

A VALIDATED ANALYTICAL METHOD FOR RANITIDINE HCL THROUGH LC-MS/MS, HPLC AND QUALITY BY DESIGN APPROACH.

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ABSTRACT

Objective: The current study aimed to develop and validate simple, precise, and highly sensitive LC-MS/MS and HPLC methodologies for the quantitative estimation of Ranitidine HCl in pharmaceutical formulations. The goal was to develop a speedy and dependable technique for routine quality control and regulatory analysis.

Methods: Chromatographic separation was achieved using an Atlantis C₁₈ column (150×4.6 mm, 5µm) under isocratic elution mode. The mobile phase consisted of 10 mmol ammonium acetate (solvent A) and acetonitrile (solvent B) in a 10:90 v/v ratio, delivered at a flow rate of 0.7 ml/min. An injection volume of 10µl* was employed, and the run time was 4 min. Ranitidine HCl was eluted at 1.993 min. The method was validated in accordance with ICH Q2 (R1) guidelines for parameters including linearity, sensitivity, accuracy, precision, and robustness.

Results: The LC-MS/MS method showed excellent linearity across a concentration range of 5-250 ng/ml with a correlation coefficient (R²) of 0.9998. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 1.0 ng/ml and 3.0 ng/ml, respectively. Recovery ranged from 95.33% to 97.93% and relative standard deviation (RSD) was 0.69%, confirming the method's accuracy and precision.

Conclusion: The validated LC-MS/MS and HPLC methods are simple and sensitive for the quantification of ranitidine HCl. Both techniques demonstrated robust performance with high accuracy and precision. Their successful application to a commercial formulation confirms their suitability for pharmaceutical quality control and supports regulatory compliance in the routine analysis of ranitidine HCl products.

Keywords: Analytical method development, Method validation, ICH, Ranitidine HCl, QbD

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INTRODUCTION

Ranitidine hydrochloride (RAN HCl) is a competitive histamine H₂-receptor antagonist widely prescribed for the treatment of duodenal ulcers, gastro-oesophageal reflux disease (GERD), erosive esophagitis, and Zollinger-Ellison syndrome [1]. As a structural derivative of substituted furan, it inhibits gastric acid secretion by blocking histamine action on parietal cells. Due to its long-standing safety and efficacy, it was included in the WHO model list of essential medicines.

RAN HCl is a white-coloured powdered drug substance, widely recognized for its favourable solubility in organic solvents such as acetonitrile (ACN) and methanol. These physicochemical properties make it highly suitable for formulation development and analytical applications, particularly in chromatographic and mass spectrometric techniques [2]. The chemical structure of RAN HCl is shown in (fig. 1).

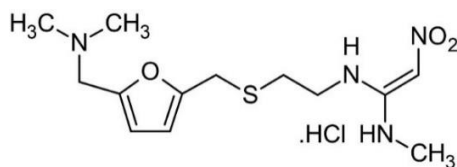


Fig. 1: Ranitidine HCL chemical structure

In 2019, the discovery of N-nitrosodimethylamine (NDMA), a probable human carcinogen classified as Group 2A by the International Agency for Research on Cancer (IARC), in ranitidine-containing formulations raised significant concerns about the drug's stability and safety [3]. Investigations by health agencies such as the

United States Food and Drug Administration (USFDA) and the European Medicines Agency (EMA) linked the presence of NDMA to the intrinsic instability of the ranitidine molecule, especially under conditions involving high temperature or the presence of nitrites and amines [4-6].

The global impact was swift and significant. The FDA, in April 2020, requested the immediate withdrawal of all ranitidine products from the U. S. market due to unacceptable levels of NDMA that could increase over time even under normal storage conditions [7]. Likewise, the EMA recommended a precautionary suspension of ranitidine products across the European Union [8]. Regulatory agencies in Canada, Australia, India, and several Asian and Gulf countries followed similar action, prompting a worldwide re-evaluation of ranitidine's safety profile [9]. These events underscored the critical need for reliable, highly sensitive analytical techniques to detect trace levels of NDMA in pharmaceutical preparations.

Traditional methods such as UV spectrophotometry and standard High Performance Liquid Chromatography (HPLC) were found inadequate in detecting NDMA at levels as low as the FDA-recommended limit of 96 ng/day [10]. Consequently, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) emerged as the gold standard for NDMA detection due to its superior sensitivity, selectivity, and suitability for trace analysis [11].

Simultaneously, the application of Quality by Design (QbD) principles, as outlined in ICH guidelines Q8 through Q11, has revolutionized pharmaceutical development, including analytical method design [12]. QbD emphasizes a science-based, systematic, and risk-driven approach by defining an Analytical Target Profile (ATP), identifying Critical Method Parameters (CMPs), and applying Design of Experiments (DoE) to optimize and validate method performance. Box-Behnken Design (BBD), integrated with Response

Surface Methodology (RSM), is widely used to identify robust operating conditions in multi-variable systems [13-15].

Although HPLC methods remain common in pharmaceutical analysis, their utility in nitrosamine detection is limited unless coupled with derivatization or fluorescence techniques [16]. In contrast, LC-MS/MS offers detection limits as low as 0.25 ng/ml for NDMA, as evidenced in several recent studies [13, 14]. Despite this, limited research has integrated both HPLC and LC-MS/MS platforms under a QbD framework for comprehensive ranitidine analysis and impurity profiling.

Accordingly, the present study aimed to develop and validate robust, stability-indicating analytical methods for the quantitative estimation of RAN HCl and associated nitrosamine impurities using both HPLC and LC-MS/MS platforms. Guided by QbD principles, the methods were optimized using BBD to establish a Method Operable Design Region (MODR). Critical factors such as mobile phase composition, flow rate, and pH were evaluated for their impact on performance [17-19]. Validation was performed in accordance with ICH Q2(R1) guidelines to ensure accuracy, precision, and regulatory compliance. The developed methods were successfully applied to commercial formulations, offering a sensitive and reliable solution for routine quality control and impurity profiling of ranitidine products.

MATERIALS AND METHODS

Chemicals and standards

For method development and validation, analytical grade active pharmaceutical ingredients (APIs) were used, each supported by a certificate of analysis confirming a purity greater than 99% and compliance with specified quality standards.

Sigma-Aldrich provided the ranitidine hydrochloride reference standard. SD Fine Chemicals provided LC-MS/MS solvents such as acetonitrile, ammonium hydroxide solution, ammonium acetate, and

methanol. The Milli-Q RO water purification system provided ultrapure water for mobile phase preparation and sample processing, which was then filtered through a 0.22 µm PVDF membrane. All additional chemicals and reagents were analytical grade and purchased from reputable commercial providers.

Preparation of standard solutions and buffer

A precise quantity of 10 mg of RAN HCL was precisely weighed and diluted in 10 ml of methanol to yield a primary stock solution with a concentration of 1 mg/ml. To achieve full dissolution, the solution was sonicated for 5 min. To create a secondary solution of 100 µg/ml, dilute 1 ml with 10 ml of methanol (Solution A). A working standard solution of 10 µg/ml was produced by diluting 1 ml of solution A to 10 ml with methanol for analytical and validation investigations [20].

The 10 mmol ammonium acetate buffer was prepared by dissolving 0.256 g of ammonium acetate in 200 ml of distilled water. To achieve full dissolution, the solution was sonicated for 10 min. The buffer pH was initially measured and found to be 6.3; thus, it was raised to 7.0 using a 25% ammonium hydroxide solution. To remove particles, the buffer was filtered through a 0.45 µm membrane filter before being used in the mobile phase [21].

Experimental design and optimization

Design-Expert® version 13 software was employed to process the experimental design for the optimization of analytical methods for RAN HCl using both HPLC and LC-MS/MS platforms. A BBD was utilized to systematically assess the influence of selected method parameters. The Independent variables included flow rate, percentage of acetonitrile, and pH, while the dependent variables were retention time, tailing factor, and peak area. The aim was to establish a robust MODR applicable across both analytical techniques [22, 23]. The coded values and levels of the independent variables used in the design are summarized in (table 1).

Table 1: BBD was used to analyse the experimental factors and observed values

Factors	Name	Unit	Type	Low (-)	Central (0)	High (+)
A	Flow rate	ml	Numeric	0.6	0.7	0.8
B	Acetonitrile	%	Numeric	85	90	95
C	pH	Unit	Numeric	6.5	7.0	7.5

The relationship between the method parameters – flow rate, acetonitrile percentage, and pH, and the responses (retention time, peak area, and tailing factor) were explored using BBD. Response surface methodology was applied to visualize individual and interaction effects, aiding in the identification of optimal conditions for LC-MS/MS analysis of RAN HCl. The model's validity and predictive strength were confirmed through Analysis of Variance (ANOVA), R² values, and lack-of-fit tests, ensuring a good fit and reliable optimization.

Instrumentation and chromatographic conditions of HPLC

The analysis was carried out using a WATERS-2695 HPLC system equipped with a UV-VIS Dual Absorbance Detector (WATERS-2487), with data acquisition performed using Lab Solutions software. An Atlantis C18 column (75 mm × 4.6 mm, 5 µm particle size) was used to achieve chromatographic separation at ambient temperature. The mobile phase consisted of 10 mmol ammonium acetate buffer and acetonitrile in a 10:90 (v/v) ratio, supplied at a flow rate of 0.7 ml/min. A UV-VIS dual absorbance detector at 230 nm was used to detect the samples. The approach achieved acceptable peak symmetry and retention for RAN HCl. The HPLC process was optimised using BBD, with a focus on three variables: flow rate, acetonitrile %, and pH.

Instrumentation of LC-MS/MS

The method was developed and validated on a Shimadzu LC-MS 8030 system with an electrospray ionisation (ESI) interface. The system had a customisable injection volume from 0.1 to 100 µl, depending on the injection mode and loop design. The system

included a SIL-20AC HT autosampler, CTO-20AC column oven, LC-20AD pump, and LCMS-8030 mass spectrometer. System control, data acquisition, and chromatographic data integration were all accomplished using LabSolutions software.

LC-MS/MS chromatographic and mass spectrometric conditions

An Atlantis C18 column (75 × 4.6 mm, 5 µm particles) facilitated chromatographic separation at room temperature. The mobile phase consisted of 10 mmol ammonium acetate combined with acetonitrile in a 10:90 (v/v) ratio, flowing at 0.7 ml/min. The injection volume was 10 µl, with a total run time of 4 min. RAN HCl eluted with a retention duration of 1.993 min.

Mass spectrometric detection was performed using a negative-polarity ESI interface in Multiple Reaction Monitoring (MRM) mode. The desolvation line (DL) and block temperatures were maintained at 250 °C and 350 °C, respectively. Argon served as the collision-induced dissociation (CID) gas at a pressure of 230 kPa, with collision energy set to +22 eV. Both the nebuliser gas (3 l/min) and the drying gas (15 l/min) were nitrogen-based. The probe's temperature remained at ambient levels. Data collection, processing, and integration were managed using the LabSolutions data system.

Method validation for RAN HCl

The developed HPLC and LC-MS/MS techniques for RAN HCl measurement were validated using ICH Q2 (R1) recommendations, which included essential factors such as specificity, sensitivity, linearity, precision, accuracy, and robustness [24-28]. Specificity

was determined by assessing the capacity to identify the analyte in the presence of possible degradants and matrix constituents. Linearity was found at six concentration ranges, ranging from 5 to 250 ng/ml, with correlation coefficients (R^2) close to one, confirming excellent linearity. Sensitivity was established by calculating the LOD and LOQ for signal-to-noise ratios of 3:1 and 10:1, respectively. Accuracy was assessed by recovery studies utilising three quality control levels in six replicates, and precision was evaluated by determining the RSD%. Robustness was assessed by purposefully altering method parameters such as flow rate, pH, and mobile phase, with no discernible effect on method performance. These validation results show that the approach is reliable, reproducible, and appropriate for routine quality control of RAN HCl.

RESULTS AND DISCUSSION

Method development and response surface optimization for HPLC and LC-MS/MS

The chromatographic conditions for both HPLC and LC-MS/MS methods were optimized using BBD in DoE software. Flow rate (A), Acetonitrile percentage (B), and mobile phase pH (C) were selected as independent variables, while retention time, tailing factor, and peak area of RAN HCl served as the response variables. The goal was to identify the optimal combination of these parameters to ensure minimal tailing, appropriate retention, and improved peak intensity.

A recent study by Patel *et al.* in 2024 also applied BBD to optimize an LC-MS/MS method for impurity profiling of Ranitidine, reporting flow rate and organic phase composition as significant factors, findings that align with our model outputs and confirm the robustness of the chosen design strategy [29].

BBD were employed to evaluate the interactive effects of three critical method parameters – flow rate, acetonitrile percentage, and pH on the analytical responses for RAN HCl using both HPLC and LC-MS/MS. A total of 17 experimental runs were generated and executed according to the BBD matrix, covering all possible combinations of factor levels for both HPLC and LC-MS/MS platforms, respectively (table 2 and 3). The dependent responses assessed for both techniques included retention time, peak area, and tailing factor. The observed results, along with predicted values, were analysed to assess model predictability and performance. Statistical validation of the experimental design was carried out using ANOVA and lack of fit test for both HPLC (table 4A, 4B and 4C) and LC-MS/MS (table 5A, 5B and 5C). These analyses confirmed the model's statistical significance and reliability, reinforcing its applicability for optimizing both chromatographic platforms [30]. Furthermore, Chen *et al.* in 2022 demonstrated the superior sensitivity and selectivity of LC-MS/MS over HPLC for Ranitidine analysis, aligning with our observed advantages of the LC-MS/MS approach [31].

Table 2: Optimization of parameters for analysis of RAN HCl using BBD for HPLC platforms

Std	Run	Factor 1: flow rate	Factor 2: ACN	Factor 3: pH	Response 1: retention time	Response 2: peak area	Response 3: tailing factor
5	1	0.5	90	6.5	3.59	173862	0.99
8	2	1.5	90	7.5	1.98	188623	1.02
11	3	1	85	7.5	2.38	213861	1.03
3	4	0.5	95	7	3.56	163782	0.98
4	5	1.5	95	7	1.95	213690	1.01
16	6	1	90	7	2.35	193961	1.03
15	7	1	90	7	2.35	183928	1.03
17	8	1	90	7	2.32	203815	1.03
10	9	1	95	6.5	2.46	223785	1.04
9	10	1	85	6.5	2.44	203892	1.02
7	11	0.5	90	7.5	3.52	163862	0.97
1	12	0.5	85	7	3.67	191262	0.99
6	13	1.5	90	6.5	1.99	213623	1.01
14	14	1	90	7	2.48	193873	1.05
2	15	1.5	85	7	1.92	213789	1.06
13	16	1	90	7	2.52	203764	1.01
12	17	1	95	7.5	2.43	193654	1.02

Table 3: Optimization of parameters for analysis of RAN HCl using BBD for LC-MS/MS platforms

Std	Run	Factor 1: flow rate	Factor 2: ACN	Factor 3: pH	Response 1: retention time	Response 2: peak area	Response 3: tailing factor
13	1	0.7	90	7	2.01	238965	1.03
3	2	0.6	95	7	2.28	250112	1.06
7	3	0.6	90	7.5	2.25	259082	0.98
12	4	0.7	95	7.5	1.98	230640	0.99
9	5	0.7	85	6.5	2.2	236098	1.23
4	6	0.8	95	7	2.53	245602	1.03
11	7	0.7	85	7.5	1.98	239087	1.01
1	8	0.6	85	7	2.27	250127	1.02
8	9	0.8	90	7.5	2.5	247912	1.08
10	10	0.7	95	6.5	1.99	236108	1.5
6	11	0.8	90	6.5	2.54	248170	1.5
17	12	0.7	90	7	2	234089	1.02
14	13	0.7	90	7	1.99	238764	1.05
15	14	0.7	90	7	1.93	239730	1.01
5	15	0.6	90	6.5	2.29	250617	1.5
2	16	0.8	85	7	2.5	245021	0.99
16	17	0.7	90	7	1.99	234609	0.97

Table 4A: Statistical validation from BBD for the response of HPLC (Retention time)

Source	Sum of squares	df	mean square	F-value	p-value	
Model	5.84	9	0.6484	110.71	<0.0001	Significant
A-Flow rate	5.28	1	5.28	901.79	<0.0001	
B-Acetonitrile	0.0000	1	0.0000	0.0021	0.9644	
C-pH	0.0036	1	0.0036	0.6168	0.4580	
AB	0.0049	1	0.0049	0.8367	0.3908	
AC	0.0009	1	0.0009	0.1537	0.7067	
BC	0.0002	1	0.0002	0.0384	0.8502	
A ²	0.5359	1	0.5359	91.50	<0.0001	
B ²	0.0009	1	0.0009	0.1460	0.7137	
C ²	0.0004	1	0.0004	0.0615	0.8112	
Residual	0.0410	7	0.0059			
Lack of Fit	0.0089	3	0.0030	0.3684	0.7809	Not Significant
Pure Error	0.0321	4	0.0080			
Cor Total	5.88	16				

Table 4B: Statistical validation from BBD for the response of HPLC (Peak area)

Source	Sum of squares	df	mean square	F-value	p-value	
Model	4.607	9	5.119	8.87	0.0044	Significant
A-Flow rate	2.345	1	2.345	40.63	0.0004	
B-Acetonitrile	9.725	1	9.725	1.69	0.2354	
C-pH	3.804	1	3.804	6.59	0.0372	
AB	1.874	1	1.874	3.25	0.1145	
AC	5.625	1	5.625	0.9747	0.3564	
BC	4.020	1	4.020	6.97	0.0335	
A ²	6.085	1	6.085	10.54	0.0141	
B ²	5.847	1	5.847	10.13	0.0154	
C ²	5.528	1	5.528	0.0958	0.7660	
Residual	4.040	7	5.771			
Lack of Fit	1.283	3	4.276	0.6204	0.6379	Not Significant
Pure Error	2.757	4	6.892			
Cor Total	5.011	16				

Table 4C: Statistical validation from BBD for the response of HPLC (Tailing factor)

Source	Sum of squares	df	mean square	F-value	p-value	
Model	0.0078	9	0.0009	3.99	0.0408	Significant
A-Flow rate	0.0036	1	0.0036	16.58	0.0047	
B-Acetonitrile	0.0003	1	0.0003	1.43	0.2700	
C-pH	0.0000	1	0.0000	0.2295	0.6465	
AB	0.0004	1	0.0004	1.84	0.2175	
AC	0.0002	1	0.0002	1.03	0.3433	
BC	0.0002	1	0.0002	1.03	0.3433	
A ²	0.0026	1	0.0026	12.08	0.0103	
B ²	0.0001	1	0.0001	0.4832	0.5094	
C ²	0.0002	1	0.0002	1.09	0.3318	
Residual	0.0015	7	0.0002			
Lack of Fit	0.0007	3	0.0002	1.21	0.4143	Not Significant
Pure Error	0.0008	4	0.0002			
Cor Total	0.0094	16				

Table 5A: Statistical validation from BBD for the response of LC-MS/MS (Retention time)

Source	Sum of squares	df	mean square	F-value	p-value	
Model	0.7882	9	0.0876	42.00	<0.0001	Significant
A-Flow rate	0.1200	1	0.1200	57.58	0.0001	
B-Acetonitrile	0.0036	1	0.0036	1.73	0.2295	
C-pH	0.0120	1	0.0120	5.76	0.0474	
AB	0.0001	1	0.0001	0.0480	0.8329	
AC	0.0000	1	0.0000	0.0000	1.0000	
BC	0.0110	1	0.0110	5.29	0.0550	
A ²	0.6217	1	0.6217	298.17	<0.0001	
B ²	0.0030	1	0.0030	1.45	0.2684	
C ²	0.0030	1	0.0030	1.45	0.2684	
Residual	0.0146	7	0.0021			
Lack of fit	0.0107	3	0.0036	3.63	0.1225	Not significant
Pure error	0.0039	4	0.0010			
Cor Total	0.8028	16				

Table 5B: Statistical validation from BBD for the response of LC-MS/MS (Peak area)

Source	Sum of squares	df	mean square	F-value	p-value	
Model	8.806E+08	9	9.785E+07	12.51	0.0015	Significant
A-Flow rate	6.747E+07	1	6.747E+07	8.63	0.0218	
B-Acetonitrile	7.744E+06	1	7.744E+06	0.9900	0.3529	
C-pH	4.101E+06	1	4.101E+06	0.5243	0.4925	
AB	88804.00	1	88804.00	0.0114	0.9181	
AC	1.902E+07	1	1.902E+07	2.43	0.1629	
BC	1.788E+07	1	1.788E+07	2.29	0.1743	
A ²	7.362E+08	1	7.362E+08	94.12	<0.0001	
B ²	3.159E+07	1	3.159E+07	4.04	0.0844	
C ²	4.133E+06	1	4.133E+06	0.5284	0.4909	
Residual	5.476E+07	7	7.822E+06			Not Significant
Lack of Fit	2.641E+07	3	8.802E+06	1.24	0.4050	
Pure Error	2.835E+07	4	7.087E+06			
Cor Total	9.354E+08	16				

Table 5C: Statistical validation from BBD for the response of LC-MS/MS (Tailing factor)

Source	Sum of squares	df	mean square	F-value	p-value	
Model	8.806E+08	9	9.785E+07	12.51	0.0015	Significant
A-Flow rate	6.747E+07	1	6.747E+07	8.63	0.0218	
B-Acetonitrile	7.744E+06	1	7.744E+06	0.9900	0.3529	
C-pH	4.101E+06	1	4.101E+06	0.5243	0.4925	
AB	88804.00	1	88804.00	0.0114	0.9181	
AC	1.902E+07	1	1.902E+07	2.43	0.1629	
BC	1.788E+07	1	1.788E+07	2.29	0.1743	
A ²	7.362E+08	1	7.362E+08	94.12	<0.0001	
B ²	3.159E+07	1	3.159E+07	4.04	0.0844	
C ²	4.133E+06	1	4.133E+06	0.5284	0.4909	
Residual	5.476E+07	7	7.822E+06			Not Significant
Lack of Fit	2.641E+07	3	8.802E+06	1.24	0.4050	
Pure Error	2.835E+07	4	7.087E+06			
Cor Total	9.354E+08	16				

Further insights into the optimization process were obtained through the 3D surface plots, which showed the individual and interactive effects of the critical method parameters for HPLC and LC-MS/MS platforms. The model graph of Retention time (fig. 2A for HPLC and fig. 2B for LC-MS/MS) illustrates that lower flow rates combined with higher acetonitrile percentages led to an

increase in retention time in both methods. This behaviour is attributed to stronger interactions between the analyte and the stationary phase, resulting in delayed elution. However, comparatively sharper and more distinct retention behavior was observed in LC-MS/MS due to enhanced sensitivity and resolution capabilities.

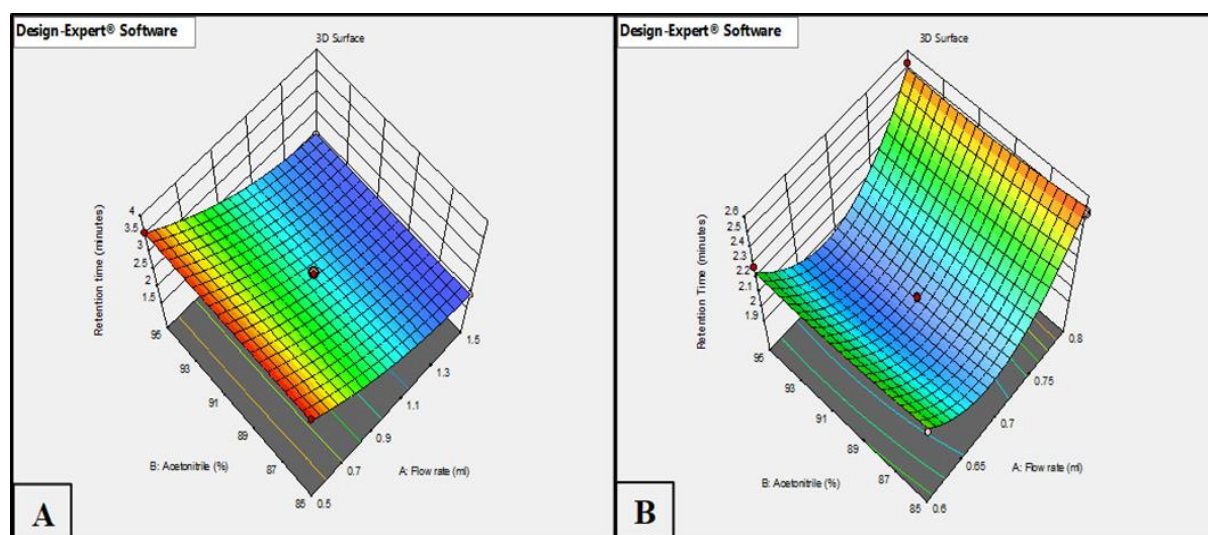


Fig. 2: Response surface plots illustrate the effect of flow rate and acetonitrile % on retention time using (A) HPLC method and (B) LC-MS/MS method

The model graphs for peak area (fig. 3A for HPLC and fig. 3B for LC-MS/MS) indicate that a lower flow rate coupled with an acidic pH significantly enhanced the peak area in both techniques. This

enhancement is likely due to improved ionization efficiency and better analyte stability, particularly in LC-MS/MS, which showed higher sensitivity at low pH compared to HPLC.

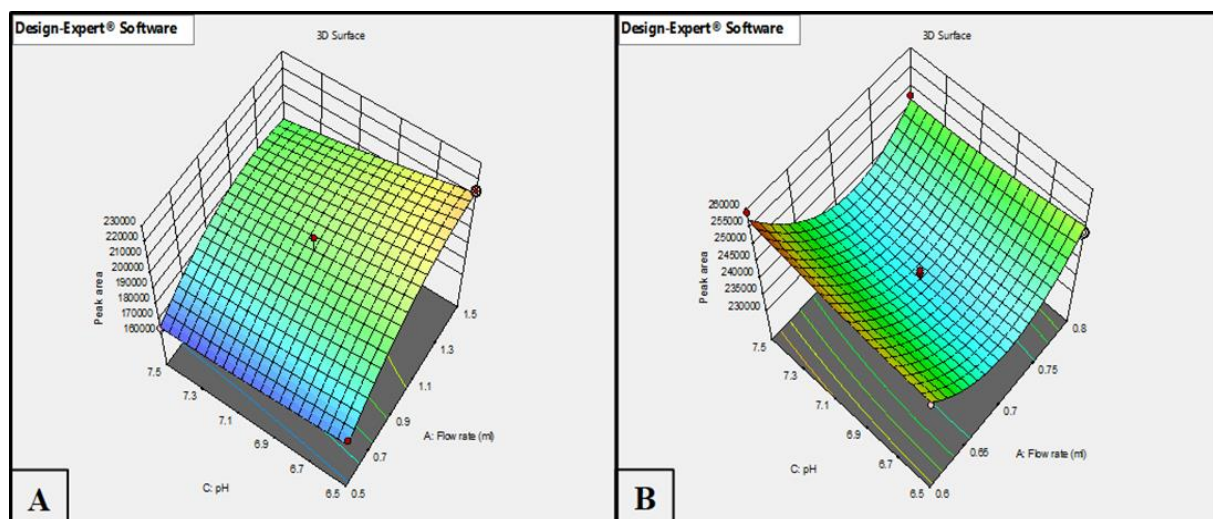


Fig. 3: Response surface plots illustrating effect of flow rate and pH on peak area using (A) HPLC method and (B) LC-MS/MS method

The model graph of tailing factor (fig. 4A for HPLC and fig. 4B for LC-MS/MS) demonstrates that an increased acetonitrile% with a lower pH resulted in higher tailing in both systems. This effect may be due to possible analyte overloading or interaction with

residual silanol groups in the column, more pronounced in HPLC. In LC-MS/MS, although the tailing was observed, the effect was slightly mitigated due to faster ion detection and enhanced sensitivity.

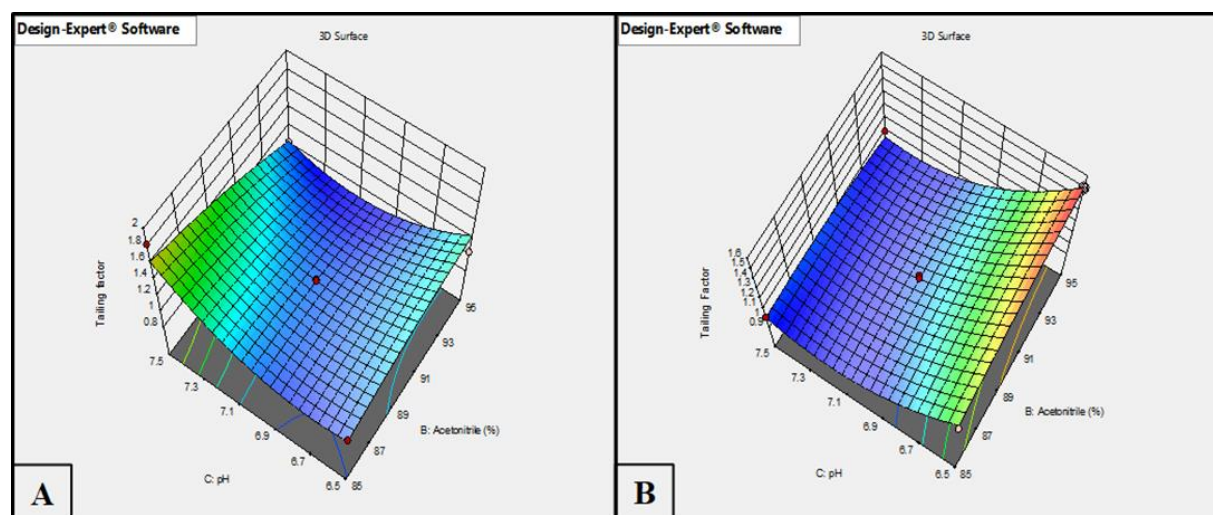


Fig. 4: Response surface plots illustrating the effect of acetonitrile % and pH on tailing factor using (A) HPLC method and (B) LC-MS/MS method

These observations affirm that careful modulation of flow rate, organic content, and pH is essential to achieve a robust and optimized analytical method. Although the trends observed during optimization were similar for both HPLC and LC-MS/MS, the enhanced sensitivity and ionization efficiency achieved via LC-MS/MS allowed for more precise adjustments based on peak area and retention behavior [32].

Method development for RAN HCl by HPLC

Preparation of stock solution

A precisely weighed amount of 10 mg of RAN HCl was transferred into a volumetric flask and dissolved in 10 ml of methanol to yield a primary stock solution with a concentration of 1 mg/ml. In order to produce a secondary solution of 100 µg/ml, 1 ml of the stock was diluted with 10 ml of methanol (Solution A). After diluting 1 ml of Solution A with 10 ml of methanol, a working standard solution of 10 µg/ml was obtained [33].

Chromatographic conditions

A Phenomenex C18 column (150 mm × 4.6 mm, 5 µm particle size) was used for chromatographic separation with a mobile phase of acetate buffer and acetonitrile in a 10:90 (v/v) ratio. The flow rate was kept constant at 1.0 ml/min, and the column temperature was fixed at 25 °C. A 20 µl sample volume was injected over an 8-minute timeframe.

The mobile phase was prepared by mixing 900 ml (90%) of HPLC-grade acetonitrile with 100 ml (10%) of the prepared acetate buffer. The mixture was degassed by ultrasonication for 15 min before use.

The typical chromatographic profile under optimized conditions is shown in fig. 5, illustrating the standard chromatogram of RAN HCl with well-resolved peaks and satisfactory symmetry. Similar optimization using QbD by Wang *et al.* in 2021 for Ranitidine also identified flow rate and pH as critical parameters influencing retention time and peak shape, supporting our DoE-based observations [34].

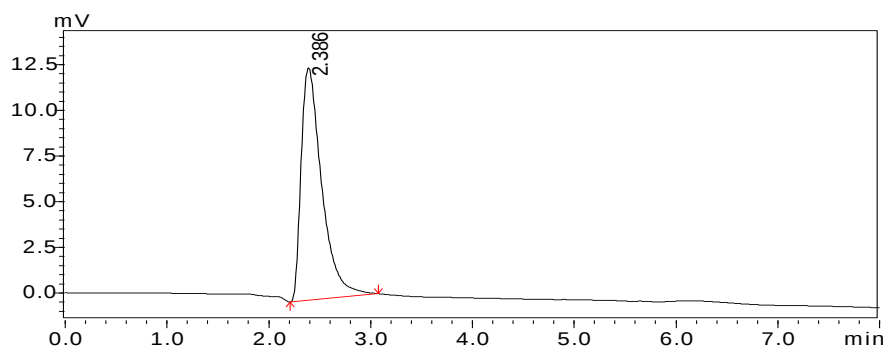


Fig. 5: Typical standard chromatogram of ranitidine hydrochloride (RAN HCl)

System suitability parameters

System suitability assessment is an important part of analytical method validation since it ensures that the chromatographic system is capable of performing accurate and reliable investigations. The optimised chromatographic settings were used to evaluate system compatibility metrics such as theoretical plates, tailing factor, LOD,

and LOQ (table 6). The computed results confirmed that the system satisfied the predefined acceptance criteria, ensuring the accuracy and reliability of the method for RAN HCl [26]. Similarly, Jain *et al.* in 2023 developed a stability-indicating HPLC method for ranitidine, reporting consistent peak response and retention time under stress conditions, which aligns with the robustness observed in our validated method [35].

Table 6: System suitability by HPLC

S. No.	Parameters	Observation	Limit
1	Theoretical Plate	6781	N>2000
2	Tailing Factor	1.02	T<2
3	LOD	1 µg/ml	
4	LOQ	3 µg/ml	

Linearity range

RAN HCl demonstrated linearity at 7 concentration levels (1-30 µg/ml). Calibration curves were generated by graphing the peak area (Y-axis) against the corresponding concentration (X-axis), and the R² value was 0.9985, demonstrating excellent linearity. The regression equation derived from the calibration curve was $y = 24,613x + 39,554$ (fig. 6). After injecting the sample into the

chromatographic apparatus, the peak areas for each component were determined. Linearity results are presented in table 7, confirming the method's suitability for accurate quantification of RAN HCl across the tested concentration range. In 2021, Kaur *et al.* established a validated HPLC method for ranitidine in pharmaceutical formulations, reporting a tailing factor under 1.2 and strong linearity ($R^2 = 0.9991$), which mirrors the performance achieved in our optimized HPLC method [36].

Table 7: Linearity table

S. No.	Concentration (µg/ml)	Area
1	1	18843
2	2	34687
3	4	68653
4	5	126306
5	10	243612
6	20	504224
7	30	735836

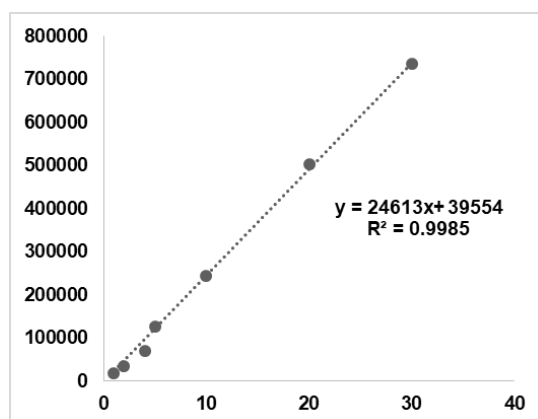


Fig. 6: Linearity plot

LOD and LOQ

The limit of detection (LOD) for RAN HCl was 1 µg/ml, with a signal-to-noise ratio of 3:1. The limit of quantification (LOQ) was set at 3 µg/ml, with a signal-to-noise ratio of 10:1. These values suggest that the developed technique has good sensitivity for reliable detection and quantification of the analyte [26].

Accuracy and precision

The method's accuracy and precision were examined at three levels of quality control: LQC, MQC, and HQC. The intraday accuracy was between 94.81% and 100.79%, with precision values (%RSD) ranging from 0.17% to 0.55%. Interday accuracy ranged between 93.24% and 99.40%, with percentage RSD values ranging from 0.17% to 0.56% (table 8). These results demonstrate that the approach is consistently accurate and precise over the measured concentration range, making it reliable for routine quantitative determination of RAN HCl [26].

Chromatography and tandem mass spectrometry

The RAN HCl was separated using an Atlantis C18 column (75 × 4.6 mm, 5 µm particle size) at room temperature. The mobile phase,

which was made up of 10 mmol ammonium acetate and acetonitrile in a 10:90 (v/v) ratio, was given at a constant flow rate of 0.7 ml/min under isocratic conditions. A 10 µl** sample volume was injected, with a run time of 4 min. RAN HCl eluted in 1.993 min, with distinct, symmetrical peaks suitable for quantitative analysis.

A precise LC-MS/MS approach was created for the quantification of RAN HCl, using optimised chromatographic and mass spectrometric conditions that lead to high sensitivity and selectivity. Negative ion mode using ESI was employed, and full scan spectra revealed prominent protonated molecular ions at m/z 313.05, 349.05, and 359.10 (fig. 7). The optimized MRM transitions selected for quantification were m/z 349.05 → 170.00 and 313.10 → 325 (fig. 8). These transitions were chosen based on sensitivity and signal stability. The molecular weight of RAN HCl is 350.86 g/mol, with [M+H]⁺ as the major adduct ion and 349.05 as the precursor ion. The developed method demonstrated excellent peak shape, high sensitivity, and a short run time, making it suitable for routine quantification using standard LC-MS/MS platforms [37]. In 2020, Zhang *et al.* validated a similar LC-MS/MS method for the simultaneous determination of ranitidine and NDMA in accordance with ICH M7 guidelines, which underscores the regulatory applicability of our validated approach [38].

Table 8: Accuracy studies

Sample (µg/ml)	Recovery (µg/ml)±SD	Recovery (%)	Intraday		Interday	
			Accuracy (%)	Precision (% RSD)	Accuracy (%)	Precision (% RSD)
LQC-2.5	2.3704± 0.004126	94.81	93.93	0.170	93.24	0.175
MQC-10	9.8425± 0.04447	98.42	97.65	0.550	97.31	0.562
HQC-20	20.1584± 0.09411	100.79	99.45	0.501	99.40	0.490

Value are expressed as mean±SD (n = 3)

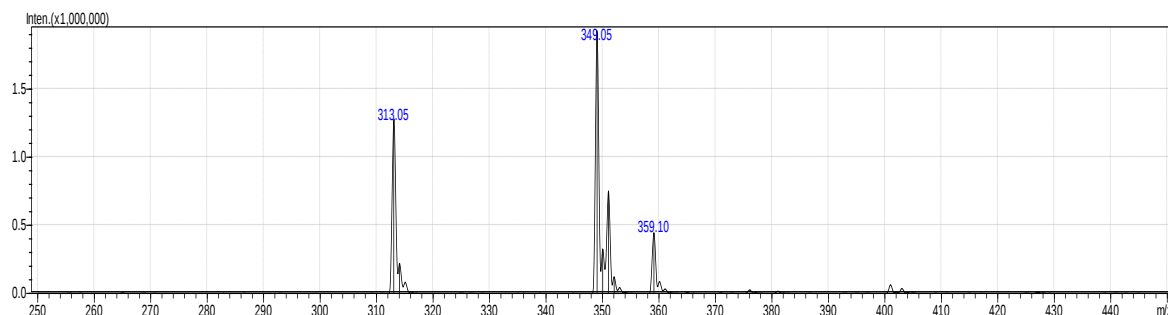


Fig. 7: Negative scan of ranitidine hydrochloride

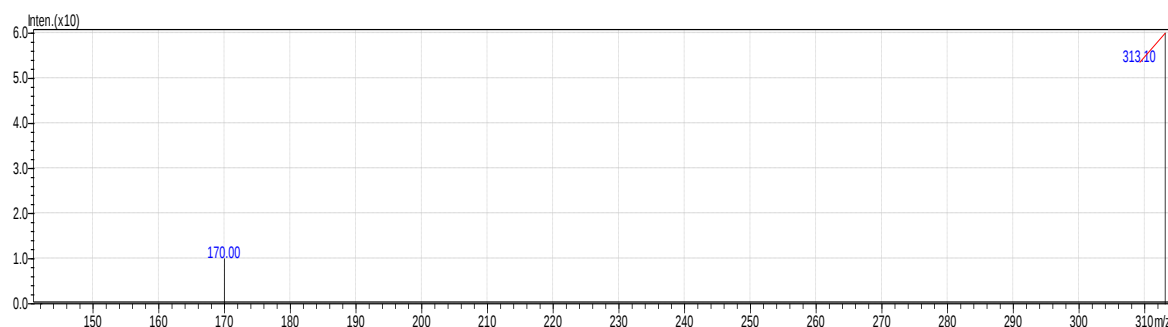


Fig. 8: MRM scan of ranitidine hydrochloride

Specificity

No interfering peaks were observed at the retention time of RAN HCl (1.993 min) in the chromatogram (fig. 9), confirming the method's selectivity and sensitivity, with no contribution from endogenous

components [39]. Similarly, in 2021, Liu *et al.* developed an LC-MS/MS method for NDMA determination in ranitidine, achieving an LOD of approximately 3 ng/ml, which closely aligns with the sensitivity obtained in our study using optimized MRM transitions [40].

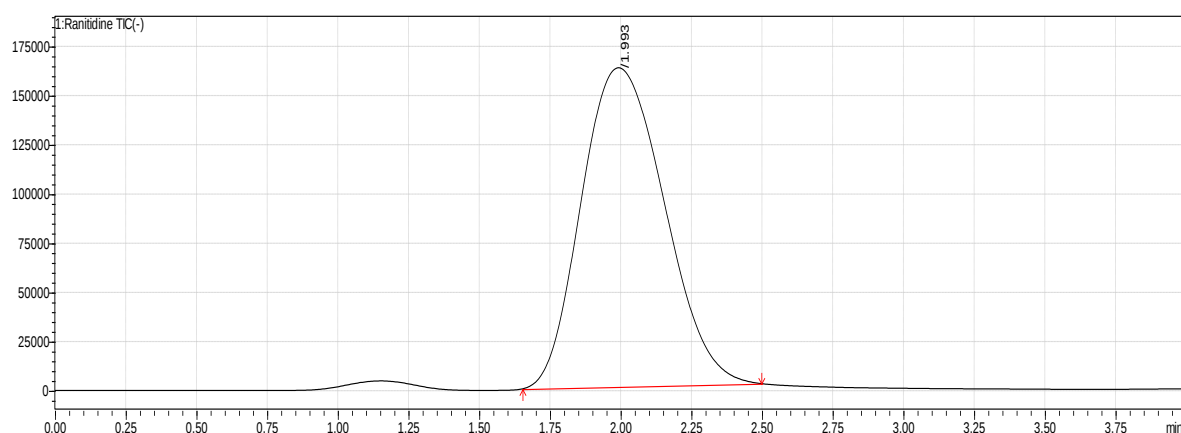


Fig. 9: Typical standard chromatogram of ranitidine hydrochloride

Linearity

Calibration curves for RAN HCl were constructed using 7 different conc. levels ranging from 5 to 250 ng/ml (table 9). A high degree of

linearity was observed for the method, as indicated by an R^2 value of 0.9998. The regression equation derived from the calibration curve was $Y = 1965.3x + 3253.3$, reflecting a clear and direct correlation between analyte concentration and peak area (fig. 10) [41].

Table 9: Linearity concentration ranges of ranitidine HCl

S. No.	Conc. (ng/ml)	Peak area
1	5	9843
2	30	54687
3	75	140653
4	125	240986
5	175	342906
6	200	390674
7	250	487620

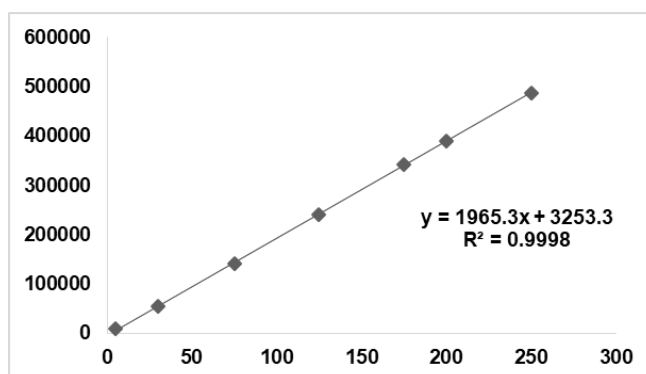


Fig. 10: Linearity plot for ranitidine

Accuracy and precision

Three quality control levels were used to measure accuracy: low (LQC), medium (MQC), and high (HQC) at concentrations of 5, 125 and 225

ng/ml, respectively. Accuracy was determined by computing the mean percentage recovery at each QC level, which ranged from 95.33% to 97.93%. Repeated study confirmed the method's reliability and reproducibility, with % RSD values ranging from 0.23% to 0.69% [42].

Table 10: Accuracy and precision table

Sample	Recovery (%)	Recovery (ng/ml)±SD	Intraday		Interday	
			Accuracy (%)	Precision (%RSD)	Accuracy (%)	Precision (%RSD)
LQC-5	95.33	4.766±0.033	94.86%	0.57	95.44%	1.80
MQC-125	97.07	121.346±0.285	97.04%	0.12	95.96%	0.04
HQC-225	97.93	220.354±0.562	98.04%	0.05	97.77%	0.13

Value are expressed as mean±SD (n = 3)

LOD and LOQ

The LOD for RAN HCl was established at 1 ng/ml, based on a signal-to-noise ratio of 3;1, while the LOQ was determined to be 3 ng/ml,

corresponding to a signal-to-ratio of 10:1. These values may vary depending on the reagents used and the purity of solvents, particularly when non-LC-MS/MS grade solvents are employed

which can affect the signal clarity. The low LOD and LOQ values indicate that the developed method exhibits high sensitivity, making it suitable for trace-level detection of RAN HCl [43].

System suitability

System suitability parameters were evaluated using a representative LC-MS/MS chromatogram of RAN HCl. The retention time was recorded at 1.993 min, with 6098 theoretical plates reflecting good column efficiency. The peak symmetry value of 1.37 indicated an acceptable peak shape. The % RSD for replicate RAN HCl injections was 0.87%, demonstrating excellent precision of the method.

Solution stability

The working calibration standards and internal standard solutions of RAN HCl were stable for up to 24 h under refrigerated conditions (2–8 °C), showing no significant changes in retention time or peak area. However, when stored at room temperature, both the RAN HCl and internal standard solutions showed stability only up to 12 h. Beyond this duration, noticeable changes in peak area and chromatographic response were observed, indicating degradation or loss of analyte integrity upon prolonged room temperature exposure [26].

CONCLUSION

RAN HCl is a widely prescribed H₂-receptor antagonist used in the treatment of gastric disorders. Due to safety concerns associated with nitrosamine impurities, particularly NDMA, there is a critical need for accurate and sensitive analytical methods for its quantification. In this study, both HPLC and LC-MS/MS methods were developed and validated as per ICH Q2(R1) guidelines to ensure precision, linearity, and robustness. The HPLC method showed optimal performance with 90% acetonitrile, 1.0 ml/min flow rate, and pH 7.0, resulting in a retention time of 2.3 min. The LC-MS/MS method, under similar mobile phase conditions but with a 0.7 ml/min flow rate, yielded a retention time of 1.99 min and exhibited excellent sensitivity and reproducibility.

The application of BBD within the QbD framework enabled efficient optimization of key parameters-flow rate, acetonitrile percentage, and pH. This systematic approach allowed the identification of a robust method operating design region, supported by ANOVA and model validation statistics. The developed method is suitable for routine pharmaceutical analysis and regulatory applications related to RAN HCl, providing a reliable platform for quality control and ensuring patient safety.

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AUTHORS CONTRIBUTIONS

All the authors contributed significantly to this manuscript. Debangana pal has conceptualized and writing the original draft of

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CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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