official methods of analysis of famotidine and ranitidine.

The H_2 receptor agonist pharmaceuticals containing ranitidine hydrochloride and famotidine are widely used to inhibit gastric acid secretion. A high-performance thin-layer chromatographic method was

developed for their in-process control and content uniformity testing[16]. HPTLC separation was performed on silica precoated plates using the USP 23 mobile phase for famotidine and toluene–methanol–diethylamine (9:1:1, v/v) for ranitidine. 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. The method is fast, accurate and cost-effective.

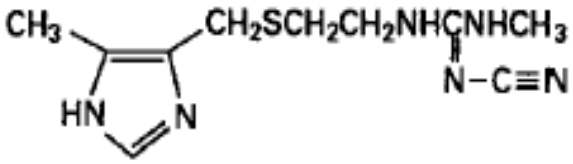
New, simple and convenient potentiometric and spectrophotometric methods were described for the determination of ranitidine[17]. The potentiometric technique was based on direct measurements of the drug cation with novel PVC matrix membrane sensors incorporating ranitidine-reineckate, tungstophosphate and tungstosilicate ion association complexes as electroactive compounds with 2-nitrophenyl phenyl ether as plasticizing solvent mediator. These sensors exhibited rapid near-Nernstian stable responses for 10^{-2} – 10^{-6} M ranitidine over the pH range 4–8, and were used in a flow-through sandwich cell for flow injection determination of the drug. The spectrophotometric method involved the formation of a yellow di(N-nitroso)ranitidine chromophore by reaction of ranitidine with excess nitrite in acetate buffer of pH 4.8 and in the presence of Cu^{2+}/Br^{-} or micelles as catalyst. Beer's law was obeyed at 450 nm over the range 0.3–12 mg ml^{-1}. Determination of ranitidine in a variety of pharmaceutical dosage forms using the proposed potentiometric and spectrophotometric methods showed an average recovery of 98.4% of the nominal values and a mean standard deviation of 0.5%. No

interferences were caused by various drug excipients and diluents. The results compared favourably with those obtained by the liquid Chromatographic method of the US Pharmacopoeia.

Ranitidine hydrochloride in tablets (T) and injections (I) was determined by ultraviolet spectrophotometry (UVS) at 313 nm and visible spectrophotometry (VS) at 615 nm, after reaction with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and ferric chloride[18]. For UVS, Beer's law was obeyed in the range 5.0 - 18.0 µg/mL. The coefficients of variation (CV) for the samples (T) were 0.36% and 0.71% and for the samples (I) were 0.51% and 0.24%. The recovery average (RA) was 99.88% for (UVS), Beer's law was obeyed in the range 1.44 - 5.76 µg/mL. The CV for (T) were 0.72% and 0.59%, and for (I) were 0.53% and 0.61%. The RA was 99.39%. The precision and accuracy of the two methods were compared.
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. There is no significant difference between the reported and proposed methods with regard to accuracy and precision.

2- Literature survey for the determination of cimetidine

Cimetidine is a histamine H_2-receptor antagonist. Chemically it is 1-cyano-2-methyl-3-[2-[(5-methyl-1H-imidazol-4-yl) methylsulfanyl] thio] ethyl] guanidine. The empirical formula for cimetidine is $C_{10}H_{16}N_6S$ and for cimetidine hydrochloride, $C_{10}H_{16}N_6SHCl$; these represent molecular weights of 252.34 and 288.80, respectively. The Chemical Structure of Cimetidine is



Cimetidine

Cimetidine contains an imidazole ring, and is chemically related to histamine. It has a bitter taste and characteristic odor. It is soluble in alcohol, slightly soluble in water, very slightly soluble in chloroform and insoluble in ether. Cimetidine hydrochloride is freely soluble in water, soluble in alcohol, very slightly soluble in chloroform and practically insoluble in ether.

USES: Cimetidine is approved by the FDA for the reduction of the secretion of gastric acid. Prescription cimetidine is used to treat and prevent ulcers, to treat gastro esophageal reflux disorder (GERD), and hypersecretory conditions including Zollinger-Ellison syndrome and multiple endocrine adenomas. In dermatology it is most commonly used to treat warts, urticaria, and mastocytosis. This medication may also be used to treat skin rashes caused by allergic conditions. Nonprescription cimetidine is used to relieve heartburn and acid indigestion.

Jantratid *et, al*[19] demonstrated the analysis of cimetidine in human plasma with HPLC using a simplified sample preparation by protein precipitation with perchloric acid. Plasma cimetidine concentration was determined by plotting peak height ratio of cimetidine to ranitidine (internal standard, IS) against cimetidine concentrations in plasma. The cimetidine and ranitidine peaks were completely separated and no interference from plasma was observed. The lower limit of quantification (LLOQ) of the method was established at 0.1 µg/mL with a precision of 4.3% and a relative error of 1.9%. The average analytical recovery was >90% over the range of cimetidine concentrations (0.1-15.0 µg/mL). The linearity of calibration curve was excellent (r^2 > 0.999). The within- and between-day precision and accuracy, expressed as the coefficients

of variation and relative error, were found to be less than 5%. Compared with previously reported methods, the analytical technique for cimetidine determination in human plasma presented here demonstrates comparable accuracy and precision, an acceptable analysis time, shorter and simpler sample preparation, and a reduced need for complicated equipment. The method presented here is simple and rapid, and the precision and sensitivity are appropriate for the determination of cimetidine in plasma in pharmacokinetic studies.

A high-performance liquid chromatographic method was developed for determination of cimetidine and its main related compounds, 4-hydroxy-methyl-5-methylimidazol (MH), N-cyano-N',N''-dimethylguanidine (Carbonate), 1 -methyl-3-[2-[[(5-methyl-1H-imidazol-4-yl)methyl]-sulfonyl]ethyl] guanidine (Guanidine), 2-cyano-1-methyl-3-[2-[[(5-methyl-1H-imidazol-4-yl)methyl] sulfonyl] (Sulfoxide), and 1-[(methylamino)[[2-([(5-methyl-1H-imidazol-4-yl)methyl]sulfonyl]ethyl]amino]methylene]urea (Amide)[20]. Chromatographic separation was achieved on a porous graphitic carbon (PGC) column with a gradient 17:83 to 19:81 (v/v) acetonitrile-0.05 M potassium phosphate buffer containing 0.40% pentane sulfonic acid at pH 2.5. Analysis was performed at a flow-rate of 1 mL min^{-1} and the detection wavelength was 228 nm. Calibration plots were linear in the concentration ranges 0.25 to 83 µg mL^{-1} for cimetidine and Carbonate, 0.25 to 75 µg mL^{-1} for Guanidine, Amide, and Sulfoxide, and 0.25 to 100 µg mL^{-1} for MH, with correlation coefficients (R2) between 0.9990 and 0.9998. The lowest detectable concentration of cimetidine and Amide was 0.07 µg mL^{-1}; for MH, Carbonate, Guanidine, and Sulfoxide it was 0.06 µg mL^{-1}. Method repeatability (intraday) and reproducibility (interday) was always less than 2% (n=5). The proposed liquid chromatographic method was successfully used for analysis of commercially available cimetidine dosage forms; recoveries were from 99.2 to 100.8%.

Two sensitive and fast spectrophotometric methods using batch and flow-injection procedures for the determination of cimetidine (CMT) were proposed[21]. The methods were based on the formation of a green complex between this drug and Cu (II) in acetic / acetate medium of pH 5.9. The calibration graphs resulting from measuring the absorbance at 330 nm are linear over the ranges 2.5 x 10^{-6} -1.0 x 10^{-3} and 5 x 10^{-6} - 2.0 x 10^{-3} M with detection limits of 9.5 x 10^{-7} and 2.1 x 10^{-6} for batch and flow-injection methods, respectively. The methods were applied to the routine analysis of CMT in pharmaceuticals and human urine.

Three simple, accurate, and sensitive colorimetric methods for the determination of cimetidine (Cim) in pure form, in dosage forms, and in the presence of its oxidative degradates were developed[22]. These methods were indirect, involving the addition of excess oxidant [N-bromosuccinimide (NBS) for method A; cerric sulfate [$Ce(SO_4)_2$] for methods B and C] of known concentration in acid medium to Cim, and the determination of the unreacted oxidant by measurement of the decrease in absorbance of amaranth dye for method A, chromotrope 2R for method B, and rhodamine 6G, for method C at a suitable maximum wavelength (λ_{max}: 520, 528, and 525 nm, for the three methods, respectively). Regression analysis of the Beer plots showed good correlation in the concentration ranges of .2 - 4.4 µg/mL for method A, and .2-3.4 µg/mL for methods B and C. The apparent molar absorptivity, Sandell sensitivity, and detection and quantitation limits were evaluated. The stoichiometric ratio between the drug (Cim) and the oxidant (NBS or Ce^{4+}) was estimated. The validity of the proposed methods was tested by analyzing pure and dosage forms containing Cim with relative standard deviation = 1.18. The proposed methods could successfully determine the studied drug with varying excess of its oxidative degradation products, with recovery between 99.2 and 101.8, 100.2 and 102.8, and 99.8 and 102. % for methods A-C, respectively.

A novel cimetidine ion-selective electrode was prepared, characterized and used in pharmaceutical analysis[23]. The electrode incorporated PVC-membrane with cimetidine–phosphotungstate ion pair complex. The electrode exhibited a Nernstian response for cimetidine in the concentration range 1.0×10^{-5}–1.0×10^{-2} M with a slope of 58±1 mV per decade. The limit of detection was 5.0×10^{-6} M. The electrode displays a good selectivity for cimetidine with respect to a number of common foreign inorganic and organic species. It could be used in the pH range 3.0–5.5. The membrane sensor was successfully applied to the determination of cimetidine in its tablets as well as its recovery from a urine sample.

An on-line flow injection-solid-phase extraction-capillary zone electrophoresis (FI-SPE-CZE) method was developed for determination of cimetidine in human plasma[24]. Sodium dodecylsulfate (SDS) was used as dynamic chemical modifier for elimination of capillary contamination by biological macromolecules. FI on-line preconcentration and cleaning of the analyte by means of a C_{18} microcolumn was performed automatically and CZE separation was performed consecutively without interruption of the applied voltage and between-run-washing of the capillary. A detection limit of 8 μgL^{-1} ($3\times\sigma$) was achieved at a sample throughput of $12h^{-1}$. The approach was successfully used for a pharmacokinetic study of cimetidine.

The separation of cimetidine from the metabolites cimetidine amide and cimetidine sulfoxide, endogenous creatinine and the internal standard ranitidine was achieved by capillary electrophoresis in less than 5 min.[25]. All compounds were separated from cimetidine, including possible plasma ingredients, as the UV spectra of cimetidine standard and cimetidine from the plasma extract match. Plasma levels of cimetidine were determined in the range 250–3000 ng/ml in plasma

and higher concentrations were determined by dilution of the sample with blank plasma.

A titrimetric method, with N,N-dibromodimethylhydantoin as reagent, was developed for the determination of cimetidine in pure form and in dosage forms[26]. It was a simple, direct titration method using amaranth as indicator. The method was simpler than, and superior to, the existing methods for this purpose.

Cimetidine was determined in the presence of its acid-induced degradation products using a second derivative (D_2-) spectrophotometric method (method I) or a calorimetric method (method II)[27]. The former was based on D_2-value measurement at 216 nm, whilst the latter depended on charge-transfer complexation with dichlorophenol—indophenol. The two methods were proved to be stability indicating, since plots of log *C*% versus time were linear. The application to cimetidine determination in tablets and ampoules gave good results.

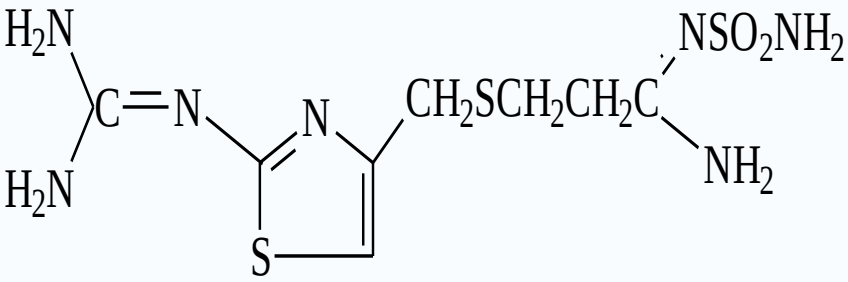
A simple and reproducible method for the determination of cimetidine in serum was described[28]. Separation and quantitation were performed by high-performance liquid chromatography using a Radial-Pak CN column with a mobile phase of 33 % acetonitrile solution containing 5 mM triethylamine (adjusted to pH 3.0 with phosphoric acid) at a flow rate of 2 ml/min and at a detection of 220 nm. Cimetidine and ranitidine as an internal standard were extracted from serum with ethyl acetate and then back-extracted into dilute acid. An aliquot of the dilute acid was analyzed in the chromatographic system. The limit of detection was as low as 0.02 μg/ml using 100 μl of serum at a signal-to-noise ration of 2. Recoveries of cimetidine and ranitidine from serum were both greater than 95%. Within-run and day-to-day reproducibility for

10.0 µg/ml samples were 2.6 % and 2.9 %, respectively. The method was applicable to pharmacokinetic studies.

3- Literature survey for the determination of famotidine

Famotidine is a histamine H_2-receptor antagonist that inhibits stomach acid production, and is commonly used in the treatment of peptic ulcer disease and gastro esophageal reflux disease. It is commonly marketed by Merck under the trade names **Pepcidine** and **Pepcid**.

The IUPAC name of famotidine is N'- (aminosulfonyl)-3-[[[2-[(diamino-methylene) amino]-4-thiazolyl]methyl]thio]propanimidamide. The empirical formula is $C_8H_{15}N_7O_2S_3$ and its molecular weight is 337.43. The structural formula is:



Certain preparations of famotidine are available in various countries. In the United States, preparations of 10 mg and 20 mg tablets, sometimes in combination with a more traditional antacid, are available. Larger doses still require a prescription.

Famotidine is given to surgery patients before operations to prevent post-operation nausea and to reduce the risk of aspiration pneumonitis.

Famotidine was developed by Merck & Co. The imidazole-ring of cimetidine was replaced with a 2-guanidinothiazole ring. Famotidine proved to be 30 times more active than cimetidine.

Famotidine is a white to pale yellow crystalline compound that is freely soluble in glacial acetic acid, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol.

Each tablet for oral administration contains either 20 mg or 40 mg of famotidine and the following inactive ingredients: hydroxypropyl cellulose, hydroxypropyl methylcellulose, iron oxides, magnesium stearate, microcrystalline cellulose, corn starch, talc, and titanium dioxide.

Each Orally Disintegrating Tablet for oral administration contains either 20 mg or 40 mg of famotidine and the following inactive ingredients: aspartame, mint flavor, gelatin, mannitol, red ferric oxide, and xanthan gum.

Each 5 mL of the oral suspension when prepared as directed contains 40 mg of famotidine and the following inactive ingredients: citric acid, flavors, microcrystalline cellulose and carboxymethylcellulose sodium, sucrose and xanthan gum. Added as preservatives are sodium benzoate 0.1%, sodium methylparaben 0.1%, and sodium propylparaben 0.02%.

X-ray powder diffractometric and Raman spectrometric methods were developed for quantitative measurement of the polymorphic forms of famotidine in their mixtures[29]. This study aimed to deduce some useful conclusions regarding quantitative polymorph analysis, which could also be utilized in industrial practice. Both form A and form B of famotidine possess specific X-ray diffraction reflections as well as characteristic Raman vibrational bands, which permits simple

determination of the phases in their mixtures. Keeping in mind that multivariate data processing by chemometric approach is thought of nowadays as superior over univariate one, the results of the two evaluation methods were compared by precision, accuracy as well as robustness. It was found that both approaches provide similar results provided analytically useful data regions are properly selected. Overcoming the common problems of quantitative X-ray powder diffractometry and solid state Raman spectrometry both permit accurate quantification of famotidine polymorphs; the latter, however, seems to be more favourable in regular laboratory practice.

A simple, economic, selective, and stability indicating spectrofluorimetric method was developed for the determination of famotidine (FMT)[30]. The method was based on its reaction with 9, 10-phenan-thraquinone in alkaline medium to give a highly fluorescent derivative measured at 560 nm after excitation at 283 nm. The fluorescence intensity - concentration plot was rectilinear over the concentration range of 50–600 ng/ml with minimum quantification limit (LOQ) of 13.0 ng/ml and minimum detection limit (LOD) of 4.3 ng/ml. The factors affecting the development of the fluorescence intensity of the reaction product were carefully studied and optimized. The method was applied for the determination of FMT in its dosage forms. The stability of the compound was studied, and the proposed method was found to be stability indicating one. The results obtained were in good agreement with those obtained by the official method. Furthermore, the method was applied for the determination of FMT in spiked and real human plasma. The mean % recovery (n=4) was found to be 99.94 ± 0.24, and 105.13 ± 0.64 for spiked and real human plasma, respectively. The composition of the reaction product as well as its stability constant was also investigated. Moreover, the method was utilized to investigate the kinetics of both alkaline and oxidative induced

degradation of the drug. The apparent first order rate constant and half life time of the degradation product was calculated. A proposal of the reaction pathway was postulated.

A simple spectrophotometric method for determination of famotidine was described[31]. The method was based on bromination of the drug with excess brominating mixture in acidic medium. The yellow colour developed was measured at 350 nm against distilled water blank. Beer's law was obeyed in the range of 40-200 µg/ml.

A dissolution test for famotidine tablets was optimized and validated using flow injection analysis (FIA)[32]. The effect of dissolution parameters such as pH, medium and stirring speed was studied, while the ruggedness of the procedure was validated. All measurements were performed using a simple direct spectrophotometric flow injection assay (λ_{max} = 265 nm) that had also been optimized and fully validated in terms of linearity, limit of detection, precision, selectivity and accuracy. Linearity was obeyed in the range 50–150% of famotidine (20–60 mg L^{-1}), while the detection limit (0.1 mg L^{-1}) and repeatability (s_r < 1.0%, n = 12) were satisfactory. The sampling rate was 30 h^{-1}. The dissolution results during quality and stability control of two batches of famotidine tablets obtained by the flow injection method were in good agreement with high-performance liquid chromatography (HPLC).

Walash *et.al*[33]. developed a sensitive and rapid polarographic method for the determination of famotidine in pure form and in certain dosage forms. 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 (DC), differential pulse (DP), and alternating current (AC) polarography. The different experimental

parameters affecting the cathodic waves were carefully investigated and optimized. Moreover, 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. There is no significant difference between the comparison and proposed method with regard to accuracy and precision.

Electrochemical studies of famotidine were carried out using voltammetric techniques: cyclic voltammetry, linear sweep and square wave adsorptive stripping voltammetry[34]. The dependence of the current on pH, buffer concentration, nature of the buffer, and scan rate was investigated. The best results for the determination of famotidine were obtained in MOPS buffer solution at pH 6.7. This electroanalytical procedure enabled to determine famotidine in the concentration range 1×10^{-9}–4×10^{-8} mol L^{-1} by linear sweep adsorptive stripping voltammetry (LS AdSV) and 5×10^{-10}–6×10^{-8} mol L^{-1} by square wave adsorptive stripping voltammetry (SW AdSV). Repeatability, precision and accuracy of the developed methods were checked. The detection and quantification limits were found to be 1.8×10^{-10} and 6.2×10^{-10} mol L^{-1} for LS AdSV and 4.9×10^{-11} and 1.6×10^{-10} mol L^{-1} for SW AdSV, respectively. The method was applied for the determination of famotidine in urine.

A simple, sensitive and specific method was developed for the determination of famotidine (FMT) in pharmaceutical preparations and biological fluids[35]. The proposed method was based on ternary

complex formation of famotidine (FMT) with EDTA and terbium chloride $TbCl_3$ in acetate buffer of pH4. Alternatively, the complex is formed via the reaction with hexamine and either lanthanum chloride $LaCl_3$, or cerous chloride $CeCl_3$ in borate buffer of pH6.2 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 rectilinear 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 $TbCl_3$, $LaCl_3$, and $CeCl_3$ 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. A proposal of the reaction pathways was presented.

A rapid and sensitive HPLC method using a monolithic column was developed for quantification of famotidine in plasma[36]. The assay enables the measurement of famotidine for therapeutic drug monitoring with a minimum detectable limit of 5 ng ml^{-1}. The method involved 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^{-1}. The coefficients of variation for inter-day and intra-day assay were found to be less than 8%.

By using different spectrophotometric methods, it was found that famotidine and palladium(II) ions form a complex, Pd(II) : famotidine

1:1, which had an absorption maximum at 345 nm. 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[37]. 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. Beer's law was verified over the famotidine concentration range from $5x10^{-5}$ – $6x10^{-4}$ M. The proposed method was found to be suitable for accurate and sensitive analysis of famotidine both as the substance (*RSD* = 1.02–1.80 %) and its dosage forms (*RSD* = 1.75–1.83 %).

A simple, rapid and sensitive spectrophotometric method had been described for the determination of aciclovir, ceftazidime pentahydrate, famotidine and isoxsuprine hydrochloride[38]. The method was based on ternary complex formation with eosin and Cu(II), and did not involve solvent extraction. The colour of the produced complex was measured at 545-548 nm. Appropriate conditions were established for the colour reaction and for the drug:Cu(II): eosin ratio to obtain maximum sensitivity. Under the proposed conditions the method was applicable over concentration range of 1-35 µg mL^{-1} with molar absorpitivities ranging from 7.283×10^{3} - 1.182×10^{5} $L.mol^{-1}cm^{-1}$ and Sandell sensitivities ranging from 2.847×10^{-3} - 9.9016×10^{-3} μgcm^{-2}. The results obtained demonstrated that the proposed method was equally accurate, precise and reproducible as the official or reported methods, and thus it was recommended for quality control and routine analysis where time, cost effectiveness and high specificity of analytical techniques are of great importance.

A simple kinetic spectrophotometric method was described for the determination of famotidine[39]. The method was based on the oxidation of the drug with alkaline potassium permanganate. The

reaction was followed spectrometrically by measuring the rate of change of the absorbance at 610 nm. The initial-rate and fixed-time (at 12 min) methods were adopted for determining the drug concentration. The calibration graphs were linear in the ranges of 2 - 10 μg mL^{-1} and 1 - 8 μg mL^{-1} using the initial-rate and fixed-time methods, respectively. The method was applied to the determination of famotidine in tablet formulations. The obtained results were compared statistically with those given by a reference spectrophotometric method.

A simple and sensitive high-performance liquid chromatographic method was developed for the quantification of famotidine in human plasma[40]. Famotidine and the internal standard 3-[({2-[(aminoiminomethyl)amino]-4-thiazolyl}-methyl)thio]-propanamide were extracted from plasma samples by cation-exchange solid-phase extraction and separated by HPLC. The limit of quantification of famotidine in plasma was 10 mg L^{-1}. The method was utilized to support clinical pharmacokinetic studies.

A simple and fast spectrophotometric procedure was developed for the determination of famotidine[41]. The method was based on the interaction of ninhydrin with primary amines present in the famotidine. This reaction produced a blue colored product which absorbed maximally at 590 nm. The effects of variables such as reagent concentration and reaction time were investigated to optimize the procedure. Beer's law was obeyed in the concentration range of 5-30 μg/mL with molar absorptivity of 6.99×10^3 L mol^{-1} cm^{-1}. The results were validated statistically. The proposed method had been applied to the determination of famotidine in tablets with satisfactory results.

Two new potentiometric methods for determination of famotidine in pure form and in its pharmaceutical tablet form were developed[42].

In the first method, the construction of plasticised poly(vinyl chloride) (PVC) matrix-type famotidine ion-selective membrane electrode and its use in the potentiometric determination of famotidine in pharmaceutical preparations were described. It was 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 was 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 (KBr). 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 was applied successfully to commercial tablet. The results obtained reveal good percentage recoveries, which are in good agreement with those obtained by the official methods.

A rapid, sensitive and robust assay procedure using liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) for the determination of famotidine inhuman plasma and urine was described[43]. Famotidine and the internal standard; 3-[{2-[(aminoiminomethyl)amino]-4-thizolyl}-methyl)thio]propanamide 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 Hypersil 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 introductions. 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^{-1} and 0.05-50 µg ml^{-1} 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^{-1} in plasma and 0.05 µg ml^{-1} in urine. The methods were utilized to support clinical pharmacokinetic studies in infants aged 0-12 months.

A simple and rapid chromatographic procedure using a specific analytical detection method (ESI tandem mass spectrophotometric detection) in combination with a fast and efficient sample work-up procedure, protein precipitation, was presented[44]. A demonstration of the entire chromatographic procedure was given for an HPLC method for the determination of famotidine in human plasma, a basic polar drug with poor solubility in organic solvents. 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 (at 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.

Three rapid and simple spectrophotometric methods were described for the determination of famotidine[45]. The methods were based on charge transfer complexation of the drug with reagents viz. chloranil, dichloro dicyano benzoquinone and dichloronitrophenol in methanol to give intensely colored products. The absorbance was measured at the wavelengths of maximum absorption viz. 458, 460 and 425 nm respectively. The effect of several variables on color 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.

A spectrophotometric stability—indicating method was presented for the determination of famotidine in the presence of its degradation products[46]. The 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

A high-performance liquid chromatographic (HPLC) method was developed for the determination of famotidine and related compounds in drug raw materials and formulations[47]. The minimum detectable amount of the available related compounds was less than 0.02% and the minimum quantifiable amount was less than 0.1%. Famotidine impurity levels were between 0.5 and 2.5% in raw materials and 0.44% in one tablet sample.

4- Literature survey for the determination of H_2-receptor antagonists: ranitidine, cimetidine and famotidine

A simple, accurate and sensitive spectrophotometric method for determination of H_2-receptor antagonists: cimetidine (CIM), famotidine (FAM), nizatidine (NIZ), and ranitidine hydrochloride (RAN) had been full developed and validated[48]. The method was based on the reaction of these drugs with *N*-bromosuccinimide (NBS) and subsequent measurement of the excess (NBS) 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. Different variables affecting the reaction were carefully studied and optimized. Under optimal conditions, linear relationships with good correlation coefficients (0.9988-0.9998) were found between ΔA values and the corresponding concentrations of the drugs in a concentration range of 8–30, 6–22, 6–25, and 4–20 μgmL^{-1} for CIM, FAM, NIZ, and RAN, respectively. Limits of detection were 1.22, 1.01, 1.08, and 0.74 μgmL^{-1} for CIM, FAM, NIZ, and RAN, 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 above mentioned drugs in bulk substance and in pharmaceutical dosage forms; percent recoveries ranged from 98.5 ± 0.9 to 102.4 ± 0.8% without interference from the common excipients. The results obtained by the proposed method were comparable with those obtained by the official methods.

A simple, accurate and sensitive spectrophotometric method had been developed and validated for determination of H_2-receptor antagonists: cimetidine, famotidine, nizatidine and ranitidine

hydrochloride[49]. 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-dimethylaminobenzaldehyde to give a red colored product (λ_{max} at 464 nm). The decrease in the absorption intensity of the colored product (Δ A), 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 (0.9990-0.9994) were found between Δ A values and the concentrations of the drugs in a concentration range of 1-20 μgmL^{-1}. The assay limits of detection and quantitation were 0.18-0.60 and 0.54-1.53 μgmL^{-1}, 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 determination of the investigated drugs in pure and pharmaceutical dosage forms (recovery was 98.3-102.6+/-0.57-1.90%) without interference from the common excipients. The results obtained by the proposed method were comparable with those obtained by the official methods.

A validated, simple and universal HPLC-UV method for the determination of cimetidine, famotidine, nizatidine and ranitidine in human urine was presented[50]. This was the first single HPLC method reported for the analysis of all four H_2- antagonists in human biological samples. This method was also utilized for the analysis of ranitidine and its metabolites in human urine. All calibration curves showed good linear regression (r(2)>0.9960) within test ranges. The method showed good precision and accuracy with overall intra- and inter-day variations of 0.2-13.6% and 0.2-12.1%, respectively. Separation of ranitidine and its metabolites using this assay provided significantly improved resolution, precision and accuracy compared to previously reported

methods. The assay was successfully applied to a human volunteer study using ranitidine as the model compound.

Three different spectrophotometric methods were established for the determination of cimetidine (I), ranitidine hydrochloride (II) and famotidine (III)[51]. The first one was a colorimetric method, it was applied for the determination of the three drugs by using sodium nitroprusside as a color reagent to produce a red colored complexes. In this method, the zero order spectrum °D was used for the determination of drug (III) at λ = 500 nm while the first derivative spectra ^{1}D were used for the determination of drug (I) and (II) at their corresponding wave lengths 523 and 510 nm and Δλ = 4 nm. The method can be considered as a stability indicating method for the determination of the three drugs in the presence of their induced hydrogen peroxide oxidative degradates. Beer's law was obeyed in the concentration range of 25-150 μgmL^{-1} for (I) and 50-500 μgmL^{-1} for (II) and (III) with mean percentage recoveries of 100.27, 99.79 and 99.15, respectively. The second method was a simple colorimetric method which was applied for the determination of drug (III). Where, 3-Methyl-2-benzo-thiazolinone hydrazone (MBTH) was used as a color reagent. It reacts with the drug to produce a bluish violet color, having two maxima at 536 and 620 nm. The percentage recoveries were 99.73 and 99.94, respectively within the concentration range of 20-120 μgmL^{-1}. The third one was a spectrophotometric method via a complex formation reaction by using cobalt II. A colorless complex was developed having λ_{max} at 319 nm with a ratio of 1:1 and a stability constant logarithm of 5.49. The percentage recovery was 99.84 within a concentration range of 10-60 μgmL^{-1}. The statistical comparison with the BP official methods and the assay validation for the three proposed methods had been applied. The results obtained showed that they could be used for the determination of the three drugs in pure and dosage forms.

A selective, precise, and accurate method was developed for the determination of cimetidine (C), famotidine (F), and ranitidine hydrochloride (R.HCl) in the presence of their sulfoxide derivatives[52]. The method involved quantitative densitometric evaluation of mixtures of the drugs and their derivatives after separation by high-performance thin-layer chromatography on silica gel plates (10 x 20 cm) with ethyl acetate-isopropanol-20% ammonia (9 + 5 + 4, v/v) as the mobile phase for both C and F and ethyl acetate-methanol-20% ammonia (10 + 2 + 2, v/v) as the mobile phase for R.HCl; R_f values for C, F, and R.HCl and their corresponding derivatives were 0.85 and 0.59, 0.73 and 0.41, and 0.56 and 0.33, respectively. Developing time was approximately 20 min. For densitometric evaluation, peak areas were recorded at 218, 265, and 313 nm for C, F, and R.HCl, respectively. The relationship between concentration and the corresponding peak area was plotted for the ranges of 5-50 microg/spot for C and 2-20 microg/spot for F and R.HCl. Mean recoveries were 100.39 +/- 1.33, 99.77 +/- 1.30, and 100.09 +/- 0.69% for C, F, and R x HCl, respectively. The proposed method was used successfully for stability testing of the pure drugs in the presence of up to 90% of their degradates, in bulk powder and dosage forms. The results obtained were analyzed statistically and compared with those obtained by the official methods.

A simple charge-transfer complexation method was described for the spectrophotometric assay of nizatidine, ranitidine, and famotidine[53]. The method was based on interaction of these drugs, as n-electron donors, with 7,7,8,8-tetracyanoquinodimethane, as the pi-acceptor, in acetonitrile to give highly colored green radical anions that are measured at 840 nm. Calibration graphs for the 3 compounds were linear over the concentration ranges of 1-6 µg/mL for nizatidine and

ranitidine and 1-7 µg/mL for famotidine, with correlation coefficients (n = 6) of about 0.999. The conditioned stability constants and the free energy changes were measured; the values obtained were generally high and negative, respectively, suggesting highly stable complexes. The proposed method was successfully applied to the determination of the drugs in pharmaceutical preparations. The assay results were in accordance with those obtained by using reference methods.

A simple and sensitive capillary electrophoresis method using UV detection was developed for the direct determination of ranitidine (RANT) and famotidine (FAMT) in serum, urine and pharmaceutical formulations[54]. 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 0.088 µg ml^{-1} for RANT and 0.16 µg ml^{-1} for FAMT.

A simple capillary zone electrophoresis (CZE) method was described for the simultaneous determination of cimetidine (CIM), famotidine (FAM), nizatidine (NIZ), and ranitidine (RAN)[55]. The analysis of these drugs was performed in a 100 mM phosphate buffer, pH 3.5. Several parameters were studied, including wavelength for detection, concentration and pH of phosphate buffer, and separation voltage. The quantitative ranges were 100-1000 µM for each analyte. The intra- and interday relative standard deviations (n = 5) were all less than 4%. The detection limits were found to be about 10 µM for CIM, 20 µM for RAN, 20 µM for NIZ, and 10 µM for FAM (S/N = 3, injection 1 s) at 214 nm. All recoveries were greater than 92%. Applications of the method to the assay of these drugs in tablets proved to be feasible.

Simple, sensitive and rapid spectrophotometric techniques had been established for the determination of cimetidine (I), famotidine (II) and

ranitidine hydrochloride (III) in presence of their S-oxide derivatives in both raw materials and in pharmaceutical formulations[56]. Hydrogen peroxide was used to enhance the formation of S-oxide compounds (oxidative derivatives). The first derivative of the ratio spectra (^{1}**DD**) technique was applied for the determination of (I) and (II). While, direct zero order (^{0}**D**), first derivative (^{1}**D**) and the second derivative of the ratio spectra (^{2}**DD**) was carried out for the determination of drug (III). Linear calibration curves were obtained for determination of (I) and (II) by applying ^{1}**DD** at 220 and 230 nm in the concentration range of 2-20 and 4-40 µg mL^{-1} with the mean percentage recoveries of 99.88 ± 0.943 (*n* = 10) and 99.88 ± 0.824% (*n* = 10) for (I) and (II), respectively. While for (III) linear calibration curves were obtained by applying ^{0}**D**, ^{1}**D** and ^{2}**DD** at 313, 334 and 255 nm, respectively. The concentration range was 2-20 µg mL^{-1} with the corresponding mean percentage recoveries of 100.13 ± 0.464, 100.01 ± 0.428 and 99.94 ± 0.439 for (I), (II) and (III), respectively. The results obtained by the proposed methods were statistically analyzed and compared with those obtained by the official British pharmacopeias methods.