ANALYTICAL TECHNIQUES FOR THE ESTIMATION OF RABEPRAZOLE IN BULK AND PHARMACEUTICAL DOSAGE FORMS: A REVIEW

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ABSTRACT
Rabeprazole an antiulcer drug in the class of proton pump inhibitors. Its chemical formula is C18H21N3O3S and its IUPAC name is 2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl] sulfinyl]-1H-1,3-benzodiazole. Rabeprazole sodium inhibits the gastric secretions and provides the antiulcer activity. Rabeprazole, a substituted Benzimidazole, inhibits gastric acid secretion, used as an ant ulcerative in treatment of duodenal ulcers, gastro esophageal reflux disease (GERD), Zollinger-Ellison syndrome etc. Literature survey reveals that Lansoprazole is estimated individually by uv spectrometry, RP-UPLC, RP-HPLC-Solid phase extraction, LC/MS/MS, Content uniformity, Stability indicating determination of process related impurities by HPLC, Direct injection Column Switching-LC, Effect of pharmaceutical excipients on aqueous stability.

KEYWORDS: Rabeprazole, RP-HPLC, LC/MS/MS, UV Spectrometric, RP-UPLC, DICS-LC.

INTRODUCTION
Rabeprazole is a substituted benzimidazole that inhibits gastric acid secretion and primarily used in the treatment of Ulcerative Gastroesophageal Reflux Disease (GERD). It is chemically 2-[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]sulfinyl]-Hbenzimidazole. Rabeprazole is officially used as PDR. Rabeprazole belongs to a class of antisecretory compounds that suppress gastric acid secretion by inhibiting the gastric H+,
K+ATPase at the secretary surface of the gastric parietal cell. Because this enzyme is regarded as the acid (proton) pump within the parietal cell, rabeprazole has been Rabeprazole blocks the final step of gastric secretion. In gastric parietal cells, rabeprazole is protonated, accumulates and is transformed to an active sulfonamide.

![Fig no 1: Rabiprazole](image)

Mass spectrometry (MS) has been described as the smallest scale in the world, not because of its size of what it weighs a molecule and a micro analytical technique that can be used selectively to detect and determine the amount of a given analyte (Watson & Sparkman, 2007; Chiu & Muddiman, 2008). MS is also used to determine the elemental composition and some aspect of the molecular structure of an analyte. Unique features of MS include its capacity for direct determination of the nominal mass of an analyte, and to produce an detect fragments of the molecule that correspond to discrete groups of atoms of different elements that reveal structure features (Watson & Sparkman, 2007). The tools of MS are mass spectrometers, and data are called mass spectra that can be displayed in many different ways, which allow the desired information about the analyte to be easily extracted (Watson & Sparkman, 2007). A MS is an apparatus which produces a beam of gaseous ions from a sample, sorts out the resulting mixture of ions according to their mass-to-charge ratios, and provides output signals which are measures of relative abundance of each ionic species present. MS are usually classified on the basis of how the mass separation is accomplished, but they all can be described as ion optical devices which separate ions according to their mass-to-charge (m/z) ratios by utilizing electric and/or magnetic force fields. The concept of MS is to form ions from a sample, to separate the ions based on their m/z ratio (this can be considered to be the same as the mass because the ion has only a single charge in most cases), and to measure the abundance of the ions. In modern MS instrumentation used in environmental analyses, all of the functions (ionization separation of the ions, rate of data acquisition, detection of the ions, and storage of the data) are under computer control. Gaseous molecules are ionized in the ion source to form molecular ions which some of that
will fragment. By various processes, ions of differing m/z values pass through the mass analyzer one at a time to reach the detector. When the ions strike the detector, they are converted into an electrical signal which, in turn, is converted into a digital response that can be stored by the computer (Sparkman, 2000). A mass spectrometer does not directly determine mass but, determines the mass of a molecule by measuring the m/z of its ion. The knowledge of the m/z of the ions enables one to determine what is present, while the measured ion intensities answer the question of how much is present. In addition, systematic interpretation of the mass spectra provides a detailed picture of the ionization process which, in turn, may be utilized in the elucidation of molecular structures. This definition of the term m/z is important to understanding of MS. It should be noted that the m/z value is a dimensionless number that is always used as an adjective, e.g. the ions with m/z 256, or the ion has an m/z value of 256. A recording of the number of ions (abundance) of a given m/z value as a function of the m/z value is a mass spectrum (Watson & Sparkman, 2007). The mass component that makes up the dimensionless m/z unit is based on an atomic scale rather than the physical scale normally considered as mass. Only ions are detected in mass spectrometer and any nonionic particles that have no charge are removed from the mass spectrometer by the continuous pumping that maintains the vacuum. The MS first must produce a collection of ions in the gas phase. These ions are separated according to their m/z values in a vacuum where the ions cannot collide with any other forms of matter during the separation process. Ions of individual m/z values are separated and detected in order to obtain the mass spectrum. Separation of ions in an evacuated environment is mandatory. If an ion collides with neutrals in an elastic collision during ion separation process, the ion’s direction of travel could be altered and ion might not reach the detector. If an ion’s collision with neutral is inelastic, sufficient energy transfer may cause it to decompose, meaning that the original ion will not be detected. Close encounters between ions of the same charge can be cause deflection in the path of each. Direct contact between ions of opposite charge sign will result in neutralization.
Ions are positively or negatively charged atoms, groups of atoms, or molecules. The process whereby an electrically neutral atom or molecule becomes electrically charged, due to losing or gaining one or more of its extra nuclear electrons, is called ionization (Chiu & Muddiman, 2008). Although both positive and negative ions can be analyzed by MS, the majority of instruments are used to investigate positive ions because in most ion sources they are produced in larger number than negative ions. (Chiu & Muddiman, 2008) There is a minimum amount of energy, characterized by the “ionization potential,” that must be provided in order for ion formation to occur. The first ionization potential of an atom or molecule is defined as the energy input required removing (to infinite distance) a valence electron from the highest occupied atomic or molecular orbital of the neutral particle to form the corresponding atomic or molecular ion, also in its ground state. When only one electron is removed the ion is called an atomic or molecular ion; often the term “parent ion” is used. The formation of parent ions may be considered as ionization without cleavage. The numerical magnitude of the ionization potential is influenced by such factors as the charge upon the nucleus, the atomic or molecular radius, the shielding effect of the inner electronic shells, and the extent to which the most loosely bound electrons penetrate the cloud of electric charge of the inner shells Because only ions can be detected in MS, any particles that are not ionic (molecules or radicals) are removed from the MS by the continuous pumping that maintains the vacuum. When only individual ions are present, they can be grouped according to their unique properties (mass and number of charges) and moved freely from one point to another. In order to have individual ions free from any other forms of matter, it is necessary to analyze them in a vacuum, which means that the ions must be in the gas phase. It is a fundamental
requirement of MS that ions be in the gas phase before they can be separated according to their individual m/z values and detected (Watson & Sparkman, 2007). Due to ionization sources such as electro spray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), MS has become an irreplaceable tool in the biological sciences. Over the past decade, MS has undergone tremendous technological improvements allowing for its application to proteins, peptides, carbohydrates, DNA, drugs, and many other biologically relevant molecules (Chiu & Muddiman, 2008).

Fig no:3 Ionization of molecule

**Instrumentation of mass spectrometric detection:** Mass spectrometry is a particularly powerful scientific technique because it can be successfully moles for a compound of mass 1000 Daltons (Da). Compounds can be identified through mass spectrometry at very low concentrations (one part in 1012) in chemically complex mixtures. The basic mass spectrometry processes of instrumentation are consisted of (1) introduction of sample; a sample which can be a solid, liquid, or vapor is loaded onto a mass spectrometry device and is vaporized, (2) ionization; sample components are ionized by one of several available methods to create ions, (3) analyzer sorting; the ions are sorted in 445 an analyzer according to their m/z ratios through the use of electromagnetic fields, (4) detector; the ions then pass through a detector where the ion flux is converted into a proportional electrical current and (5) data conversion; the magnitude of the ion/electrical signals is converted into a mass spectrum (Watson & Sparkman, 2007). MS instruments consist of three modules: an ion source, which can convert gas phase sample molecules into ions (or, in the case of ESI, move ions that exist in solution into the gas phase); a mass analyzer, which sorts the ions by their masses by applying electromagnetic fields; and a detector, which measures the value of an
indicator quantity and thus provides data for calculating the abundances of each ion present. The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

**Methodologies**

**Shashikant B. Bagade et al.,** A simple and rapid stability indicating HPLC method was developed for the Rabeprazole sodium API. Here the HPLC method which was developed was capable of separating the degradants from the API which may be developed during the storage of the API or during the synthesis of the API. So here the degradation was carried out under the different hydrolytic conditions like acid, base and neutral and the HPLC method was develop to separate the degradants from API. The method which was developed have the methanol and phosphate buffer as a mobile phase and the detection was carried out at 283 nm and at a flow rate of 1 ml/ min. The retention time of Rabeprazole sodium was 8.98±0.10 minutes. Validation of the method was also performed in which Linearity, Precision, LOD & LOQ, Accuracy and robustness was performed.

**M.Kalyan Obula Reddy et al.,** A precise and feasible high-performance liquid chromatographic (HPLC) method for the analysis of the novel antacid drug Rabeprazole in tablet dosage form has been developed. The analysis was carried out on a Symmetry C18 (4.6 x 150mm, 5 mm, Make: XTerra) or equivalent column, using a mixture of phosphate buffer (pH 5.5), methanol (30:70) as the mobile phase using a low pressure gradient mode with flow rate at 0.9ml/min and analysis was performed at wavelength 284 nm using Photo Diode Array (PDA) detector at ambient temperature. The injection volume was 20μl. The retention time of the drug was 2.657 min. The method produced linear responses in the concentration range of 20 to 60μg/ml of Rabeprazole. The LOD and LOQ values for HPLC method were found to be 2.96 and 10.1μg/ml respectively. The method was found to be applicable for determination of the drug in tablets.

**N.V.S.Ramakrishna et al.,** A simple, sensitive and selective HPLC method with UV detection (284 nm) was developed and validated for quantitation of rabeprazole in human
plasma, the newest addition to the group of proton-pump inhibitors. Following solid-phase extraction using Waters Oasis™ SPE cartridges, the analyte and internal standard (Pantoprazole) were separated using an isocratic mobile phase of 5 mM ammonium acetate buffer (pH adjusted to 7.4 with sodium hydroxide solution)/acetonitrile/methanol (45/20/35, v/v) on reverse phase Waters symmetry® C₁₈ column. The lower limit of quantitation was 20 ng/mL, with a relative standard deviation of less than 8%. A linear range of 20–1000 ng/mL was established. This HPLC method was validated with between- and within-batch precision of 2.4–7.2% and 2.2–7.3%, respectively. The between- and within-batch bias was −1.7 to 2.6% and −2.6 to 2.1%, respectively. Frequently coadministered drugs did not interfere with the described methodology. Stability of rabeprazole in plasma was excellent, with no evidence of degradation during sample processing (autosampler) and 3 months storage in a freezer. This validated method is sensitive, simple and repeatable enough to be used in pharmacokinetic studies.

R.Ramdev et al., The aim of this work is to develop and validate the derivative spectrophotometric method for determination of the proton pump inhibitor rabeprazole sodium in pharmaceutical formulations. The technique was applied using water (pH 10.0) as diluent. The first-order derivative spectra were obtained at N=5, Δλ=4.0 nm, and determinations were made at 304 nm. The method showed high specificity in the presence of formulation excipients and good linearity in the concentration range of 6.0 to 18.0 µg/mL⁻¹. The intra- and interday precision data demonstrated the method has good reproducibility [Relative Standard Deviation ((RSD)=1.0 interdays)]. Accuracy was also evaluated and results were satisfactory (mean recovery of 99.15%). The detection and quantitation limits were 0.055 and 0.17 µg/mL⁻¹, respectively. The method was demonstrated to be adequate for routine analysis in quality control.

Cassia V. Garcia et al., The aim of this work is to develop and validate a dissolution test for rabeprazole sodium coated tablets using a reverse-phase liquid chromatographic method. After test sink conditions, dissolution medium and stability of the drug, the best conditions were: paddle at 75 rotations per minute (rpm) stirring speed, HCl 0.1 M and borate buffer pH 9.0 as dissolution medium for acidic and basic steps, respectively, volume of 900 ml for both. The quantitation method was also adapted and validated. Less than 10% of the label amount was released in the acid step, while more than 95% was achieved over 30 min in the basic one. The dissolution profile for tablets was considered satisfactory. The dissolution test
developed was adequate for its purpose and could be applied for quality control of rabeprazole tablets, since there is no official monograph.

**Jinchang Huangd et al.,** A simple and sensitive liquid chromatography/tandem mass spectrometry method, employing electrospray ionization, has been developed and validated to quantify rabeprazole in human plasma using omeprazole as the internal standard. The method was validated to demonstrate the specificity, lower limit of quantification, accuracy, and precision of measurements. Selected reaction monitoring was specific for rabeprazole and omeprazole (the internal standard, IS); no endogenous materials interfered with the analysis of rabeprazole and IS from blank plasma. The assay was linear over the concentration range 0.2–200 ng/mL using a 2 µL aliquot of plasma. The correlation coefficients for the calibration curves ranged from 0.9988–0.9994. The intra- and inter-day precision, calculated from quality control samples, were less than 6.65%. A mixture of methanol and water (50:50) was used as the isocratic mobile phase, with 0.1% of formic acid in water, that did not affect the stability of rabeprazole or IS. A simple sample preparation method of protein precipitation with methanol was chosen. The method was employed in a pharmacokinetic study after oral administration of 20 mg rabeprazole to 24 healthy volunteers. Copyright © 2005 John Wiley & Sons, Ltd.

**Shan Ren et al.,** The chemical stability of a proton-pump inhibitor, rabeprazole sodium, was evaluated in simulated intestinal fluid (pH 6.8) containing various ‘Generally Recognized As Safe (GRAS)’-listed excipients, including Brij® 58, Poloxamer 188, Cremophor RH40, Gelucire 44/14 and PEG 6000. After incubation at 37 and 60 °C, the amounts of rabeprazole and its degradation product, thioether-rabeprazole, were quantitated by HPLC analysis. The main degradation product was separated and characterized by LC/MS. The degradation of rabeprazole followed first-order kinetics. In the absence of any excipients, the rate constants \( k \) obtained at 37 and 60 °C were 0.75 and 2.78 h\(^{-1}\), respectively. In contrast, the addition of excipients improved its stability. Among several excipients tested in this study, Brij® 58 displayed the greatest stabilizing effect. For instance, at 37 and 60 °C, Brij® 58 reduced the \( k \) values to 0.22 and 0.53 h\(^{-1}\), respectively. The stabilizing mechanisms of these hydrophilic polymeric excipients with optimal HLB values could be partially explained in terms of their solubilizing efficiency and micellar formation for thioether-rabeprazole. In conclusion, rabeprazole formulations that contain suitable excipients would improve its stability in the intestinal tract, thereby maximizing bioavailability.
Sonu Sundd Singh et al., A rapid, simple and sensitive high-performance liquid chromatography–ultra violet (HPLC–UV) method with column switching between sample pre-treatment column and analytical column was developed for the quantitation of rabeprazole in human plasma; on a Bio-Sample Analysis system (Co-sense® for BA) from Shimadzu Corporation, Kyoto, Japan. Zaleplon was used as an internal standard. The method was validated as per USFDA guidelines for the concentration range of 20.0–1200.0 ng/mL and the correlation coefficient were found to be better than 0.999. Recovery of rabeprazole as well as the internal standard from human plasma was more than 90.0%. Rabeprazole was stable in human plasma for 4 months at −70 ± 5 °C and for 20.0 h at ambient temperature. In the auto sampler, the drug was stable for 24.0 h at 4 °C. The method was specific as there were no interfering peaks in the human plasma eluting at the retention times of the rabeprazole and the internal standard. The frozen plasma samples containing rabeprazole were stable to three freeze thaw cycles. The bioanalytical method was rugged in terms of inter- and intra-day accuracy and precision. The method was simple, specific, sensitive, precise, accurate and suitable for bioequivalence and pharmacokinetic studies. It was successfully applied to the pilot bioequivalence study of 20 mg rabeprazole tablet of German Remedies Ltd. (A division of Cadila Healthcare Ltd.), India versus Pariet tablet of Eisai Ltd. & Janssen-Cilag Ltd., Japan in male human subjects.

Yong Zhang et al., An analytical method based on liquid chromatography coupled with tandem mass spectrometry detection has been developed and validated for the determination of rabeprazole in human plasma usingomeprazole as the internal standard. The analyte and internal standard was extracted with n-hexane–dichloromethane–isopropanol (20:10:1, v/v) and chromatographed isocratically on a Diamonsil C18 analytical column. Methanol was used as the mobile phase to avoid decomposition of rabeprazole. The drug was detected in the selected reaction monitoring mode using an atmospheric pressure chemical ionization source. The method was linear within the range 2.0–800 ng/ml. The lower limit of quantification was 2.0 ng/ml. The intra- and inter-day precision, calculated from quality control (QC) samples, was less than 9.8%. The accuracy was within ±1.1%. The method herein described was employed in a pharmacokinetic study after an oral administration of 20 mg rabeprazole to 18 healthy volunteers.

S. Elumalai et al., The aim of the present work was to develop simple, shorter and effective HPLC method with UV detection (285nm) and subsequent validation for the content
uniformity determination of Rabeprazole Sodium in marketed tablet samples. The method uses isocratic mobile phase of 0.1M sodium phosphate buffer (pH adjusted to 6.5 with sodium hydroxide solution) and acetonitrile 65:35 compositions on reverse phase Lichrosphere RP-100 C8 column. The RSD was observed to 0.21 percentage and linearity range of (LOQ) 0.025 – 150 percentage of label claim established with 0.9999 correlation, 8 different brands marketed samples were successfully analysed for content uniformity and compared the results with the USP and other guidelines for acceptance criteria. The developed method was found precise, linear, rugged and robust for validated parameters. The method can be used for assay and the content uniformity determination of Rabeprazole Sodium in its tablet dosage form.

Mallikarjuna Gouda M et al., Rabeprazole sodium (RSM) is a proton pump inhibitor used against peptic ulcer disease to suppress excess acid secretion in the stomach. Physico chemical characterization studies showed that RSM has showed a melting point of 137°C. The solubility of drug RSM followed the order distilled water> pH 9.0> pH 8.0> pH 7.5> pH 2.5. The analytical method developed for the estimation of RSM in bulk fluids showed maximum absorbance λmax of 272.2 nm in distilled water between 200 nm and 400 nm. Linearity studies indicated that estimation of RSM between 2.00 μg/ml to 10.00 μg/ml was found to be linear with regression equation of y = 0.034*X -0.00267; (r² = 0.99961). The accuracy, precision studies showed that the recovery of drug from bulk fluids and dosage form are highly accurate and precise with minimum error. The above analytical parameters indicated that the developed UV Spectrophotometric method for RSM was simple, accurate, precise and reproducible.

K Karunakaran et al., Purpose: To develop a reverse phase ultra-performance liquid chromatographic (RP-UPLC) method for the estimation of rabeprazole sodium in tablet formulations. Methods: Chromatographic separation was achieved on a Waters Acquity BEH C18 (50 x 2.1 mm, particle size 1.7 μm) column using an isocratic method with mobile phase composed of acetonitrile and phosphate buffer (pH 7.4) in the ratio of 35:65 (v/v). The flow rate was 0.4 ml/min, temperature of the column was maintained at ambient, injection volume was 5 μL and detection was made at 280 nm. The run time was as short as 2 min. Comparison of system performance with conventional HPLC was made with respect to analysis time, efficiency and sensitivity. Results: The developed method was linear for rabeprazole sodium from 0.03 - 30 μg/ml and the linear regression obtained was > 0.999. Precision, evaluated by
intra- and inter-day assay,s had relative standard deviation (R.S.D) values within 1.5 %. Recovery data were in the range 98.0 - 101.5 % with R.S.D. values < 1.5 %.

CONCLUSION

The method is precise, accurate, linear, robust and fast. The short retention time of 1.49 min allows the analysis of a large number of samples in a short period of time and, therefore, should be cost-effective for routine analysis in the pharmaceutical industry.

REFERENCE

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