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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND NATEGLINIDE IN BULK AND COMBINED TABLET DOSAGE FORM

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ABSTRACT

An accurate, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Metformin and Nateglinide in bulk and combined pharmaceutical tablet dosage forms. Metformin and Nateglinide were separated by using a Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size, Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of Methanol: 0.1% Orthophosphoric acid (64:36% v/v). The flow rate was set to 1ml/min with the responses measured at 224nm. The retention time of Metformin and Nateglinide was found to be 2.808min and 3.880min respectively with resolution of 5.68. Linearity was established for Metformin and Nateglinide in the range of 20-100µg/ml for Metformin and 60-140µg/ml for Nateglinide with correlation coefficient 0.999. The percentage recovery was found to be is 100.30% for Metformin and 100.21% for Nateglinide respectively. Validation parameters such as specificity, linearity, precision, accuracy and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present and in combined tablet dosage form.

Keywords: Metformin and Nateglinide, RP-HPLC, Validation, Accuracy, Robustness.

1. INTRODUCTION

Nateglinide is chemically 3-phenyl-2-[(4-propan-2-yl cyclohexane carbonyl) amino] propanoic acid (Fig. 1) with molecular formula C19H27NO3. It acts by blocking adenosine triphosphate sensitive potassium channels of beta cells of pancreas, causes membrane depolarization results in calcium influx and their by stimulation of insulin secretion. Metformin HCl is chemically N, N-Dimethyl imidodicarbonimidic diamide hydrochloride with molecular formula C4H11N5.HCl. The main mechanism of metformin HCl was lowering glucose intestinal absorption, inhibition of hepatic glucose production, and improving glucose uptake and utilization. It was found that very few articles are available in detailed literature survey on simultaneous estimation of nateglinide and metformin HCl by reversed-phase high-performance liquid chromatography (RP-HPLC) in pure and dosage form. The resting literature was found on analytical and bioanalytical methods by HPLC, LC– MS/MS, RP-LC, high-performance thin-layer chromatographic, and ultraviolet (UV) spectrophotometric estimations, in combination with glinides (nateglinide, repaglinide, and mitiglinide) and metformin HCl.

2. METHODOLOGY

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Metformin and Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.6ml of Metformin and 1ml of Nateglinide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

2.1. Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

2.2. Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: 0.1% Orthophosphoric acid in proportion 64:36 v/v respectively.

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2.3. Optimization of Column:

The method was performed with various C18columns like Symmetry, X terra and ODS column. Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

2.4. METHOD VALIDATION PARAMETERS

2.4.1. SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Metformin and Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.6ml of Metformin and 1ml of Nateglinide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.4.2. SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Metformin and Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.6ml of Metformin and 1ml of Nateglinide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Metformin and Nateglinide sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.6ml of Metformin and 1ml of Nateglinide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

2.4.3. PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Metformin and Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20ppm of Metformin and 60ppm of Nateglinide):

Pipette out 0.2ml of Metformin and 0.6ml of Nateglinide in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – II (40ppm of Metformin and 80ppm of Nateglinide):

Pipette out 0.4ml of Metformin and 0.8ml of Nateglinide in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – III (60ppm of Metformin and 100ppm of Nateglinide):

Pipette out 0.6ml of Metformin and 1ml of Nateglinide in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – IV (80ppm of Metformin and 120ppm of Nateglinide):

Pipette out 0.8ml of Metformin and 1.2ml of Nateglinide in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

Preparation of Level – V (100ppm of Metformin and 140ppm of Nateglinide):

Pipette out 1ml of Metformin and 1.4ml of Nateglinide in to a 10ml volumetric flask and make the volume upto mark by using diluent and sonicate for air entrapment.

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Inject each level into the chromatographic system and measure the peak area.Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

2.4.4. PRECISION REPEATABILITY

Accurately weigh and transfer 10 mg of Metformin and Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.6ml of Metformin and 1ml of Nateglinide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

2.4.5. INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

DAY 2:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10mg of Metformin and Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.3ml of Metformin and 0.5ml of Nateglinide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

2.4.6. ROBUSTNESS:

Accurately weigh and transfer 10 mg of Metformin and Nateglinide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.6ml of Metformin and 1ml of Nateglinide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions: The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Methanol: 0.1% Orthophosphoric acid (64:36% v/v) was taken in the ratio and 69:31, 59:41 instead of 64:36 remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

3. RESULTS AND DISCUSSION

3.1. System suitability for Metformin

Table 1. system suitability for Metformin and Nateglinide

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.816	65358	4536	**5.36**	1.08
2	Nateglinide	3.893	8658746	658985	5.69	1.42

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

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3.2. SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Metformin and Nateglinide in drug product.

Table 2. Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Metformin	2.813	65684	4365		1.08	5632.4	1
2	Nateglinide	3.886	8659824	659824	5.69	1.42	6859.2	1
3	Metformin	2.813	65985	4329		1.09	5682.3	2
4	Nateglinide	3.886	8645872	658266	5.68	1.43	6824.1	2
5	Metformin	2.813	65784	4426		1.08	5692.8	3
6	Nateglinide	3.886	8657847	6589412	5.69	1.43	6895.4	3

3.3. LINEARITY

Table 3: Linearity study of Metformin:

Concentration µg/ml	Average Peak Area
20	24759
40	47859
60	70898
80	93985
100	116698

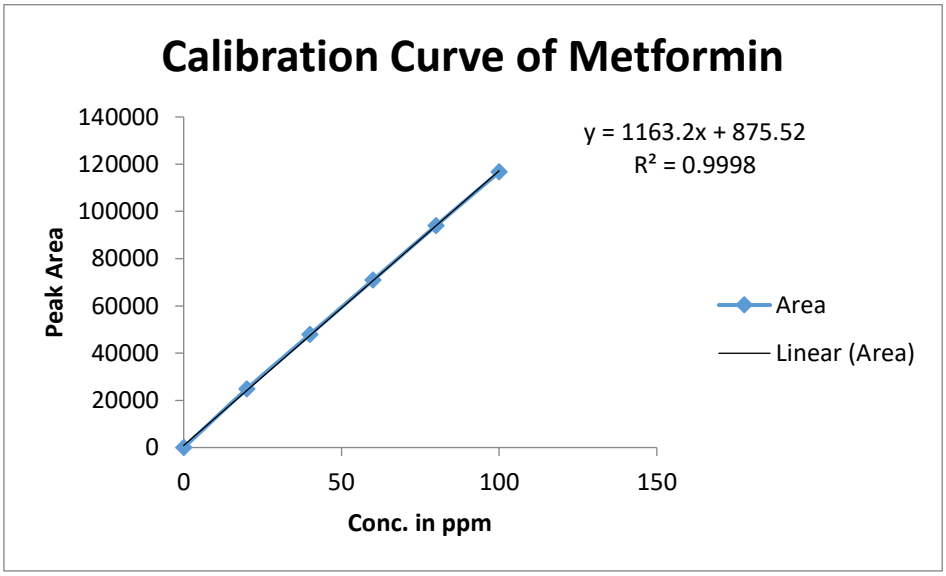

Figure1: Calibration graph for Metformin

Table 4: Linearity study of Nateglinide

Concentration µg/ml	Average Peak Area
60	4928578
80	6687842
100	8389878
120	10085847
140	11769854

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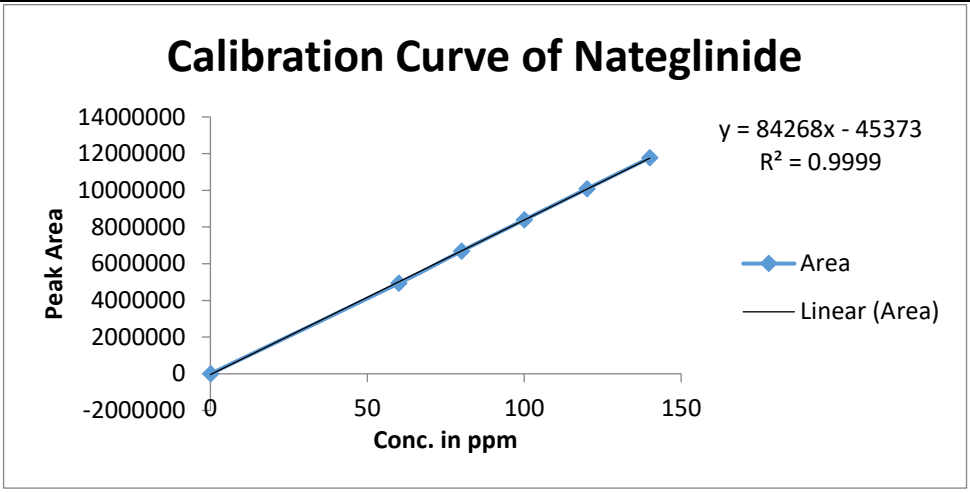

Figure2: Calibration graph for Nateglinide

Table 5: Results of repeatability for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	3.003	654426	61521	8474	1.1
2	Metformin	3.005	659862	61937	8262	1.2
3	Metformin	3.007	650837	62018	8117	1.1
4	Metformin	3.008	651433	61893	7917	1.2
5	Metformin	3.005	652752	61867	8011	1.1
Mean			653862			
Std. Dev			3626.323			
% RSD			0.554601			

Table-6: Results of method precision for Nateglinide:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Nateglinide	3.851	3028371	381736	6881	3.851
2	Nateglinide	3.852	3009188	380138	9363	3.852
3	Nateglinide	3.854	3067464	386615	7844	3.854
4	Nateglinide	3.853	3076611	380183	9746	3.853
5	Nateglinide	3.851	3011912	379471	7883	3.851
Mean			3038709			
Std. Dev			31463.69			
% RSD			1.035429			

3.4. ACCURACY:

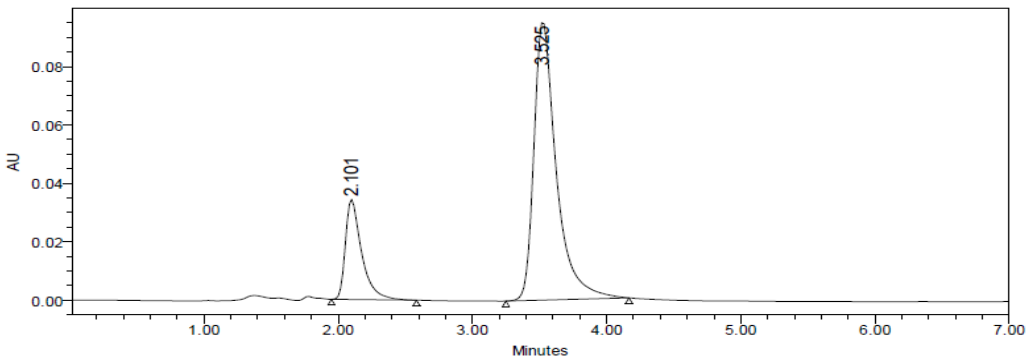

Fig-3: Chromatogram showing accuracy-50% injection

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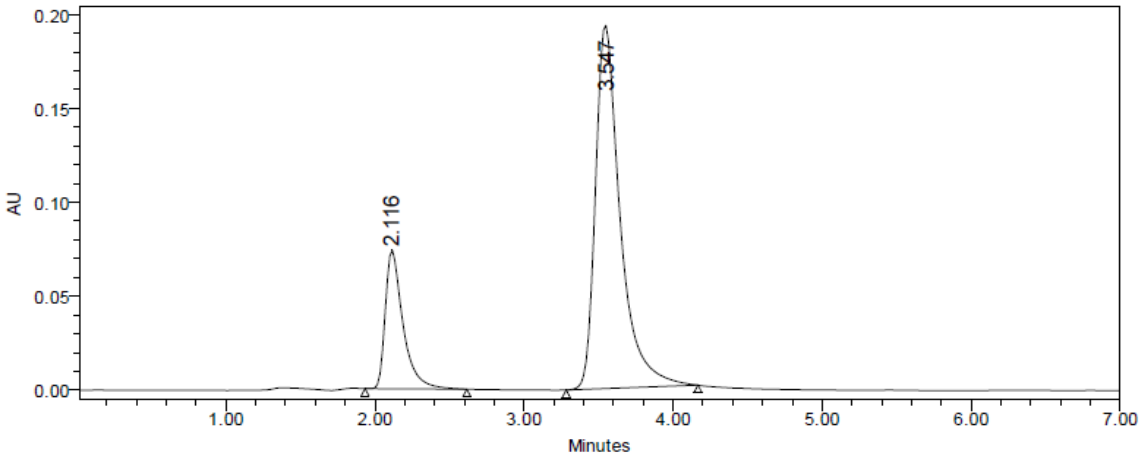

Fig 4: Chromatogram showing accuracy-100% injection

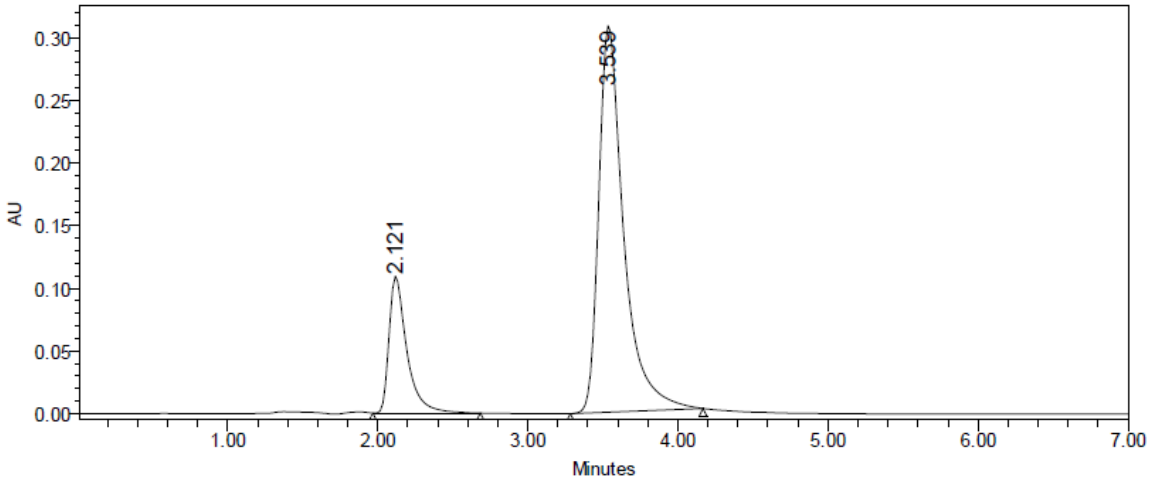

Fig-5: Chromatogram showing accuracy-150% injection

3.5. LIMIT OF DETECTION

Metformin:= 0.97µg/ml Nateglinide:= 2.06µg/ml

3.6. LIMIT OF QUANTITATION

Metformin=2.91µg/ml

Nateglinide= 6.18µg/ml

3.7. ROBUSTNESS

Table7-: Results for Metformin and Nateglinide Robustness

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5895.3	1.12
2	*Actual	5685.4	1.08
3	10% more	5964.2	1.16
S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6785.2	1.46
2	*Actual	6895.7	1.42
3	10% more	6982.4	1.49

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4. CONCLUSION

The study is focused to develop and validate HPLC methods for estimation of Metformin and Nateglinide in bulk and tablet dosage form.

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.

The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Metformin and Nateglinide

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