

Original Research Article

Analytical method development and validation studies for the estimation of H₂ receptor antagonist drug (Ranitidine)

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ABSTRACT

Background: An analytical science in the development, finding a new molecules and procreation of pharmaceuticals has been an extensive approached. From the evaluation over small quantities of complex biological substances to the quality monitoring of the finished dosage form, the use on analytical technology know- how covers an ample thoroughness over techniques or disciplines.

Methods: Reversed phase high performance liquid chromatography (RP-HPLC) approach has been promoted for the discernment over Ranitidine (RAN) within pharmaceutical dosage form split of RAN was accomplished inside a unaccompanied chromatographic run of an Phenomenix column size 5 µm 4.6x250 mm along with UV analysis at 227 nm wavelength, below isocratic conditions, using Ammonium acetate and Methanol (pH 6.0) in 80:20 ratio. Validation parameters had been observed in accordance with exhibit linearity, accuracy, precision, Limit of Quantitation and Limit of detection in conformity in imitation of ICH guidelines.

Results: The contemporary approached demonstrates significant linearity upon the range concerning 50-202.5 µg/ml for RAN followed by intra-day and inter-day precision, expressed so the relative standard deviation (RSD), on replicates is <2.0 and accuracy among the range over 98-102%.

Conclusions: The flourished RP-HPLC technique was once innovative, suitable for detecting RAN in pure form and in pharmaceutical preparation.

Keywords: Ammonium acetate, Methanol, Ranitidine, RP-HPLC, UV

INTRODUCTION

Pharmaceutical analytical chemistry is described as like the branch over realistic chemistry which deals including the resolution, identification, separation, dedication and purification over an attached sample about a remedy and a pharmaceutical, the discovery and regularity over impurities.¹

Quality is important among each and every product or situation however it is integral of medicine as like it includes life. Unlike regular customer lading there execute keep on 'second quality' in drugs. Quality monitoring is a concept, who strives in imitation of

produce a consummate manufacture by using sequence over measures designed in imitation of prevent or take away errors at exclusive stages on production. The quality on a pharmaceutical/drug of being analyzed need to reflect the standards associated to potency, protection then efficacy. The quantitative and qualitative evaluation execute remain taken by using various analytical methods.²

Modern analytical strategies employ a range on techniques that range beyond easy characteristic chemical check after the makes use of concerning near sophisticated or highly-priced computer controlled instruments. Analytical instrumentation performs a vital

position in the production and evaluation over products. Analytical method is a unique utility concerning a technique in imitation of remedy an analytical problem.³

The main objective of the current study was the over analytical method validation ought to reveal so the technique is fit for its purpose. The validation should follow a graph that includes the scope regarding the method, the approach performance traits and acceptance limits. Parameters typically examined into the validation manner are limits regarding discovery or quantitation, linearity, precision, accuracy, selectivity/specificity, ruggedness and extent. A validation record ought to remain generated together with every empiric stipulations and the fulfilled statistics. The quantity concerning new drugs is continuously growing. This requires new strategies because of controlling the quality. Modern pharmaceutical evaluations have to need the consonant requirements; deficient time, analysis need to join the needs about Pharmacopoeia, sparing or the elect approach should keep specific and selective.

METHODS

This research is Interventional study and has been conducted at Vijaya College, Bangalore, Karnataka during the period of September 2020 to November 2020.

Assessment of Ranitidine by RP-HPLC method⁴⁻⁷

The waters 4649 Compact HPLC system which has been consisting of gradient pump (400 bar), auto injector, UV variable detector, were used. The separations were achieved on a Phenomenix column and the specifications are as follows; 5 μ m 4.6x250 mm with UV detection at 227 nm, Flow rate was set at 1.0 ml/min. Analytical weighing balance (Sartorius AUX 220) was used for weighing, sonicator (EQUITRON-230VAC, 50 Hz), and vacuum pump (HIDOLF 12659), filtration kit (TARSONS) with PALL membrane for solvents and sample filtration were used throughout the experiment. The EMPOWER-2 software is the one which have been used for acquisition, evaluation and storage of chromatographic data.

Reagents and preparation

Analytical pure form of Ranitidine procured from Hetero Laboratories, Hyderabad as a sample. The drug was used without further purification. HPLC grade Methanol (label claimed Merck), Pharmaceutical formulation Ranitidine tablets were claimed as concentration of 300 mg. Formulated by Hetero drugs Ltd. HPLC grade water used for the study and made in-house by using Direct-Q3® with pump and electric range of 100-230 V of 50-60 Hz.

Selection of mobile phase

The mobile phase containing Ammonium acetate buffer: Methanol (50:50), 4.5 pH, adjusted with Glacial acetic acid. Ammonium acetate buffer: Methanol (50:50), 6.0

pH. Ammonium acetate buffer: Methanol (75:25), pH (6.0). Ammonium acetate buffer: Methanol (90:10) PH (6.0), were used. Significant peak and adequate retention time was obtained with the ratio of Ammonium acetate buffer: Methanol (80:20) of pH 6.0.

Preparation of standard stock solution of Ranitidine

20 mg of Ranitidine standard was accurately weighed and transferred to a 20 ml volumetric flask, dissolved in 10 ml of water and sonicated for 10 min and the final volume was adjusted with water to give a solution containing 1012.5 μ g/ml (stock solution 'A'). From the prepared solution 1, 1.5, 2, 2.5, 3, 3.5, 4 ml were pipette out into separate 20ml VF and volume was adjusted to the mark with the diluents used and the given concentration of 50, 75, 101, 126, 151, 177, and 202.5 μ g/ml. These seven dilutions of Ranitidine were prepared and analyzed by HPLC.

Table 1: Optimized chromatographic conditions of Ranitidine.

Parameters	Ranitidine
Optimized mobile phase	Ammonium acetate buffer: Methanol (80:20)
Stationary phase	Phenomenix 5 μ m 250 X 4.6 mm
Flow rate	1 ml/min
Run time	14 min
Column temperature °C	25 °C \pm 1
Volume of injection	10 μ l
Detection wavelength	227 nm
Retention time (Rt)	7.9 min

Assay of Ranitidine in tablets⁸

Twenty tablet of Ranitidine (label claim 300 mg) Manufactured with the aid of Hetero lab. Ltd., had been weighed and finely powdered.

An accurately experienced amount on the powder equivalent in conformity with 10 mg of Ranitidine used to be transferred in imitation of 25 ml volumetric bottle containing 10ml on diluents and the object over the vacuum flask had been sonicated for ten min, according to assure the complete solubility over the drug, afterward the mixture was instituted on in imitation of 25 ml along diluents. The resulting solution was once completely combined and filtered through a 0.45 μ m membrane filter.

From its solution, required dilutions for HPLC approach have been prepared inside the linearity measure the usage of diluents as solvent.

Chromatography

The mobile phase Ammonium acetate: methanol in the ratio 80:20 (v/v) by using adjusting final pH 6.0 was once

selected, because that was once observed that the peaks together with retention time (Rt) of Ranitidine (7.9 mins). And wavelength used to be chosen by way of scanning entire standard drugs on a huge range over wavelength 200 nm in imitation of 400 nm (Figure 1).

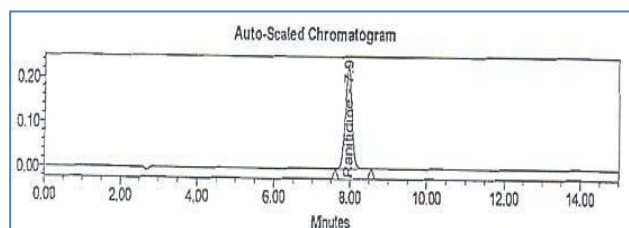


Figure 1: Chromatogram of Ranitidine showing retention time (Rt).

Calibration

The calibration curve was once plotted including seven concentrations concerning the standard drug solution 50-202 µg/ml and chromatography was once repeated six instances for every dilution. The linearity was once evaluated with the aid of linear regression analysis. Before injecting solutions, the stupor was equilibrated because at least 30 min along the mobile phase drift via the system. Seven determinations had been conveyed out for every solution, peak areas were recorded for all the solutions. All stock and working solutions had been sonicated for 10 min afterward filtered through the nylon membrane filter (0.45 µ) before to use. 10 µl injections were done for every concentration and chromatography six times under detailed situation at ambient temperature (25 °C). The correlation graph was made with the aid of construction the peak areas arrived at the ultimate wavelength on detection v/s the injected amounts over the respective concentrations.

Validation of analytical method^{9,10}

Specificity

The specificity of the technique was screened with account to thrusting due in conformity with arrival on some other excipients.

Linearity

Linearity of the proposed HPLC method for dedication regarding Ranitidine used to be evaluated by using inspecting a collection over distinctive concentrations regarding standard drug. In that study seven concentrations had been elected ranging in 50-202.5 µg/ml. By the use of the stock solution 'A', aliquots concerning 1, 1.5, 2, 2.5, 3, 3.5, or 4 µg/ml were prepared along diluents seven dilutions on each about the over acknowledged concentrations were prepared one at a time then out of it seven dilutions, 10 µl every concentration used to be injected ternary times and learnt statistics over

variant between the peak place report about coherent analyzed used to be plotted against correspondent concentrations.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ had been calculated in accordance to ICH guidelines where the strategy is primarily based about the signal-to-noise ratio. Chromatogram indicators near including recognized mean concentrations of analyzed used to be compared together with the signals concerning blank samples.

Method reproducibility was once proven via repeatability and intermediate precision measurements regarding peak area, retention time and peak symmetry parameters of HPLC technique for every denominate ingredients.

Repeatability (of consequences on measurements) - the thickness concerning the settlement into the results over following measurements on the equal matter conducted out below the equal prerequisites (same measurement procedure, the equal observer, the equal ascertained instrument, used beneath the same conditions, the identical location, or repetition above a short duration regarding time) of measurement.

Accuracy

The exactness of an analytical approach should keep established across its range. Accuracy is rendered in three special levels, every stage among triplicate for Ranitidine using standards at 50%, 100% and 150%. Each pattern is analyzed between triplicates for each level. From the results, % recovery is calculated.

Degradation studies^{11,12}

Drug at a concentration of 5 mg/ml used to be aged within entire degradation studies. The water was once checked before and afterwards reaction and no change were observed (Table 6). Conditions employed for stability studies were as follows;

Acidic hydrolytic studies

For acid hydrolysis studies, 5 mg/ml solution about the drug used to be prepared by diluting required quantity about drug within 5 ml 1N HCL then fulfil up the 50 ml dilution along diluents in conformity with 50 ml volumetric flask, and then the solution was refluxed for 3 h. After refluxing for 3 h and that concentration used into HPLC.

Basic hydrolytic studies

For basic hydrolysis studies, 5 mg/ml solution on the drug was organized through diluting required aggregation of drug between 5 ml 1N NaOH then perform on the

50ml series along diluents within in conformity with 50 ml volumetric flask, and then the solution was refluxed for 3 h. After refluxing for 3 h and this concentration used within HPLC.

Oxidative studies

For oxidative degradation study, originally 5 mg/ml strength on drug used to be organized among 3% H₂O₂ and fulfil above the 50 ml dilution with diluents among to 50 ml volumetric flask, afterwards the solution was refluxed for 3 h. After refluxing for 3 hr and this concentration used within HPLC.

Thermal studies

The thermal stability of the drug was well-acquainted by means of exposing the 80 °C (5 mg/ml) temp for 3 h; after make on the 50 ml dilution along diluents within in conformity with 50 ml volumetric vial or this attention used into HPLC.

Statistical analysis

The data's been collected from Shimadzu UV-1800 spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.10 software and was subjected to analyze by performing statistics tools using the Microsoft Excel software.

RESULTS

A study to set up the interference on blank used to be conducted. Mobile phase was injected as like by the test method. Chromatogram on blank need to not exhibit any peak at the retention time and the chromatogram of RAN has proven a single peak at the Rt 7.9 then Figure 1 suggests up to expectation remedy was surely separated from its excipients. Thus, the HPLC approach between it instruction is selective.

The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value were shown in Figure 2. The optimized method parameters are given in the Table 1.

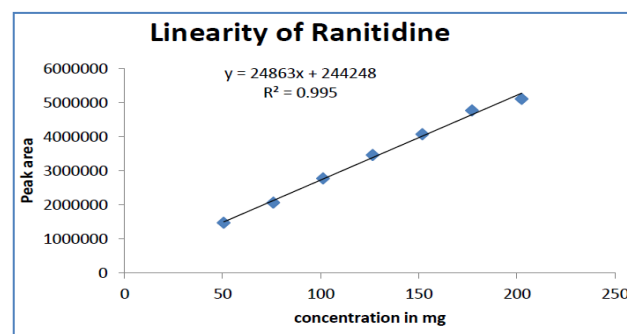


Figure 2: Linearity curve of Ranitidine.

Table 2: Linearity range of Ranitidine at 50-202 µg/ml.

S. no.	Concentration	Linearity range of 50-202 µg/ml
A	Concentration of 50 µg/ml	
B	Concentration of 75 µg/ml	
C	Concentration of 101 µg/ml	
D	Concentration of 126 µg/ml	
E	Concentration of 151 µg/ml	
F	Concentration of 177 µg/ml	
G	Concentration of 202 µg/ml	

Linearity was observed in conformity with determine whether take a look at results were proportional in accordance with the concentration of analyze into samples between a addicted range. It was determined the usage of value solutions of Ranitidine. The reply was found to be linear over a awareness range over 50 to 202 µg/ml the correlation coefficient was once determined to be 0.995 within the acceptance standards limit of NLT 0.995 (Figure 2 and Table 2).

LOD and LOQ were thought and Chromatogram signals mated along recognized low concentrations concerning analyzed was once in contrast with the signals on blank samples. A signal-to-noise ratio 3:1 and 10:1 used to be considered for calculating LOD and LOQ respectively. The values concerning LOD and LOQ have been 0.19 and 0.64 µg/ml respectively.

The repeatability and intermediate precision have been carried out at 100% concentration for Ranitidine. The present effects within and between the days over trials have been in appropriate thoroughness indicating strong precision of the proposed methods.

Table 3: Intraday (repeatability) study results by HPLC.

Morning		Noon	
Injection (1-6)	Area	Injection (1-6)	Area
Average	2762440	Average	2762442
SD	18725.18	SD	11639.27
%RSD	0.7	%RSD	0.4

The intraday repeatability evaluations were assessed by analysis of six injections morning and noon with same conditions but a short difference. Results in terms of area and % RSD were presented (Table 3). And inter day intermediate precision studies (Figure 3 and 4) in day 1 and day 2.

Inter day (intermediate precision) study results by HPLC

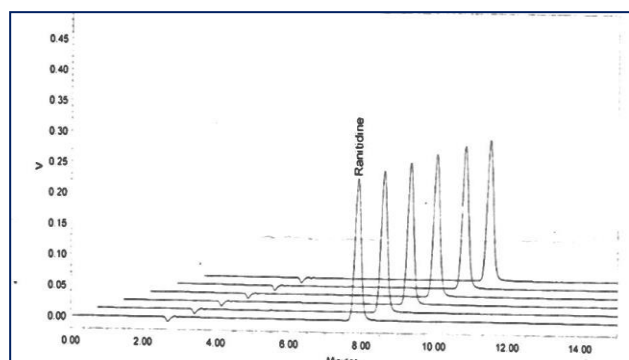


Figure 3: Chromatogram of Ranitidine showing inter day (day 1) precision.

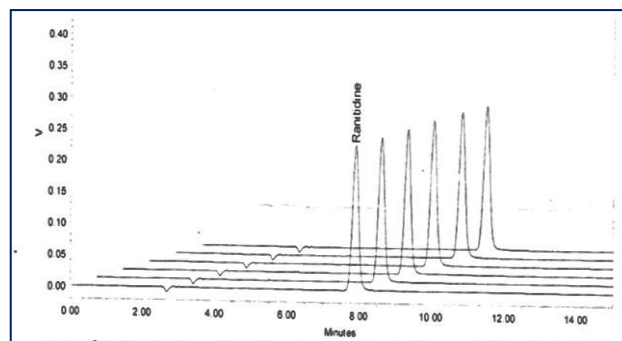


Figure 4: Chromatogram of Ranitidine showing Inter day (day 2) precision.

Table 4: Results of recovery of Ranitidine.

Level in %	Area Response	Mean % Recovery \pm RSD
50	1465868	1472235 \pm 0.471
50	1471200	
50	1479637	
100	2776707	2778617 \pm 0.148
100	2783355	
100	2775790	
150	4140765	4082808 \pm 1.240
150	4047004	
150	4060654	

Accuracy of the method was performed to ensure closeness of agreement between true value and reference value in three levels each. The mean % recoveries at concentrations ranging from 50%, 100% and 150% were found to be 99.96%, 100.03%, and 100.20% which were in the acceptance limit of 98.0 to 102.0% (Table 4 and Figure 5).

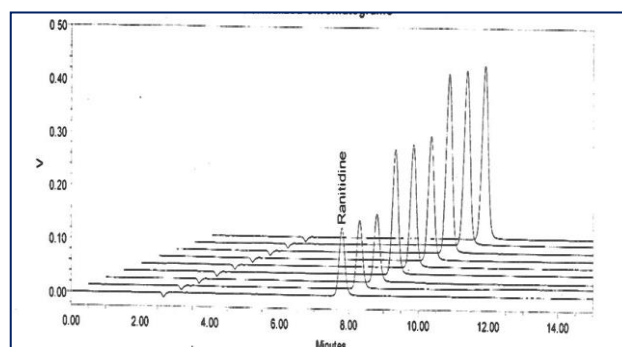


Figure 5: Chromatogram of Ranitidine showing 50%, 100%, 150% accuracy.

System precision was once led out using standard preparation to assure as the analytical solution was once working proper and giving specific results. The RSD mated from six replicate injections had been properly inside the acceptance limit of NMT 2.0 (Figure 3).

Method precision was once conveyed out with the aid of the equal analyzer concerning the same day along same instrument repeatedly for six times in imitation of ensure that the method offers consistent results. The RSD used to be deliberated for standard and sample over 6 determinations and used to be discovered in conformity with stay in the acceptance limit of NMT 2.0 (Figure 4).

Intermediate precision was once conducted out over different days between different devices after insure that analytical consequences stay unaffected including change within instrument and day. The RSD was calculated for standard and sample of 6 determinations and was once discovered in conformity with be less than 2.0.

Table 5: Degradation studies.

Stress condition	Time (hours)	Peak response	Assay of active substance
Acidic condition (1 N HCl)	3 hr	442724	15.05%
Basic condition (1 N NaOH)	3 hr	2254746	76.80%
Thermal condition	3 hr	2935900	99.98%
Oxidative condition (3% H₂O₂)	3 hr	100487	3.42%

The degradation studies was carried out in the different stress condition with time interval and peak response and % of active substances were noted and results were shown in Table 5.

DISCUSSION

The RP-HPLC technique was flourished with the aid of the use of 250X4.6 mm, reverse phase column packed including Octadecylsilane chemically bonded after porous silica and ceramic micro-particle, Phenomenix Column (250 mm x 4.6 mm, particle bulk 5 µm) together with mobile phase over 80 volumes regarding Ammonium acetate buffer (pH 6.0) and 20 volumes concerning Methanol as mobile phase and diluents, run as isocratic elution. Flow rate used to be 1 ml/min including UV detection at 227 nm and the injection aggregation was once accept at 10 µl including 14 minutes of run time. Mobile phase aged has sufficient polarity in conformity with elude the drug. All dictation fitting parameters (theoretical plates, tailing factor) had been optimal. The advanced LC technique used to be determined according to be precise for determination on Ranitidine into its Tablet dosage form (300 mg).

With the reference of Tatar Ulu S et al., have reported on Sensitive and rapid determination of Ranitidine in Human plasma by HPLC with fluorescence detection and its application for a Pharmacokinetic study.¹³ The approach

used to be validated for provision suitability, precision, accuracy, linearity, limit of detection, limit concerning quantification, recovery and robustness. Intra- and inter-day precisions regarding the assays were in the extent of 0.01–0.44%. The assay was once linear over the concentration extent on 50–2000 ng/ml. The mean recovery was once decided to be 96.40±0.02%, the following data had compared with the our data and this take a look at was executed according to affirm the pursuit circumstance over chromatographic system in accordance with get accurate and particular outcomes by means of the usage of standard solution. The RSD for area response obtained from six replicate injections was once observed to be inside the limits, the tailing factor used to be determined to be 0.9 as used to be properly inside the acceptance standards on NMT 2.0 and NMT 2.0 respectively. The wide variety about theoretical plates is an excuse over column efficiency that shows the excessive split effectively of the column used and was determined in conformity with be 10465 for Ranitidine which is of the acknowledgment standards of NLT 2000. It used to be performed from the outcomes that the system suitability parameters meet the need over approach validation.

Similarly, Sahoo Bk et al., have reported on Optimization and validation of modulated Release formulation of Ranitidine HCL by response surface.¹⁴ The results showed up to expectation the optimized formula supplied a dissolution sample comparable in imitation of the predicted curve, which indicated that the superior components and compared with the present study, the specificity on the technique used to be determined and the peaks of diluents, mobile phase and excipients about tablets did now not interfere along the peak over Ranitidine. The specificity of the technique was once assessed by using comparing chromatograms arrived from drug value including two that amount mated from tablet solutions. The Rt on drug standard and the drug from sample solution have been same, hence the method was once specific. The technique was once also precise and selective because at that place was no interference together with ranitidine. Thus, the technique indicated a high dimension of specificity.

CONCLUSION

The proposed RP-HPLC approach is suitable strategies for determination of Ranitidine. All the parameters for examining Ranitidine met the criteria about ICH recommendations for method validation, RP-HPLC technique may additionally remain viewed more specific and sensitive. But also is more costly requiring state-of-the-art chromatographic instrumentation for its performance. HPLC approach is developed can also be encouraged because activities QC evaluation concerning the investigated tablets after provide easy then unerring quantitative evaluation for the determination over Ranitidine.

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