RESEARCH PAPER

Development and QbD-Based Optimisation of an RP-HPLC Method for Quantitative Estimation of Rotigotine

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ABSTRACT

An easy, accurate, and strong RP-HPLC technique was designed and tested on the quantitative determination of Rotigotine. A C18 column was subjected to isocratic conditions, and a mobile phase of water containing 0.1% triethylamine and acetonitrile (60:40 v/v) was used to separate the mixture chromatographically. A Quality by Design (QbD) method based on Central Composite Design (CCD) was used to optimise the method and measure the effects of flow rate and pH on retention time and tailing factor. The optimised conditions led to the retention time of about 7.54 minutes with a tailing factor of about 1.2. The procedure has been confirmed following ICH standards with a large linearity of 2-10 µg/mL ($R^2 = 0.9996$), high accuracy (99.58-101.66%), and good precision (percentages RSD = 2). The limit of detection and quantification was also established at 0.21 µg/mL and 0.65 µg/mL, respectively. The method developed was observed to be specific, sensitive, and reproducible, and therefore can be used in routine quality control analysis of Rotigotine.

Keywords: Rotigotine, QbD, RP-HPLC, Analytical Method Development, Method Validation

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1. Introduction

Rotigotine is a non-ergoline dopamine agonist that is commonly utilised in the treatment of nervous system disorders like Parkinson's and restless leg syndrome. It serves its pharmacological action by acting on dopamine receptors, thus imitating the endogenous dopamine of the central nervous system [1]. Due to its need to produce constant drug delivery and constant plasma concentrations, rotigotine has received significant interest in pharmaceutical research, especially in the process of conducting transdermal and controlled-release drug delivery systems [2]. In this regard, proper and standardised quantification of rotigotine is necessary in the formulation development, quality control, and stability determination. The development of analytical methods is crucial to the provision of safety, efficacy, and quality of pharmaceutical products [3]. Reverse-phase high-performance liquid chromatography (RP-HPLC) is the most commonly used of the mentioned techniques because of high sensitivity, accuracy, reproducibility and ability to perform routine analysis [4]. Nevertheless, the establishment of a powerful analytical technique involves a meticulous optimisation of chromatographic factors in terms of mobile phase composition, pH, flow rate and detection wavelength in order to reach sufficient resolution and sensitivity.

Several analytical methods have been reported for the estimation of Rotigotine. A study by Paramita Saha and Murali Monohar Pandey (2021) developed a DoE-based RP-HPLC method that demonstrated excellent chromatographic performance with a retention time of 7.65 minutes and a high theoretical plate count of 11206. The method showed strong linearity ($R^2 = 0.9995$) over a range of 25–600 ng/mL, with low LOD (9 ng/mL) and LOQ (12 ng/mL), and was successfully applied to nanocrystal-based studies, including dissolution and nasal permeation [5]. Similarly, Susan Mohamed, Roberto Riva, and Manuela Contin (2017) reported a highly sensitive UHPLC-MS/MS method with a very low quantification limit of 50 pg/mL and excellent recovery (96.9%), which was successfully applied for plasma analysis in clinical settings. [6]. Earlier, P. Geetha Swarupa *et al.* (2015) developed a stability-indicating RP-HPLC method with a short retention time of 2.691 minutes, demonstrating high accuracy (100.33% recovery) and precision (%RSD < 2%), making it suitable for routine quality control. [7].

Despite these innovations, there are still some shortcomings in the current methods. Most of the reported methods involve complicated experimental designs, expensive instrumentation such as UHPLC-MS/MS, or are designed to work with biological samples and more sophisticated formulations, which are not necessarily available in the standard quality control lab. Some techniques have longer run times, increased solvent usage, or are not simple in regard to method conditions and routine analysis reproducibility. The identified factors indicate the necessity of a straightforward, affordable, fast and efficient RP-HPLC procedure that can be easily adopted in order to conduct a regular analysis of pharmaceuticals [8]. Moreover, it is necessary to validate analytical methods according to the International Council of Harmonisation (ICH) guidelines Q2(R2) to provide reliability, accuracy, and reproducibility [9]. The most commonly used validation parameters are specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), which have been used to verify the appropriateness of the method for the purpose applied [10]. The maximum absorption wavelength (λ_{max}) calculated with the help of UV-visible spectroscopy is also essential to reach maximum sensitivity [11]. Moreover, the implementation of the Quality by Design (QbD) principles will improve the robustness of the methods as the impact of important analytical parameters is assessed in a systematic manner.

In the present study, an attempt has been made to develop and validate a simple, precise, accurate, and cost-effective RP-HPLC method for the quantitative estimation of Rotigotine. The proposed method offers advantages in terms of simplicity of mobile phase, optimised chromatographic conditions, and suitability for routine analysis without the need for advanced instrumentation. The method was validated according to ICH guidelines and is intended to serve as a reliable analytical tool for quality control and pharmaceutical evaluation of Rotigotine in bulk and dosage forms.

2. Materials and Methods

2.1 Materials

Rotigotine was received as a gift sample. Merck (India) purchased HPLC-grade acetonitrile and triethylamine (TEA). Potassium dihydrogen phosphate and orthophosphoric acid were bought from SD Fine Chemicals (India) of analytical grade. A Milli-Q purification system was used to prepare the HPLC-grade water. All the chemicals and reagents involved in the study were of analytical or HPLC grade and used in their original form. The membrane filters (0.45 µm) were applied to both the filtration of the mobile phase, and samples were bought from Millipore.

2.2 Determination of λ_{max} using UV Spectroscopy

The UV-vis spectrophotometry was used to determine the maximum absorption wavelength (λ_{max}) of Rotigotine. First, the primary stock solution (1000 ppm) was made by weighing 10 mg of Rotigotine accurately and transferring it into a 10 mL volumetric flask. This was dissolved in a proper solvent, and the volume was adjusted to the mark to get a concentration of 1000 ppm (1000 ug/mL). Based on this stock solution, a secondary stock solution of 100 ppm was obtained by adding the right aliquot to a volumetric flask and subsequently diluting it using the same solvent. Further dilution was done to achieve a 10 ppm working solution. The spectral analysis was performed on the 10 ppm solution to make sure that it had the right absorbance in the range of the instrument that could be detected. The working solution that was prepared was scanned in the UV-visible spectrophotometer within the wavelength range of 200-400 nm against the relevant blank solvent. The wavelength with the highest absorbance was noted as the λmax of Rotigotine and used to further analytical and chromatographic analysis [12, 13].

2.3 Chromatographic Conditions

The quantification of DMB was carried out using an HPLC system (Shimadzu, Japan) equipped with a photodiode array (PDA) detector. Chromatographic separation was achieved on a C-18 column (250 mm × 4.6 mm i.d., 5 µm; Chemsil, India). The HPLC system was operated and controlled using LabSolutions software (version 6.124) for data acquisition, integration, and analysis. The mobile phase was filtered through a 0.45 µm membrane filter and degassed before use. The flow rate was maintained at 0.8 mL/min, and the injection volume was set at 20 µL. The column oven temperature was maintained at 25°C throughout the analysis. Detection was carried out at recorded λ_{max} using the PDA detector. The total run time for each chromatographic analysis was fixed at 10 minutes to ensure complete elution of analytes with adequate resolution.

2.4 Optimisation by QbD

A Quality by Design (QbD) approach was used to optimise the RP-HPLC method to estimate Rotigotine in order to achieve robustness, reliability, and consistent performance in the analysis [14]. To achieve an acceptable retention time, good peak symmetry, and acceptable column efficiency, an Analytical Target Profile (ATP) was originally defined. According to the preliminary experiments, two critical method variables

(CMVs), i.e. flow rate (A) and pH of the buffer (B), have been chosen based on their important effect on chromatographic behaviour. The variables were experimented on three levels, which consisted of the flow rate of 0.6, 0.8, and 1.0 mL/min and pH of 4.5, 5.0 and 5.5. A Central Composite Design (CCD) was used to determine the effect of these variables and interactions on the chosen responses, which are retention time (Rt) and tailing factor (TF). The overall 13 experimental runs were carried out according to the design matrix, and the data obtained were analysed with statistical programs to produce quadratic polynomial equations and response surface plots (Table 1). The analysis of variance (ANOVA) was used to determine whether the model and individual factors were significant or not. It was concluded on the basis of desirability criteria that the optimum conditions were attained with minimum retention time and acceptable tailing factor, and a design space was defined that would help verify the robustness of the method under small changes in the experimental conditions [15].

Table 1: Central Composite Design (CCD) experimental runs showing the effect of flow rate (A) and pH (B) on retention time and tailing factor of Rotigotine.

Run	Flow Rate (mL/min)	pH	Retention Time (min)	Tailing Factor
1	0.8	5.5	7.30	1.28
2	0.8	5.0	7.54	1.20
3	0.6	4.5	8.05	1.38
4	1.0	5.5	6.65	1.30
5	0.6	5.0	7.90	1.32
6	0.8	5.0	7.55	1.21
7	0.8	5.0	7.53	1.19
8	0.8	4.5	7.75	1.30
9	1.0	4.5	6.90	1.28
10	1.0	5.0	6.70	1.25
11	0.8	5.0	7.54	1.20
12	0.8	5.0	7.55	1.22
13	0.6	5.5	7.70	1.35

2.5 Analytical Method Development

The separation was done by chromatography in an isocratic mode utilising a mobile phase made up of water with 0.1% TEA and acetonitrile at a proportion of 60:40 (v/v). Optimisation of the method conditions was then further optimised with a QbD method in which the critical method variables, like flow rate and pH of the aqueous phase, were analytically tested using a CCD. These variables were investigated to have a positive impact on the important responses of the chromatographic method, such as retention time and tailing factor, to obtain an optimal separation. The overall conditions under which the final method was to be used were determined based on the QbD optimisation to provide sufficient resolution, sufficient symmetry of the peaks, and sufficient analytical performance.

2.6 Analytical Method Validation

The developed RP-HPLC method for the quantitative estimation of Rotigotine was validated in accordance with the International Council for Harmonisation (ICH) Q2(R2) guidelines to ensure reliability, accuracy, and reproducibility of the method. The validation parameters evaluated included system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) [16].

2.6 1 System Suitability

Analysis was conducted after system suitability testing to ensure that the performance and suitability of the chromatographic system were good. Optimised chromatographic conditions were used to inject a standard solution of Rotigotine six times. Parameters that were determined included retention time, theoretical plate count, tailing factor and percentage relative standard deviation (%RSD) of the peak area. When the percentage of relative standard deviation of peak area was less than 2%, the tailing factor was acceptable and adequate theoretical plates were obtained. The chromatographic system was deemed to be suitable and indicated a good column efficiency and reproducibility.

2.6.2 Specificity

Specificity of the method was determined by injecting blank solution, standard solution and sample solution to test possible interference at the retention time of Rotigotine. The chromatograms were precise in ensuring the non-occurrence of interfering peaks that could result from solvents. The procedure was deemed selective when no significant interference was found at the retention time of Rotigotine, showing selective analysis of the analyte.

2.6.3 Linearity

Linearity of the method was determined by making standard solutions of Rotigotine over an appropriate

concentration range. All concentrations were injected 3 times, and the calibration curve was plotted between the area of the peak and the concentration. Least squares were used to obtain the regression equation and the correlation coefficient (R^2). The procedure was regarded as linear in case the correlation coefficient was established to be over 0.99, which demonstrated a linear and direct dependence between concentration and detector response [17].

2.6.4 Accuracy

The accuracy of the method was assessed by carrying out recovery studies at three levels, which are usually 80%, 100%, and 120% of the nominal concentration, which is the standard addition method. Sample solutions that had been analysed were then added a known amount of Rotigotine standard, and the percentage recovery was determined. It was deemed to be accurate where the recovery values fell within the acceptable range of 98-102%, indicating that the method can be used to analyse quantitatively [18].

2.6.5 Precision

The developed method was tested for precision, which was determined as repeatability (precision within a day) and intermediate precision (precision in between days and between analysts). Intraday accuracy was measured by comparing various replicates of different concentrations of Rotigotine on the same day, whereas interday accuracy was measured on different days under the same experimental conditions. By-analyst accuracy was established where different analysts analysed using identical parameters of instrumentation and method. The percentage root-square deviation of the peak areas was estimated, and a value below 2% was considered reasonable accuracy and reproducibility of the procedure [19].

2.6.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and limit of quantification were used to determine the sensitivity of the method. The standard deviation of the response (σ) and the slope (S) of the calibration curve were used to estimate LOD and LOQ as LOD = 3.3 σ/S and LOQ = 10 σ/S. These values reflected the lowest possible concentration of Rotigotine that could be determined and measured with an acceptable degree of precision and accuracy.

3. Results and Discussion

3.1 Determination of λ_{max} using UV Spectroscopy

The UV spectrum of Rotigotine showed a distinct and sharp absorption peak at 234 nm within the scanned range of 200–400 nm. This wavelength was identified as the maximum absorption wavelength (λ_{max}) of the drug. The prominent peak at 234 nm indicates strong absorbance and good sensitivity at this wavelength. Therefore, 234 nm was selected for further analytical and quantitative studies of Rotigotine.

3.2 Optimisation by Central Composite Design

The RP-HPLC method was optimised using a QbD approach with a Central Composite Design (CCD) to study the effect of flow rate (A) and pH (B) on retention time and tailing factor. A quadratic model was found to be most suitable for both responses. For retention time, the model was highly significant ($p < 0.0001$) with excellent fit ($R^2 = 0.9985$). Both flow rate and pH significantly influenced the response, while the interaction term was not significant. Quadratic terms indicated curvature in the response surface. Despite a significant lack of fit, the model showed good predictability. The quadratic equation obtained was:

RT: $7.54793 + -0.571667 * A + -0.135 * B + 0.015 * AB + -0.267759 * A^2 + 0.0622414 * B^2$

For the tailing factor, the quadratic model was also significant ($p = 0.0001$) with good agreement between R^2 (0.9582), adjusted R^2 (0.9283), and predicted R^2 (0.7698). Flow rate was significant, while pH and interaction were not. Quadratic terms were significant, and lack of fit was non-significant, confirming model adequacy. The equation obtained was:

TF: $1.21 + -0.0366667 * A + -0.005 * B + 0.0125 * AB + 0.06 * A^2 + 0.065 * B^2$

3.3 Response Surface and Optimisation Analysis

The 3D surface plots illustrate the effect of flow rate (A) and pH (B) on chromatographic responses. In Figure 1A, retention time decreases with an increase in flow rate, while pH shows a moderate influence, confirming that flow rate is the dominant factor affecting retention behaviour. Figure 1B represents the effect on the tailing factor, where optimal peak symmetry is observed at moderate levels of both flow rate and pH, while extreme conditions result in increased tailing due to peak distortion. The overlay plot (Figure 1C) demonstrates the design space, highlighting the region where both responses meet the desired criteria. The yellow region indicates the optimised zone, where retention time and tailing factor are within acceptable limits. The optimised condition was found at a flow rate of approximately 0.8 mL/min and pH around 5.0, yielding a retention time of about 7.54 minutes and a tailing factor close to 1.2. These plots confirm the reliability of the QbD approach and the suitability of the selected conditions for achieving optimal chromatographic performance.

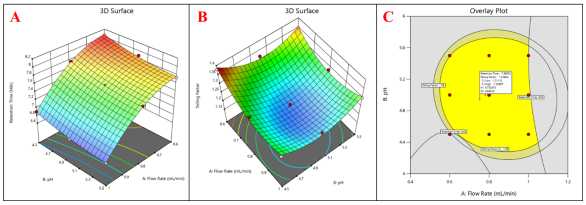

Figure 1: (A) 3D response surface plot showing the effect of flow rate (A) and pH (B) on retention time of Rotigotine. (B) 3D response surface plot illustrating the influence of flow rate and pH on the tailing factor. (C) Overlay plot indicating the design space and optimised region, highlighting the combination of variables that yield acceptable retention time and tailing factor.

3.4 Analytical Method Development

The developed RP-HPLC method showed satisfactory chromatographic performance. The standard solution of Rotigotine produced a sharp and well-defined peak. The retention time was observed at approximately 7.5 minutes. The peak area was found to be 3,476,160, indicating good detector response (Figure 2A). The chromatogram showed a stable baseline. No interfering peaks were observed at the retention time of the drug. The peak was symmetrical and well resolved. The retention time remained consistent during repeated injections. These results confirm the specificity and reliability of the method. Therefore, the developed method is suitable for routine quantitative analysis of Rotigotine.

3.5 Analytical Method Validation

3.5.1 System suitability

System suitability parameters were evaluated before sample analysis. The TF was found to be 1.2, indicating acceptable peak symmetry. The theoretical plate count was observed to be more than 2000, demonstrating good column efficiency. The retention time was consistent during replicate injections. The %RSD of peak area was within acceptable limits. These results confirm that the chromatographic system was suitable for analysis and complied with standard analytical requirements.

3.5.2 Linearity

The linearity of the developed RP-HPLC method was established over the concentration range of 2–10 µg/mL. The mean peak area increased proportionally with increasing concentration, indicating a direct relationship between analyte concentration and detector response. The calibration curve showed good linear regression with the equation y = 24937x + 127534 and a correlation coefficient (R^2) of 0.9996, demonstrating excellent linearity of the method (Figure 2B).

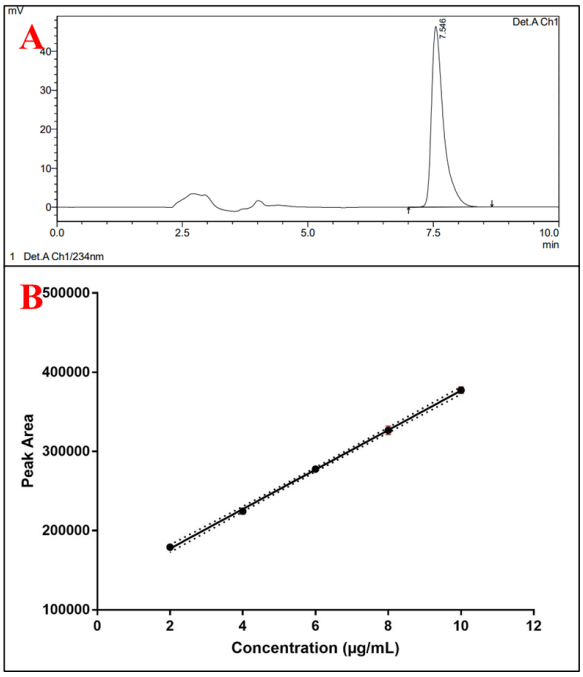

Figure 2: (A) Representative RP-HPLC chromatogram of Rotigotine showing a sharp and well-resolved peak at a retention time of approximately 7.5 minutes. (B) Calibration curve of Rotigotine depicting linearity between peak area and concentration over the studied range.

3.5.3 Accuracy

The recovery study results demonstrated that the developed RP-HPLC method is accurate for the estimation of Rotigotine. The percentage recovery values at 4.8, 6, and 7.2 µg/mL were found to be 99.58%, 100.49%, and 101.66%, respectively. The recovery values were within the acceptable limit of 98–102%, indicating good accuracy of the method. The results also showed consistent response at all concentration levels with minimal variation (Table 2). These findings confirm that the method is reliable and suitable for the quantitative determination of Rotigotine.

Table 2: Accuracy study of Rotigotine by recovery method at different concentration levels showing peak area values, average response, calculated concentration, and percentage recovery.

Concentration	PA 1	PA 2	PA 3	Average	Concentration	% Recovery
4.8	2493 74	2482 74	2425 44	24673 0.67	4.78	99.58
6	2740 21	2793 72	2802 53	27788 2.00	6.03	100.49

7.2	3103 63	3093 52	3104 73	31006 2.67	7.32	101.66

3.5.4 Precision

The precision of the developed RP-HPLC method for Rotigotine was evaluated in terms of interday, intraday, and inter-analyst studies at three concentration levels (4.8, 6, and 7.2 µg/mL). For interday precision, the %RSD values were found to be 1.49%, 1.21%, and 0.20%, respectively. Intraday precision showed %RSD values of 0.89%, 1.16%, and 0.74% for the respective concentrations. Similarly, inter-analyst precision demonstrated %RSD values of 0.79%, 1.22%, and 0.89%. All %RSD values were below 2%, indicating good repeatability and intermediate precision of the method. The low variability in peak areas confirms the reproducibility and reliability of the developed method for routine quantitative analysis of Rotigotine (Table 3).

Table 3: Precision study of Rotigotine showing inter-day, intra-day, and inter-analyst variability at different concentration levels, including peak area, mean, standard deviation, and %RSD values.

Parameters	Concentration	PA1	PA2	PA3	Average	STDEV	%RSD
Inter-day	4.8	2493 74	2482 74	2425 44	24673 0.67	3667. 24	1.49
	6	2740 21	2793 72	2802 53	27788 2.00	3372. 61	1.21
	7.2	3103 63	3093 52	3104 73	31006 2.67	617.9 1	0.20
Intra-day	4.8	2471 20	2508 65	2469 80	24832 1.67	2203. 70	0.89
	6	2754 20	2818 65	2781 40	27847 5.00	3235. 53	1.16
	7.2	3084 50	3129 65	3098 70	31042 8.33	2308. 70	0.74
Inter-Analyst	4.8	2459 80	2498 74	2482 65	24803 9.67	1956. 76	0.79
	6	2742 10	2809 65	2775 40	27757 1.67	3377. 61	1.22
	7.2	3079 85	3134 20	3098 75	31042 6.67	2759. 18	0.89

3.5.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the developed RP-HPLC method was evaluated by determining the LOD and LOQ. The LOD was found to be 0.21 µg/mL, indicating that the method is capable of detecting very low concentrations of Rotigotine. The LOQ was determined to be 0.65 µg/mL, demonstrating that the method can accurately and precisely quantify the drug at low concentration levels. The low LOD and LOQ values confirm the high sensitivity of the method and establish its suitability for routine analytical as well as bioanalytical estimation of Rotigotine.

4. Conclusion

A reliable and efficient RP-HPLC method for the estimation of Rotigotine was successfully developed and validated using a QbD approach. The application of Central Composite Design enabled systematic optimisation of critical parameters, ensuring robust chromatographic performance. The method exhibited excellent linearity, accuracy, precision, and sensitivity in accordance with ICH guidelines. The optimised conditions provided a well-resolved peak with acceptable retention time and tailing factor. Overall, the developed method is simple, cost-effective, and suitable for routine quality control and pharmaceutical analysis of Rotigotine in bulk and dosage forms.

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