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Development and Validationof Environmental Conscious High Performance Liquid Chromatography Method for Determination for Ranitidine Hydrochloride in Solid Dosage Form.

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ABSTRACT

A simple, specific and accurate reverse phase liquid chromatographic method has been developed for the estimation of Ranitidine HCl from tablet formulation. The drug is official with Indian Pharmacopeia, United States Pharmacopeia British Pharmacopoeia, however these Compendial procedures does not involve aqueous solvent as diluents. The separation was carried out using 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (10 µm) column and the mobile phase consisted of (methanol: ammonium Acetate- 85:15) in isocratic mode. The flow rate was 1.00 ml/min and effluent was monitored at 322 nm. The new method for the assay of Ranitidine hydrochloride tablet was validated as per ICH guidelines for Linearity, accuracy, precision, Robustness, and Specificity. The described is linear (Correlation coefficient is 0.999). The method is found to be accurate between concentrations 80 % to 120 % of target concentration (112 µg/mL) of Ranitidine. The precision, ruggedness and robustness values were also within the prescribed limits (<1% for system precision and <2% for other parameters). The method was found reproducible as per the predefined criteria. The method was successfully used for quantitative determination of Ranitidine HCl from tablet dosage form.

Keywords: Ranitidine HCl; HPLC; Green Chemistry; Method Development; Method Validation; Indian Pharmacopeia.

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Introduction

Ranitidine hydrochloride is official in Indian Pharmacopeia [1], United States Pharmacopeia [2] and British Pharmacopeia [3]. Ranitidine hydrochloride is a histamineH2-receptor antagonist that inhibits stomach acid production. It is commonly used in treatment of peptic ulcer disease (PUD) and gastro oesophageal reflux disease (GERD). Ranitidine hydrochloride is subject to degradation upon aging and that such degradation is accelerated by moisture and light. For dealing with such stability problem of ranitidine, tablets are film coated with suitable polymers. The drug is freely soluble in water as specified in Indian Pharmacopeia [1], United States Pharmacopeia [2] and British Pharmacopeia [3].The objective of this research work was to initiate green initiative in method development which will lead to safer environment [4-5]. As specified Ranitidine HCl Indian Pharmacopeia [1] monograph, mobile phase and diluents is methanol: 0.1 M ammonium acetate (85:15). The diluents required for one sample preparation is 525 mL excluding standard preparation. The retention time of Ranitidine is about 3 to 4 minutes. The requirement of Methanol as diluents is more than as mobile phase. 'Assuming production of 1 batch/formulation/day, there are 9490 batches of Ranitidine HCl are produced per year in India. Hence, in a year at least 5694 L of methanol consumed only for assay of Ranitidine Hydrochloride tablets by Indian Pharmacopeia [1] method. To optimize method for solvent reduction, suitablediluents is to reduce the organic content from the diluents or to replace it by safe solvent compared to existing solvent. In this context the present work reports a development of environmental conscious quantitative method for the determination of Ranitidine HCl from tablet formulations [6-8].

EXPERIMENTAL

Reagents

Methanol, Ammonium Acetate, Acetic Acid, Hydrochloric Acid, Sodium Hydroxide and Hydrogen Peroxide were used of Analytical grade reagent. Purified water was used from Millipore's Milli-Q.

Reference standards

Ranitidine Hydrochloride reference standards and bulk sample was obtained from GlaxoSmithKline Pharmaceuticals Ltd.

Instruments details

Mettler Toledo XP 105 Delta Range analytical balance was used to weigh the required materials, Mettler Toledo Seven Multi pH meter was used to adjust the pH of solutions. Waters Alliance chromatographic system with 2690 separation module, and 2487 dual wavelength detector was used. Agilent 1100 series chromatographic system was used to assess the robustness of the method.

Preparation of standard solution

Reference solution and Resolution solution preparation

56 mg of Ranitidine hydrochloride RS weighed in 100 mL volumetric flask, dissolved in water, sonicated for 5 min, make up volume by water this solution was labelled as solution A.

5mL of solution A was further diluted to 25 mL by water. This solution was labelled as solution B.20 mg of Ranitidine s oxide solution was weighed in 100 mL volumetric flask, dissolved in water, sonicated for 5 min, make up volume by water. This solution was labelled as solution C.2 mL of solution A and 5 mL of solution C was diluted to 100 mL with water.

Solution B was used as Reference solution and solution C was resolution solution. Before injecting, each solution was filtered through 0.45 µ filter by discarding first 5mL.

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Test solution preparation

Weighed and finely powdered 20 tablets using mortar and pestle. Weighed 1.5 g of the powder in 500 mL volumetric flask, added 400 mL of the Water, sonicated this solution for 20 minutes and made up to volume with Water, centrifuged 5 to 10 mL of this stock solution for 10 minutes at 2500 RPM, diluted the supernatant of centrifuged solution with the Water to obtain a solution containing the equivalent of 0.012 percent (w/v) of ranitidine. Before injecting the working solution was filtered through 0.45 µm nylon membrane filter to avoid column deterioration.

Blank preparation

Water was filtered through 0.45 µm nylon membrane filter and injected to HPLC system.

HPLC Instrumentation

Waters HPLC with 2690 separation module and 2487 dual wavelength detector. Ranitidine HCl and Ranitidine S-Oxide impurity peaks were resolved on stainless steel column with 25 cm length, 4.0 mm internal diameter, packed with octadecyl silane bonded to porous silica with 10 µm particle size. The chromatographic conditions are listed under **Table 1**.

Table 1.Chromatographic Conditions.

Sr.No	Parameter	Condition
1	Mobile phase	A mixture of 85 volumes of methanol and 15 volumes of 0.1 M ammonium acetate
2	Column Temperature	Ambient
3	Flow rate	2 ml per minute
4	Detection Wavelength	322 nm
5	Injection volume	20 µL

Validation

The developed method was validated as per ICH guidelines ICH Q2 (R1) [9] and USP-NF, General Chapters for Validation of compendial procedures [10-11].

Specificity

The Blank, Resolution, Placebo and Sample solutions were prepared in Water at the working concentration level. These individual solutions were transferred to HPLC vials; each vial was chromatographed using HPLC chromatographic conditions. Any peak response due to blank, and placebo was recorded. The retention time of Ranitidine HCl and Ranitidine S-Oxide impurity was recorded. For example typical chromatogram of sample solution is shown as **Figure1**.

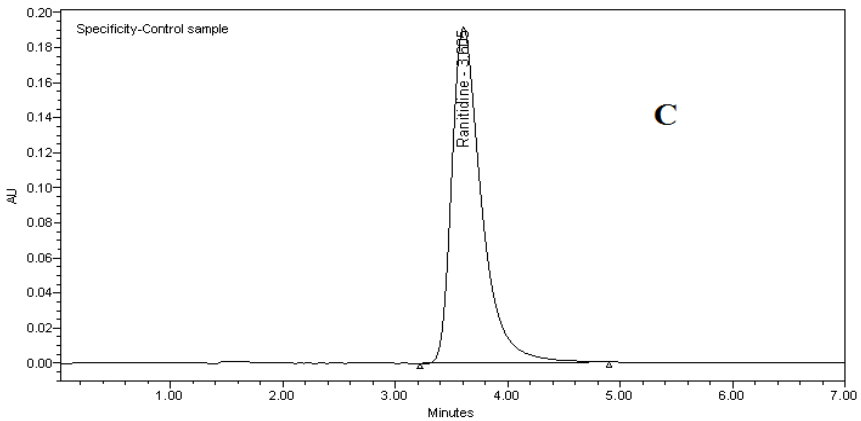

Figure1: Typical chromatogram of sample solution chromatogram.

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Linearity

Linearity solutions were prepared by quantitative dilutions of the stock solution in Water from 89.6 µg/mL to 133.4µg/mL each solution was chromatographed. The graph of peak area Vs Concentration in µg/mL was plotted and equation of regression line was determined. The slope, intercept and correlation coefficient of the regression line was reported. For example linearity plot of concentration and area of Ranitidine HCl is shown as **Figure2**.

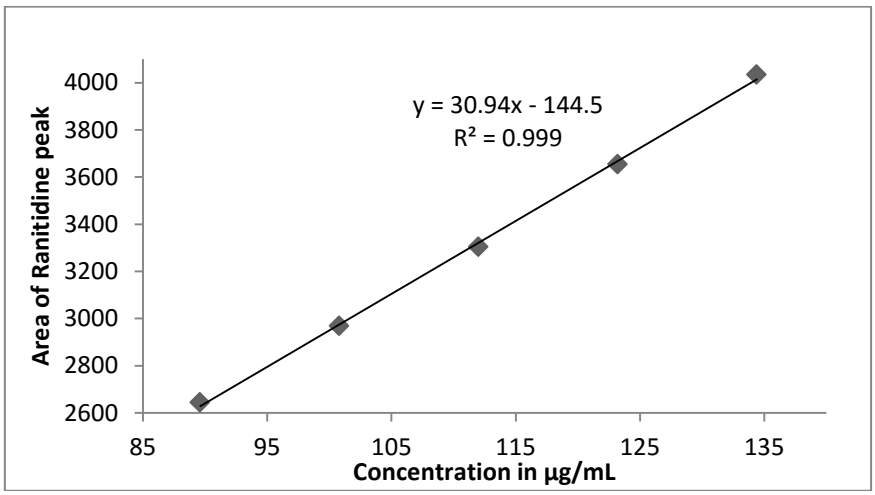

Figure2: Linearity of Ranitidine HCl in Water.

Slope = 34.66, Correlation coefficient=0.999
Acceptance criteria- Correlation coefficient is NLT 0.999

Accuracy

Placebo was weighed equivalent to target weight of the sample except Ranitidine HCl. The placebo was weighed in three different volumetric flasks. Each of these volumetric flasks was spiked with Ranitidine HCl in 3 concentration levels at 80%, 100% and 120% of the target concentration level. These samples were prepared in triplicate. Each level was analysed against the standard solution at working concentration level. Individual recovery and mean recovery was determined at each concentration level.

Precision

System precision

Ranitidine HCl standard solution at working concentration level was prepared as described methodology section and injected in six replicates into chromatograph. The peak area responses were recorded for each injection and the mean, standard deviation and relative standard deviation was calculated.The relative standard deviation of the results for System Precision experiment was less than 0.4% for all six replicate injections.

Intermediate precision

Ranitidine HCl standard solution and Samples in six replicates were prepared as described methodology section. Six replicate sample preparations were performed by first analyst on different day on different HPLC system and another six replicate sample preparations were performed by second analyst on different day on different HPLC system. The peak area responses were recorded for each sample against freshly prepared standard solution. Percentage assay of each sample preparation, mean, standard deviation and relative standard deviation was calculated.The relative standard deviation and pooled relative standard

deviation of the results for intermediate precision experiment was less than 0.4% for all six replicate preparations.

Robustness

Intermediate precision solutions were used for the robustness study. For these solutions different chromatographic conditions along with sample preparation conditions were varied within acceptable ranges. The peak area responses were recorded for each sample against freshly prepared standard solution. Percentage assay of each sample preparation, mean, standard deviation and relative standard deviation was calculated.

Solution Stability

Sample and Standard solution were prepared as described in the methodology section. These solutions were analysed against freshly prepared standard after keeping the sample solution at room temperature for 72 h. Initial results were compared with results at 72 h. Percentage assay of each sample preparation, mean, standard deviation and relative standard deviation was calculated.

RESULTS AND DISCUSSION

Specificity

There was no interference of water, placebo, Ranitidine S-Oxide impurity and other degradation products at the retention time of Ranitidine HCl principle component. Peak purity of the Ranitidine HCl principle component was found complying. Resolution between Ranitidine HCl principle component and co-elution peaks was found greater than 1.5 [9-11].

Linearity

Correlation coefficient determined from concentration 89.6 µg/mL to 133.4 µg/mL was found greater than 0.999. The method was found linear from 89.6 µg/mL to 133.4 µg/mL concentration levels [9-11].

Range

Range was defined once linearity, precision, and accuracy had been established. The results are precise, accurate and linear in the range of 80% to 120% of working concentration of Ranitidine HCl [9-11].

Accuracy

Accuracy was determined at three different concentration levels from the working concentration level. The results are accurate in the range of 80% to 120% of working concentration of Ranitidine HCl [9-11].

Precision

System precision: The relative standard deviation of the results for System Precision experiment was less than 0.4% for all six replicate injections [9-11].

Intermediate precision: The relative standard deviation and pooled relative standard deviation of the results for Intermediate Precision experiment was less than 0.4% for all six replicate preparations [9-11].

Robustness

There was no significant difference in the results of Ranitidine HCl obtained by the normal method and those obtained by carrying out deliberate changes in the method. The deliberate changes did not affect the system suitability criteria [9-11].

Solution Stability

There was no significant difference in the two ob-served values for standard preparation. There was no significant change in the results for test preparation.Hence the solution for standard preparation and testpreparation are stable for 72 h at room temperature [9-11].

CONCLUSION

The suggested method was found specific, linear, accurate and robust. The suggested method can be successfully used to estimate Ranitidine HCl from tablet formulations.

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