

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR METFORMIN HYDROCHLORIDE AND NATEGLINIDE IN BULK AND COMBINED DOSAGE FORM

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ABSTRACT

Objective: To develop an accurate, precise and linear RP-HPLC method for simultaneous quantitative estimation of Metformin hydrochloride and Nateglinide in tablets and validate as per ICH guidelines.

Methods: The method used a reverse phase column, Inertsil C18-ODS 3V (250×4.6 mm, 5 μm), a mobile phase comprising of phosphate buffer (pH 4.0): Acetonitrile: methanol (30:60:10) flow rate of 1.0 ml/min and a detection wavelength of 221 nm using a UV detector.

Results: The developed method resulted in elution of Metformin hydrochloride at 2.45 min and Nateglinide at 4.21 min. The calibration curves were linear ($r^2=0.999$) in the concentration range of 60-140 μg/ml and 14.4-33.2 μg/ml for Metformin hydrochloride and Nateglinide respectively. The percentage recoveries were found to be 99.59-101.36 for Metformin hydrochloride and 98.43-101.38 for Nateglinide. The LOD was found to be 2.18 μg/ml and 1.55 μg/ml for Metformin hydrochloride and Nateglinide respectively. LOQ was found to be 8.52 μg/ml and 4.69 μg/ml for Metformin hydrochloride and Nateglinide respectively.

Conclusion: A simple, accurate, precise, linear and rapid RP-HPLC method was developed for simultaneous quantitative estimation of Metformin hydrochloride and Nateglinide in bulk and pharmaceutical formulation and validated as per ICH guidelines. Hence, the method holds good for the routine analysis of Metformin hydrochloride and Nateglinide in various pharmaceutical industries as well as in academics.

Keywords: Metformin hydrochloride, Nateglinide, RP-HPLC, Method development, Validation

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INTRODUCTION

Metformin hydrochloride (fig. 1) is 1, 1-Dimethyl biguanide monohydrochloride. It is the first-line medication for the treatment of type 2 diabetes. It is a biguanide antihyperglycemic agent used for treating noninsulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain.

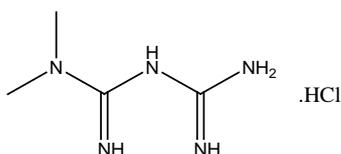


Fig. 1: Structure of metformin hydrochloride

Nateglinide (fig. 2) is 2-(4-isopropylcyclohexancarboxamido)-3-phenylpropanoic acid. It is an oral antihyperglycemic agent used for the treatment of NIDDM. It belongs to the Meglitinide class of short-acting insulin secretagogues. It lowers blood glucose by stimulating the release of insulin from the pancreas, by closing ATP-dependent potassium channels in the membrane of the β cells. This depolarizes the β cells and causes voltage-gated calcium channels to open. The resulting calcium influx induces fusion of insulin-containing vesicles with the cell membrane and insulin secretion occurs. Nateglinide is extensively metabolized in the liver and excreted in urine (83%) and feces (10%).

A detailed literature survey revealed that there was a spectrophotometric method for simultaneous estimation of Metformin with other combination [1] and Nateglinide in tablet

dosage form [2], and HPTLC method [3] have been established for simultaneous analysis of Metformin hydrochloride and Repaglinide. There were various RP-HPLC methods have been developed for the determination Metformin hydrochloride in combination with other drugs [4-11]. However, there was no RP-HPLC Method has been reported for simultaneous estimation of Metformin hydrochloride and Nateglinide, we presented easy, rapid, accurate and specific HPLC method for simultaneous estimation of RP-HPLC assay procedure for the analysis of Metformin hydrochloride and Nateglinide in bulk and tablet dosage form. The developed method was validated as per ICH guidelines [12].

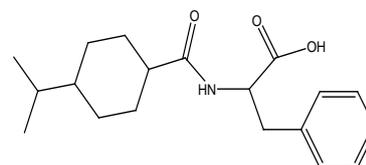


Fig. 2: Structure of nateglinide

MATERIALS AND METHODS

Pharmaceutical grade Metformin hydrochloride and Nateglinide were supplied as a gift sample by Chandra labs, Hyderabad and marketed formulation (NETOP PLUS, Metformin-500 mg, and Nateglinide-120 mg) was purchased from the local market. Methanol, Orthophosphoric acid (OPA), Acetonitrile and HPLC grade water were obtained from Merck. All solvents used in this work are HPLC grade. RP-HPLC Shimadzu (LC 20ATVP) model with Spin chrome (LC SOLUTIONS) software was employed in this method. Analytical column used for the separation of analytes is Inertsil ODS C18 (250×4.6 mm, 5μ).

Methods

Selection of wavelength

Standard solutions of Metformin hydrochloride and Nateglinide were prepared at the concentration of 10 µg/ml scanned by UV/Vis spectrophotometer at the range of 200-400 nm. UV spectrums of Metformin hydrochloride (fig. 3) and Nateglinide (fig. 4) were shown below. The isosbestic point selected for simultaneous estimation was 221 nm (fig. 5).

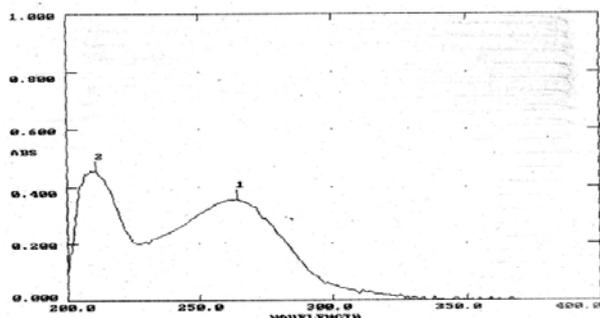


Fig. 3: UV spectrum of Nateglinide

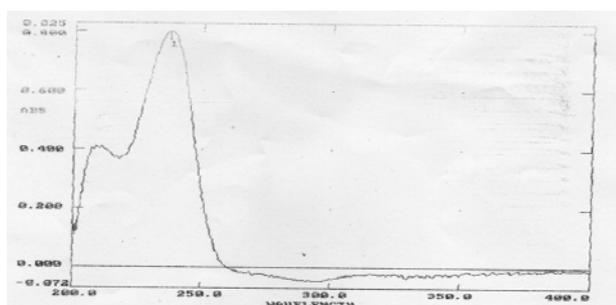


Fig. 4: UV spectrum of metformin hydrochloride

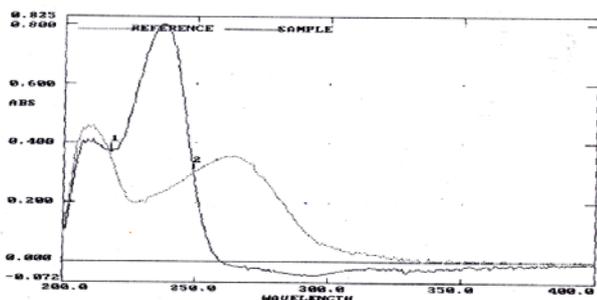


Fig. 5: UV overlaps spectrum of Nateglinide and Metformin hydrochloride

Chromatographic conditions

The developed method used a reverse phase C18 column, Waters Inertsil ODS 3V (250x4.6 mm, 5µ), a mobile phase of phosphate buffer (pH 4.0): Acetonitrile: methanol (30:60:10), flow rate of 1.0 ml/min and a detection wavelength of 221 nm using a UV detector.

Preparation of phosphate buffer

Accurately 2.7 g of potassium dihydrogen phosphate (KH₂PO₄) was weighed and dissolved in water and volume was made up to 1000 ml with water. The P^H was adjusted to 4.0 by using Orthophosphoric acid. The buffer was filtered to remove all fine undissolved particles.

Preparation of mobile phase

A mixture of 30 volumes of phosphate Buffer, 60 volumes of Acetonitrile and 10 volumes of methanol was prepared. The mobile phase was sonicated for 10 min to remove gasses.

Diluent

The mobile phase was used as diluent.

Preparation of standard solutions

Standard stock solutions of Metformin hydrochloride and Nateglinide were prepared by dissolving 100 mg of Metformin and 24 mg of Nateglinide insufficient mobile phase. After that, the solution was filtered and sonicated for 5 min and diluted to 100 ml with mobile phase. Further dilutions were prepared in 5 replicates of 100 µg/ml of Metformin and 24 µg/ml of Nateglinide, which was made by adding 1 ml of stock solution to 10 ml of mobile phase. This has been treated as 100 % target concentration.

Preparation of sample solution

20 tablets each containing 500 mg of Metformin hydrochloride and 120 mg of Nateglinide were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Primary stock solutions of Metformin hydrochloride and Nateglinide (µg/ml) were prepared by dissolving a weight equivalent to 500 mg of Metformin hydrochloride and 120 mg of Nateglinide and dissolved in sufficient mobile phase. After that, the solution was filtered by syringe filter and sonicated for 5 min and dilute to 100 ml with mobile phase. Further dilutions were prepared in 5 replicates of 100 µg/ml of Metformin hydrochloride, and 24 µg/ml of Nateglinide was made by adding 1 ml of stock solution to 10 ml of mobile phase. That has been treated as 100 % target concentration.

RESULTS AND DISCUSSION

Method development

Different chromatographic conditions were tried for better separation and resolution. Inertsil C18-ODS 3V (250x4.6 mm, 5 µm) column was found satisfactory. Peak purity of Metformin hydrochloride and Nateglinide was checked using UV detector and 221 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A number of solvents in the different ratio over a wide range of pH were tried, but either peak shape was broad or resolution was not good. Repeated trials to obtain good, sharp peak with an efficient resolution between two peaks of Metformin hydrochloride and Nateglinide done on a C18 column in isocratic HPLC gave satisfactory results. The run time was good in isocratic trial with mobile phase consisting of phosphate buffer (pH 4.0): Acetonitrile: methanol (30:60:10) and C18-ODS 3V (250x4.6 mm, 5 µm) column, flow rate 1.0 ml/min and detection wavelength 221 nm gave the satisfactory results in terms of retention time, resolution, symmetry and sensitivity. A typical RP-HPLC chromatogram for simultaneous determination of Metformin hydrochloride and Nateglinide from standard preparation and from pharmaceutical formulation was shown in (fig. 6 and 7).

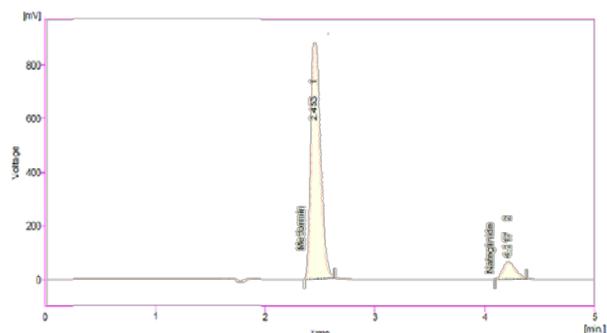


Fig. 6: Typical chromatogram of standard solution

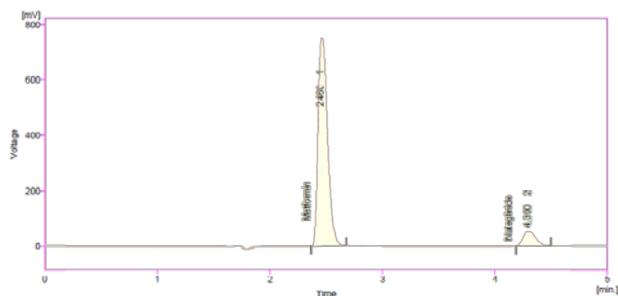


Fig. 7: Typical chromatogram of sample solution

Method validation

The developed RP-HPLC method was validated for parameters like system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness according to ICH guidelines.

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor

were evaluated. The system suitability parameters were tabulated in table 1. All the parameters were found to be within the limits.

Precision

Method precision

The precision of the method was verified by precision method studies. The sample solution was prepared at working concentration and analysis was carried out at replicating. The sample solutions of Metformin hydrochloride and Nateglinide were prepared as per the test method and injected 6 times into the column. The results of precision were tabulated in table 2. The average was taken, and % RSD was calculated and reported. % RSD values were within the limits, and the method was found to be precise.

Linearity

The linearity of the test solutions for the assay method was prepared from Metformin hydrochloride and Nateglinide standard stock solution at five concentration levels from 50% to 150% of assay concentration. The peak area versus concentration data was treated by least-squares linear regression analysis (fig. 8 and 9). The results have shown an excellent correlation between peak areas and concentration within the concentration range of 60–140 µg/ml for Metformin hydrochloride, 14.4–33.2 µg/ml for Nateglinide (tables 3-4). The correlation coefficients were found to be 0.999 for both the drugs, which meet the method validation acceptance criteria and hence the method was said to be linear for both the drugs.

Table 1: Results of system suitability studies

Parameters	Acceptance limits	Metformin hydrochloride	Nateglinide
Retention time (min)	-	2.453	4.217
Resolution	NLT 2	-	14.232
Theoretical plates	NLT 2000	8489	7583
Tailing factor	NMT 2	1.706	1.556

min: minutes

Table 2: Method precision data

n	Metformin hydrochloride		Nateglinide	
	Rt	Peak area	Rt	Peak area
Injection 1	2.459	3984.852	4.253	399.316
Injection 2	2.456	3983.028	4.243	396.285
Injection 3	2.453	3986.756	4.227	380.420
Injection 4	2.457	3983.864	4.210	390.391
Injection 5	2.459	3955.954	4.300	385.999
Injection 6	2.460	3915.924	4.367	385.427
Average	2.456	3968.396	4.267	389.640
SD	0.004	28.176	0.058	7.132
% RSD	0.164	0.710	1.35	1.83

Rt: Retention times, # n: number of injections=6

Linearity chart for Metformin hydrochloride

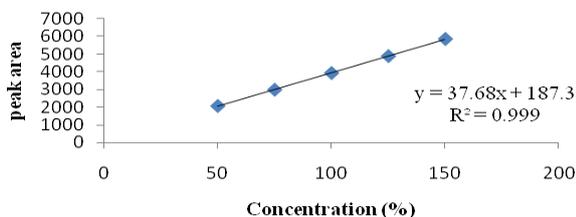


Fig. 8: Linearity chart for Metformin hydrochloride

Accuracy

The accuracy of the method was determined by recovery studies by the determination of % mean recovery of both the drugs at three different levels (80 %, 100 % and 120%). At each level, three

determinations were performed. The percentage recovery and mean percentage recovery were calculated for the drug was shown in table 4. The observed data were within the required range, which indicates good recovery values and hence the accuracy of the method developed.

Linearity chart for Nateglinide

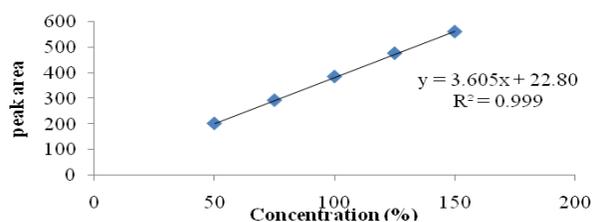


Fig. 9: Linearity chart for Nateglinide

Table 3: Linearity data for Metformin hydrochloride and Nateglinide

% Level	Metformin hydrochloride concentration ($\mu\text{g/ml}$)	Metformin peaks hydrochloride area	Nateglinide concentration ($\mu\text{g/ml}$)	Nateglinide peak area
50	60	2088.713	14.4	201.895
75	80	3002.121	19.2	292.713
100	100	3941.042	24	385.139
125	120	4890.891	28.8	476.353
150	140	5854.513	33.2	560.788
Correlation Coefficient	0.9998		0.9996	
Slope	37.68		3.60	

Table 4: Results of accuracy

Level (%)	Metformin hydrochloride		Nateglinide	
	% Recovery	% Mean	% Recovery	% Mean
80	98.59	99.59	100.74	100.42
80	99.63		99.90	
80	100.57		100.63	
100	99.48	100.46	99.53	99.30
100	100.54		98.43	
100	101.36		99.95	
150	101.20	100.09	101.38	101.14
150	99.08		100.78	
150	100.01		101.27	

Table 5: Results of robustness

Parameter	Metformin hydrochloride		Nateglinide	
	Plate count	Tailing	Plate count	Tailing
Less flow rate (0.8 ml/min)	8828	1.765	7336	1.500
More flow rate (1.2 ml/min)	8789	1.706	7114	1.556
Less wavelength 216 nm	8828	1.765	7180	1.519
More wavelength 226 nm	8451	1.667	7123	1.538

Table 6: LOD and LOQ

Sample name	LOD($\mu\text{g/ml}$)	LOQ($\mu\text{g/ml}$)
Metformin hydrochloride	2.18	8.52
Nateglinide	1.55	4.69

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered, and the system suitability parameters were evaluated. The solutions prepared as per the test method and injected at different variable conditions like flow rate (0.8, 1.2 ml/min.) and wavelength (219 nm, 223 nm), system suitability parameters were compared with that of method precision. The results were tabulated in table 5. At the flow rate of 1.0 ml/min shows, a sharp peak with good resolution and rest of the flow rates were found to be not satisfactory. The method passed all system suitability parameters indicating that the method was robust.

Detection limit and quantification limit

Limit of detection (LOD) which represents the concentration of the analyte at S/N ratio of 3 and limit of Quantification (LOQ) at which S/N was 10 were determined experimentally for the proposed methods and results were given in table 6. Hence, the detection limits and quantitation limits of the drugs were given S/N ratios of 3 and 10 respectively.

CONCLUSION

The proposed RP-HPLC method was found to be simple, specific, accurate, precise, robust, rapid and economical. This method gives good resolution between all the two compounds with a short analysis time. The proposed RP-HPLC method can be useful for

routine analysis of Metformin hydrochloride and Nateglinide in the tablet dosage form.

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CONFLICT OF INTERESTS

Declare none

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