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RESEARCH ARTICLE

Analytical Quality by Design (AQbD) Approach for the Development of a Robust RP-HPLC Method for Metformin and Nateglinide in Pharmaceutical Formulations

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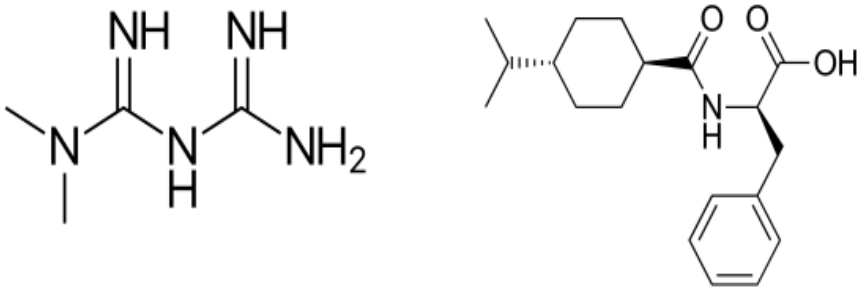
Abstract: ***Background***: ***Objective:*** As part of this study, the RP-HPLC method for measuring metformin hydrochloride and nateglinide at the same time was created. ***Methodology:*** The specificity, accuracy, precision, linearity, LOD, LOQ, sturdiness, and ruggedness of the new method were all checked to make sure they met ICH guidelines. The intraday and interday accuracy results were good enough to show that the suggested method worked and could be used again. The assay trial showed that the estimated amounts of metformin HCl and nateglinide in the tablet dosage form were not changed by interference from other substances. ***Results:*** This shows that the established method works. The suggested method worked because the recovery of normal drugs that were added ranged from 100.07 to 100.82% for metformin HCl and from 101.0 to 102.22% for nateglinide. Between 10 and 80 PPM of metformin HCl and 10 to 80 PPM of nateglinide, a good linear relationship was found. It was found that metformin HCl and nateglinide had correlation values of 0.9991 and 0.9997, respectively. The LODs for metformin HCl were 0.710 PPM, and for nateglinide they were 0.380 PPM. The LOQ for nateglinide was found to be 1.16 PPM, and for metformin HCl it was found to be 2.15 PPM. ***Conclusion:*** The results proved that the established RP-HPLC method was straightforward, linear, accurate, robust, and durable, and that it could be easily used for routine quality control analysis of both nateglinide and metformin HCl concurrently, from both the pharmaceutical formulation and the bulk drug.

Keywords: RP-HPLC, Metformin, Nateglinide, Method development, Validation, ICH.

INTRODUCTION

Making reliable analytical methods is one of the most important steps in making sure the quality of medicinal formulations [1]. High-performance liquid chromatography (HPLC) has become one of the most popular and powerful ways to analyze drugs, both qualitatively and quantitatively [2]. This is because it has great clarity, accuracy, precision, and repeatability. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is the method of choice because it can be used to analyze a wide range of chemicals with different polarities [3, 4]. Metformin hydrochloride and nateglinide are two diabetes medicines that are often given together to better treat Type 2 Diabetes Mellitus (T2DM). Nateglinide, which is a D-phenylalanine derivative, helps pancreatic β-cells release insulin. Metformin, which is a biguanide derivative, generally slows down the production of glucose in the liver and makes insulin work better [5-7]. When used in clinical practice, combination treatment is often chosen because it helps reduce both fasting and post-meal blood glucose levels. In the pharmaceutical business, it is very important to make sure that these combination formulations are safe, effective, and of good quality. It is critical to come up with an easy, quick, precise, and accurate RP-HPLC method for measuring metformin and nateglinide at the same time [8-10]. A validated analytical method not only ensures the quality of the formulation, but it also makes routine analysis,

stability studies, and batch-to-batch consistency review easier. Method validation is a very important step in making sure that the suggested analytical procedure can be used as planned, as explained by the International Council for Harmonization (ICH) (ICH Q2(R1)) [11-13]. To make sure the method works every time, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), stability, and system adaptability are all carefully checked. The purpose of this work is to create and test a new RP-HPLC method for measuring nateglinide and metformin in pharmacy dosage forms at the same time. The suggested method can be used for regular quality control checks in drug labs because it is simple, cheap, accurate, and easy to do again and again [14, 15].



Metformin **Nateglinide**

Figure 1: Nateglinide and Metformin

MATERIALS AND METHODS

Aspen Biopharma Labs Private Limited, Hyderabad, provided a gift sample of pharmaceutical-grade metformin HCI, while Beeman Pharmaceuticals, Mumbai, provided a gift sample of nateglinide. We used Natigone-M, a pharmaceutical dosage form from Care Formulation Lab in India, in this investigation. We purchased them from the local market. Merck provides acetonitrile and HPLC-grade water, while Research Lab Fine Chem. Industries in Mumbai provides orthophosphoric acid and potassium dihydrogen orthophosphate. Every solvent utilized in this project is of HPLC quality. Utilized was the RP-HPLC System Lab Corporation, Japan (LC-P-4000). The analytical column Kromasil C-18 is utilized to separate the analytes.

METHODS

Selection of wavelength: A UV spectrophotometer was used to scan a standard solution of metformin HCl and nateglinide at a concentration of 10 PPM between 200 and 400 nm. The UV spectra of nateglinide (Figure 3) and metformin HCl (Figure 2) were displayed below. 216 nm was chosen as the isobestic point for simultaneous estimate (figure 4) [16, 17].

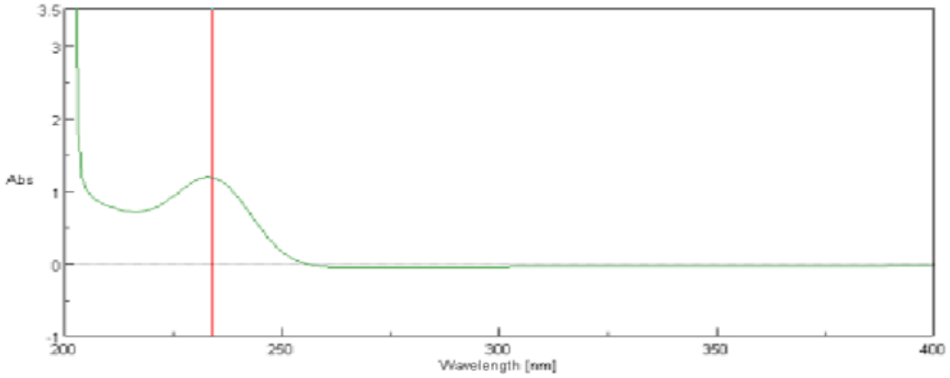


Figure 2: UV Spectrum for Metformin HCl

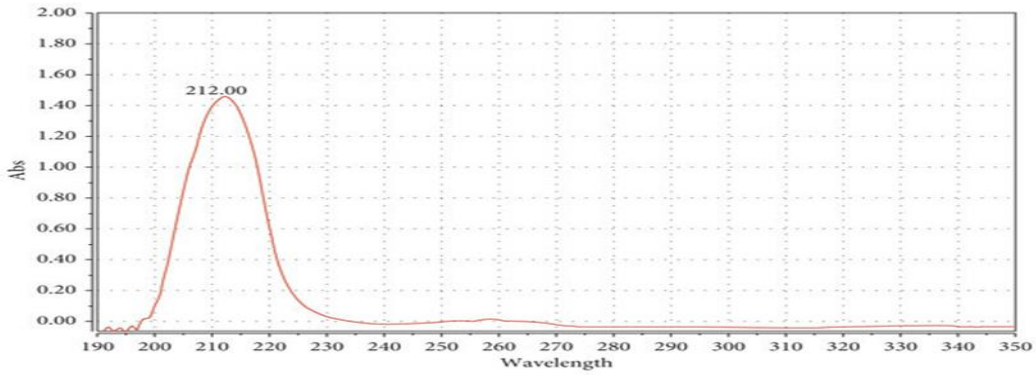


Figure 3: UV Spectrum for Nateglinide

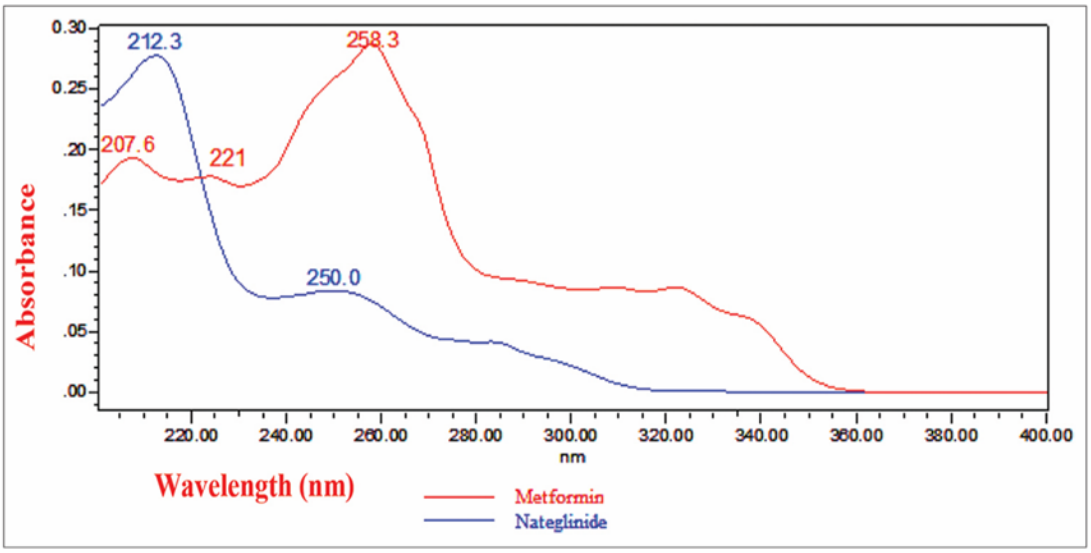


Figure 4: UV Spectrum Metformin HCl and Nateglinide Combination for isobestic point

Chromatographic conditions:
The developed method employed a reverse phase Kromasil C18 column, a mobile phase of potassium dihydrogen phosphate: acetonitrile (30:70) with orthophosphoric acid to adjust the pH, a flow rate of 1.1 ml/min, an ambient temperature, a run time of 7 min, and a UV detector with a detection wavelength of 216 nm [18, 19].

Preparation of Mobile Phase:
Potassium dihydrogen orthophosphate was weighed out exactly and dissolved in 200 milliliters of water. It was then sonicated for 15 minutes, and 200 milliliters of HPLC water were added to thin it out. Next, lower the pH with orthophosphoric acid and filter the mixture through a 0.45 µm filter to make a buffer. Finally, the best mobile phase mix is 30 volumes of buffer and 70 volumes of acetonitrile [20, 21].

Standard Stock Solution Preparation:
About 500 mg of Metformin HCI and 60 mg of Nateglinide were carefully weighed, moved to a different 100 ml volumetric flask, dissolved in the mobile phase, and diluted to a volume with the same solvent mixture. This made a stock solution with 5000 PPM of Metformin HCI and 600 µl/ml of Nateglinide. A 10 ml volumetric flask was filled with 1 ml of the solution in question, and the amount was changed with diluents. The amount of nateglinide in the body is 60 PPM, and the amount of metformin HCI is 500 PPM [22, 23].

Preparation of Sample Solution
Twenty tablets weighing 500 mg of metformin HCI and 60 mg of nateglinide each were weighed out and then crushed into a fine powder in a mortar. The powder was then mixed equally and put into a 100 ml volumetric flask, where it was mixed with 70 ml of mobile phase. The mobile phase was added to the solution until it reached a total volume of 100 ml after 30 minutes of sonication. The amount of metformin HCI used was 500 PPM, and the amount of nateglinide used was 60 PPM. A Whatman filter paper was used to filter the blend in the end [24].

RESULT AND DISCUSSION:

Method Development
To improve separation and resolution, various chromatographic settings were used. The Kromasil C18 column was deemed adequate. A UV detector was used to assess the peak purity of metformin HCI and nateglinide, and 216 nm was deemed sufficient for both medications' detection with sufficient sensitivity. Several solvents with varying ratios throughout a broad pH range were attempted; however, the resolution was poor or the peak shape was broad. Satisfactory results were obtained from repeated trials on a C18 column to get a good, crisp peak with an efficient resolution between two peaks of nateglinide and metformin HCI. With a mobile phase that contained phosphate buffer (pH 3.0): acetonitrile (30:70), a run speed of 1.1 ml/min, and a detection wavelength of 216 nm, the isocratic trial produced adequate results in terms of retention time, resolution, and sensitivity. Figures 5 and 6 display the atypical RP-HPLC chromatograph for metformin HCI and nateglinide in both standard preparation and pharmaceutical formulation.

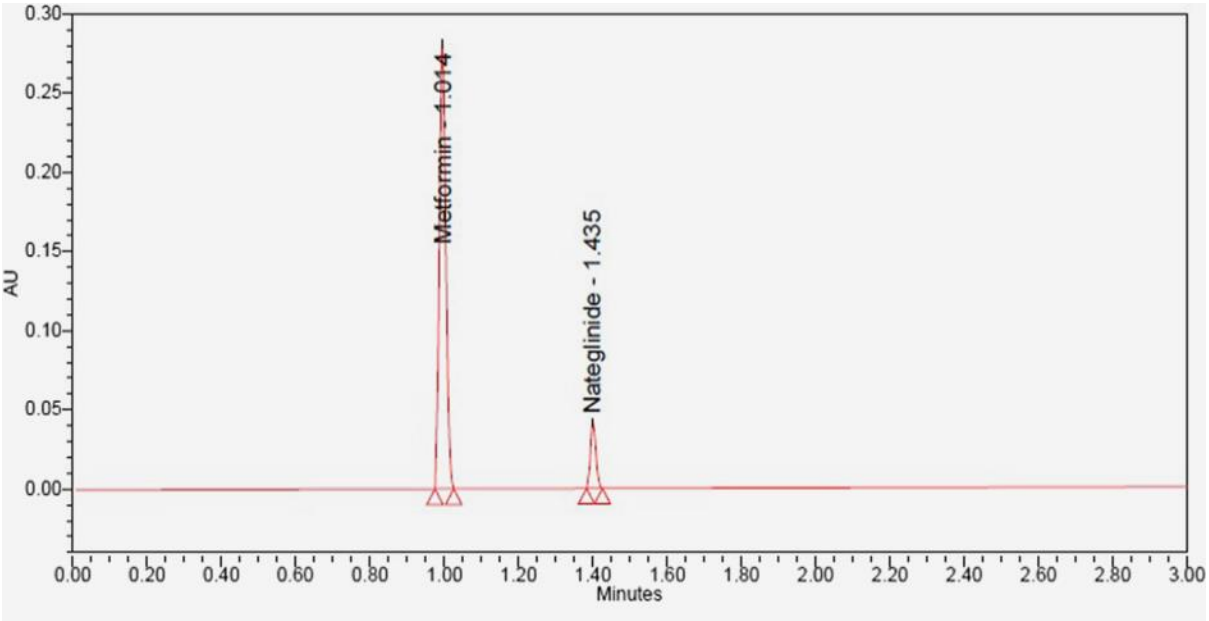


Figure 5: Chromatograph for standard solution of Metformin HCI andNateglinide

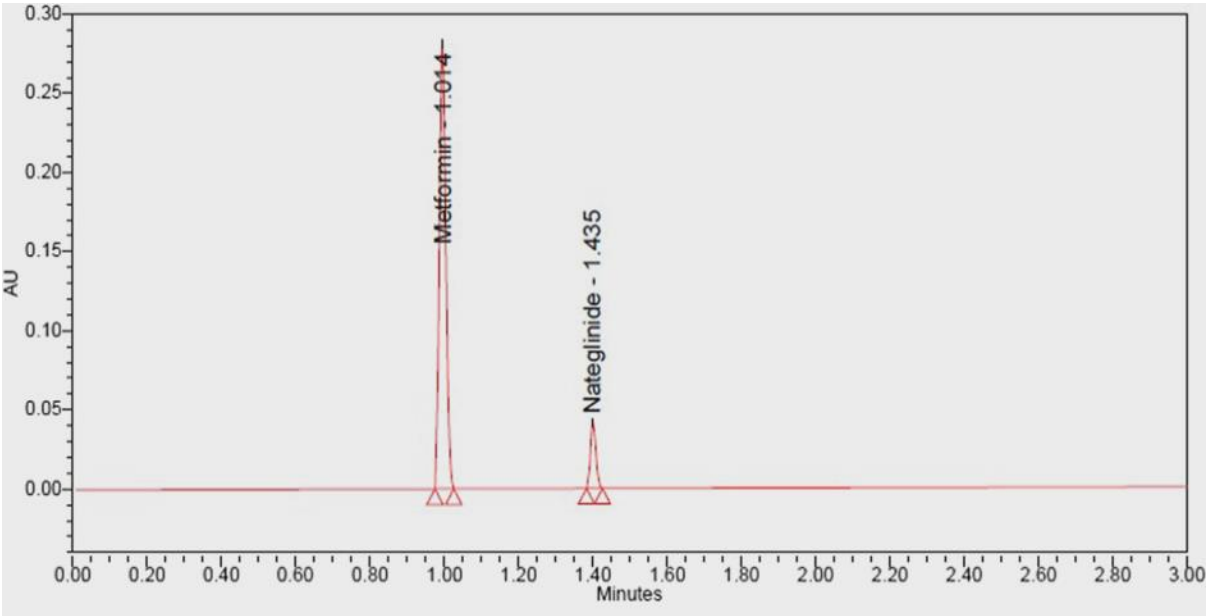


Figure 6: Chromatograph for sample solution of Metformin HCI and Nateglinide

Method validation

Validation of the developed RP-HPLC technology was carried out in accordance with the recommendations of the International Council for Harmonization (ICH). The criteria that were validated included accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ), and system sustainability.

System Suitability

In accordance with the procedure, standard solutions were made and added to the chromatographic apparatus. Asymmetric factor, resolution, and theoretical plates were among the system appropriateness parameters that were assessed. It was in Table 1 that the system suitability characteristics were detailed. All of the parameters were found to be within the acceptable range, as was discovered.

Table 1: System suitability parameter

Sr. No	Parameters	Acceptance limit	Metformin HCl	Nateglinide
1	Retention Time (min)	-	2.37	4.20
2	Resolution	NLT2	2.5	4.1
3	Theoretical Plates	NLT2000	5850	5380
4	Tailing Factor	NLT2	1.05	1.11

Precision

Three estimates of the corresponding responses were made to conduct the intraday and interday precision study of metformin HCI and nateglinide. The results are shown in table 2 as relative standard deviation.

Table 2: Intraday and Interday variability for Metformin HCI and Nateglinide

Sr. No	Drug	Intraday recision				Interday Precision			
		Trial	Area	SD	RSD	Trial	Area	SD	RSD
1	Metformin HCI	1	2952362.91	0.2484	0.24	1	2952805.43	0.0503	0.27
		2	2952619.20			2	2954280.86		
		3	2964876.23			3	2955920.57		
2	Nateglinide	1	151074.91	2.135	2.00	1	149355.43	1.770	1.78
		2	151168.00			2	144287.37		
		3	144037.54			3	149289.37		

Linearity

There was a linear relationship between peak area and concentration, as demonstrated by the calibration curves, for metformin HCI in the range of 10 PPM and for nateglinide in the range of 10–80 PPM that was used. Metformin HCI and nateglinide had regression coefficients (r^2) of 0.998 and 0.996, respectively, which maintained a strong association near unity. Plotting the concentration vs. the average area graph revealed a straight line going through the hall locations. Therefore, the suggested RP-HPLC technique for determining metformin HCI and nateglinide was shown to be linear in accordance with ICH requirements.

Accuracy

The standard deviation had to be less than 2.0% to be accepted. With the placebo spiking approach, accuracy was assessed with conventional medications at three distinct concentration levels (multilevel recovery). It is demonstrated in Table 3 that the approach that was provided is accurate for the simultaneous quantification of metformin HCI and nateglinide from their combination medicinal product in the presence of their degradation products and excipients. The value of the relative standard deviation (RSD) was less than 2%, and the recovery of standard pharmaceuticals that were added was 100.07-100.86% for metformin HCI and 101.00-102.22% for nateglinide.

Table 3: Accuracy data of Metformin hydrochloride and Nateglinide

Sr. No	Drug	Level%	Percent recovery (%)	RSD%
1	Metformin HCI	115	100.86	1.012
		125	100.56	
		150	100.07	
2	Nateglinide	115	101.22	1.020
		125	102.66	
		150	101.00	

Ruggedness

Several analysts conducted the examination using several chemical and solvent brands to assess the robustness of the suggested RP-HPLC procedure. The overall RSD for results from various analysts is within acceptable bounds. As a result, it was determined that the procedure for determining Nateglinide and Metformin HCI was robust (Table 4).

Table 4: Data for Ruggedness

Sr. No	Drug	Area	Percent recovery (%)	SD	RSD
1	Metformin HCI	2957221.20	100.04	0.1123	0.1122
		2961667.14	100.19		
		2955112.74	99.97		
2	Nateglinide	122939.03	98.37	0.0680	0.069
		123104.29	98.50		
		122981.71	98.40		

Robustness

The robustness study's findings demonstrated that there was no discernible change in either component's elution sequence or resolution. After analysis, the RSD of both components was found to be well within the 2% range. The suggested method was able to provide data of acceptable quality because the plate count and tailing factor were well below the acceptable USP limits (Table 5).

Table 5: Data for Robustness

Sr. No	Drug	Test Parameter	% RSD	Plate Count	Tailing
1	Metformin HCI	At low pH	1.50	8456	1.775
		At high pH		8124	1.701
2	Nateglinide	At low pH	1.36	7566	1.542
		At high pH		7105	1.501

Limit of Detection and Limit of Quantification:

The slope of the calibration plots and the standard deviation of the y-intercept were applied in order to independently calculate the limit of detection (LOD) and the limit of quantification (LOQ) (Table 6). Both levels of detection and quantification are referred to as limits.

Table 6: Limit of Detection and Limit of Quantification

Sr. No	Drug	LOD (PPM)	LOQ (PPM)
1	Metformin HCI	0.710	1.16
2	Nateglinide	0.380	2.15

CONCLUSION

Among the elements that are carefully assessed to ensure the reliability of the technique are accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), robustness, and system adaptability. All of these factors are evaluated to ensure that the method is reliable. Therefore, the purpose of this work is to develop and validate a new RP-HPLC method for simultaneously assessing metformin and nateglinide in pharmaceutical dosage forms. Because the proposed method is intended to be straightforward, inexpensive, accurate, and repeatable, it is suited for use in pharmaceutical laboratories for the purpose of performing routine quality control analysis. The findings that were obtained indicate that the RP-HPLC method that was recommended for the simultaneous assessment of metformin HCI and nateglinide is straightforward, linear, accurate, and reliable. Analysis of the combination tablet dose formulation has demonstrated the usefulness of the established procedure. In light of this, it is possible to quantitatively determine these compounds by employing the approach that has been suggested in the process of developing a combined tablet dosage.

DECLARATIONS:

Consent for publication:

All the authors approved the manuscript for publication.

Competing interests:

All authors declare no competing interests.

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