ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ANTI-DIABETICS IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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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.

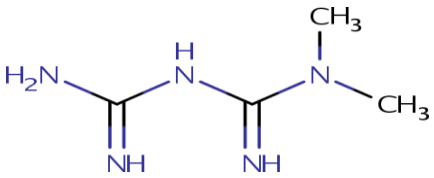
Keywords: Metformin and Nateglinide, RP-HPLC, Validation, Accuracy, Robustness.

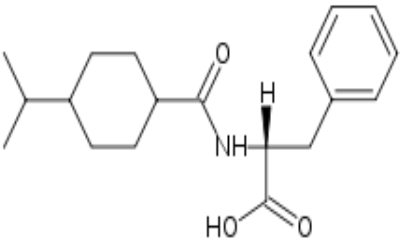
INTRODUCTION

Metformin is a class of organic compounds known as biguanides. These are organic compounds containing two N-linked guanidines and it has a IUPAC is 1-carbamimidamido-N,N-dimethylmethanimidamide.

Nateglinide is a amino-acid derivative that lowers blood glucose levels by stimulating insulin secretion from the pancreas. This action is dependent upon functioning beta-cells in the pancreatic islets. Nateglinide interacts with the ATP-sensitive potassium (K+ATP) channel on pancreatic beta-cells and its IUPAC is 3-phenyl-2-[(4-propan-2-yl cyclo hexane carbonyl)
amino] propanoic acid.

Structure of Metformin





Structure of Nateglinide

MATERIALS AND METHODS

INSTRUMENTS USED

Instruments used for experiments is HPLC is WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. And chemicals used is Metformin (Pure), Nateglinide (Pure) Water and Methanol for HPLC and Acetonitrile for HPLC

METHOD VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase: Accurately measured 640ml of Acetonitrile (64%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

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.

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.

ROBUSTNESS: The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For 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.

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.

PRECISION

REPEATABILITY

Preparation of Metformin and Nateglinide Product Solution for Precision: 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.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.

RESULT AND DISCUSSION

System suitability parameters:

Table-: 1 Results of system suitability parameters for Metformin and Nateglinide

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Metformin	2.816	65358	4536		1.08	5689.6
2	Nateglinide	3.893	8658746	658985	5.69	1.42	6892.4

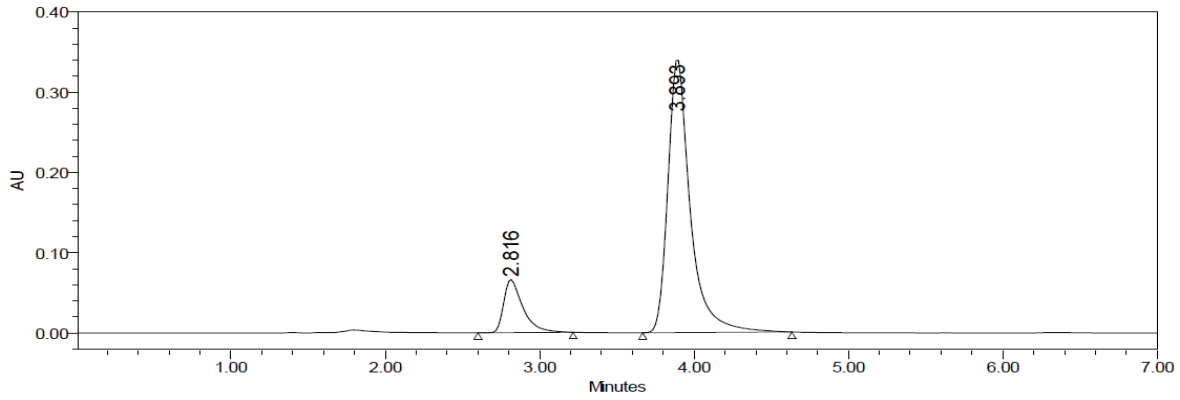


Figure:1 chromatogram for system suitability

METHOD VALIDATION PARAMETERS:

Assay (Standard):

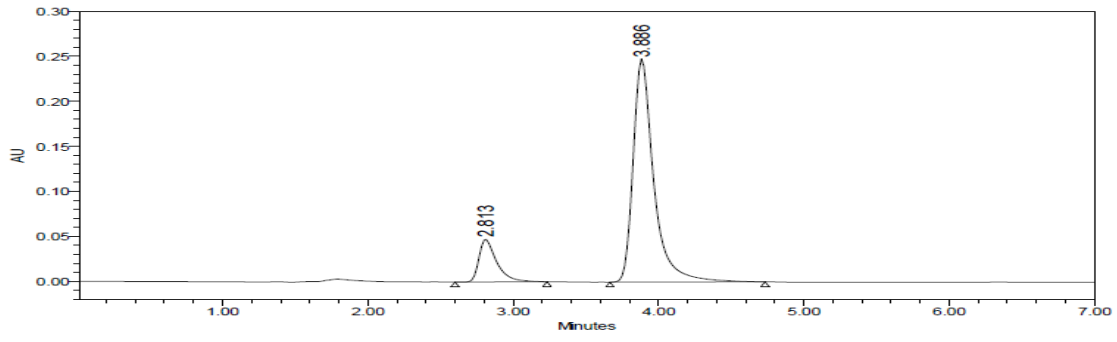


Fig-: 2 Chromatogram showing assay of standard injection-1

Table-: 2.0 Showing assay standard Results

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

Table-: 2.1 Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Metformin	60mg	59.84	99.68%
2	Nateglinide	500mg	499.63	99.46%

Precision

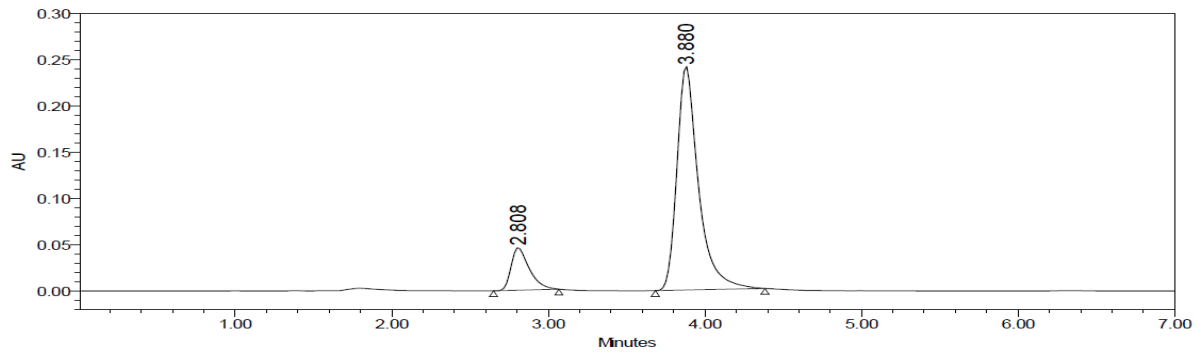


Fig:3 chromatogram for standard injection -1

Table-:3.0 Results of method precision for Metformin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.808	65898	4365	5682.2	1.08
2	Metformin	2.808	65487	4375	5628.6	1.09
3	Metformin	2.808	65324	4395	5649.7	1.08
4	Metformin	2.808	65982	4328	5638.4	1.09
5	Metformin	2.808	65248	4371	5698.3	1.08
6	Metformin	2.808	65734	4391	5682.7	1.09
Mean			65612.17			
Std. Dev			304.8425			
% RSD			0.464613			

Table-:3.1 Results of method precision for Nateglinide:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Nateglinide	3.880	8659824	658784	6859.4	1.42	5.68
2	Nateglinide	3.880	8658547	657489	6824.6	1.43	5.69
3	Nateglinide	3.880	8659824	652368	6829.3	1.42	5.68
4	Nateglinide	3.880	8659875	658745	6892.7	1.43	5.69
5	Nateglinide	3.880	8658745	658213	6875.2	1.42	5.68
6	Nateglinide	3.880	8659862	652354	6859.8	1.42	5.69
Mean			8659446				
Std. Dev			623.2924				
% RSD			0.007198				

Intermediate Precision/Ruggedness:

Table-:4.0 Results of Intermediate precision for Metformin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Metformin	2.808	66895	4468	5784.2	1.09
2	Metformin	2.808	66986	4523	5835.1	1.09
3	Metformin	2.808	66258	4475	5864.4	1.10
4	Metformin	2.808	66457	4514	5864.6	1.09
5	Metformin	2.808	66539	4489	5784.9	1.10
6	Metformin	2.808	66298	4565	5748.5	1.10
Mean			66572.17			
Std. Dev			304.536			
% RSD			0.457452			

Table-:4.1 Results of Intermediate precision for Nateglinide

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Nateglinide	3.882	8758568	669583	6982.4	1.43	
2	Nateglinide	3.882	8756982	665984	6935.3	1.44	5.69
3	Nateglinide	3.882	8746925	665345	6984.7	1.44	
4	Nateglinide	3.882	8723654	665325	6952.8	1.43	5.70
5	Nateglinide	3.882	8754982	669852	6898.9	1.44	
6	Nateglinide	3.882	8754698	665874	6976.5	1.43	5.69
Mean			8749302				
Std. Dev			13188.56				
% RSD			0.150738				

Accuracy Standard

Table-:5.0 Results of Accuracy standard values:

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Metformin	2.860	65359	4358		1.09	5698.5	1
2	Nateglinide	3.949	8659825	659862	5.68	1.42	6859.4	1
3	Metformin	2.860	65874	4395		1.08	5672.4	2
4	Nateglinide	3.949	8659875	653485	5.68	1.43	6824.2	2
5	Metformin	2.860	65398	4382		1.08	5683.1	3
6	Nateglinide	3.949	8674587	6587458	5.69	1.42	6875.6	3

Table-:5.1 Accuracy (recovery) data for Metformin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	35921.67	30	30.134	100.446%	100.30%
100%	70894.33	60	60.205	100.341%	
150%	105654.7	90	90.093	100.103%	

Table-:5.2 Accuracy (recovery) data for Nateglinide

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	4276302	50	50.208	100.416%	100.21%
100%	8484717	100	100.148	100.148%	
150%	10160609	150	150.091	100.060%	

LINEARITY:

The linearity range was found to lie from 20-100ppm of Metformin, 60µg/ml to 140µg/ml of Nateglinide and chromatograms are shown below.

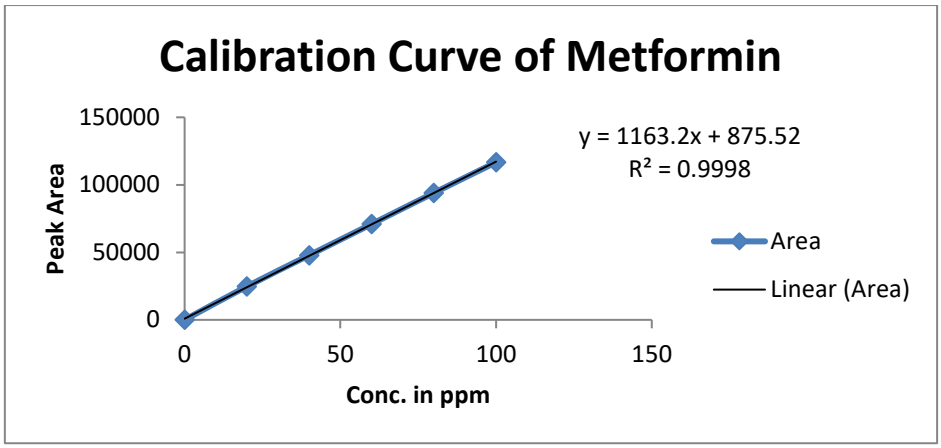


Figure : 4.0 calibration graph for Metformin

Linearity Results: (for Metformin)

S.No	Linearity Level	Concentration (ppm)	Area
1	I	20	24759
2	II	40	47859
3	III	60	70898
4	IV	80	93985
5	V	100	116698
Correlation Coefficient			0.999

Linearity Results: (for Nateglinide)

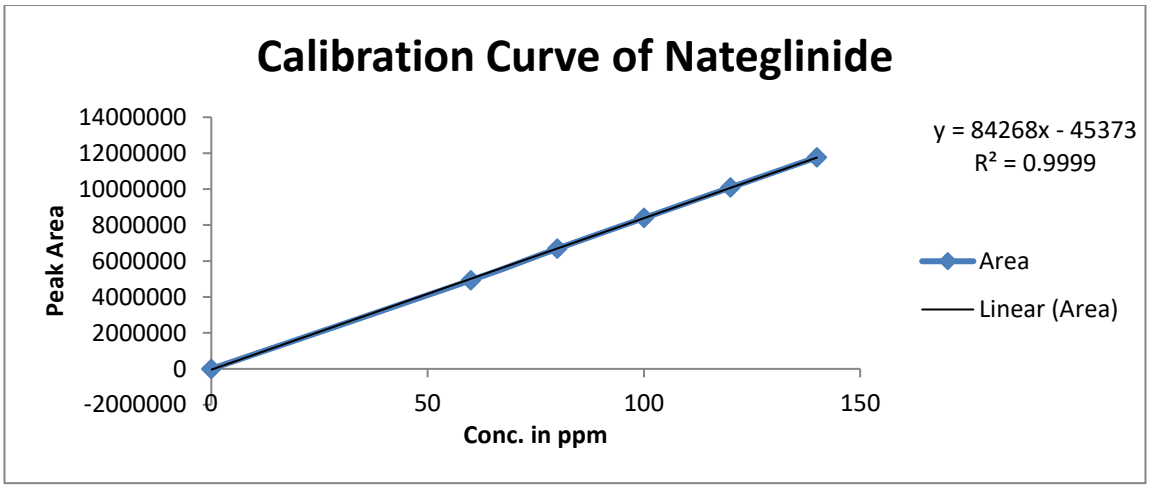


Figure:4.1 Calibration graph for Nateglinide

S.No	Linearity Level	Concentration(ppm)	Area
1	I	60	4928578
2	II	80	6687842
3	III	100	8389878
4	IV	120	10085847
5	V	140	11769854
Correlation Coefficient			0.999

ROBUSTNESS:

The standard and samples of Metformin and Nateglinide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table-:6.0 System suitability results for Metformin:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	5784.6	1.06
2	1.0	5685.4	1.08
3	1.1	5869.5	1.09

Table-:6.1 System suitability results for Nateglinide:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	6698.3	1.46
2	1.0	6895.7	1.42
3	1.1	6983.6	1.49

CONCLUSION

The examination is engaged to create and approve HPLC strategies for assessment of Metformin and Nateglinide in mass and tablet measurement structure.

For routine expository reason it is attractive to set up techniques fit for dissecting tremendous number of tests in a brief timeframe period with great power, exactness and accuracy with no earlier detachment steps. HPLC technique produces enormous measure of value information, which fill in as profoundly ground-breaking and helpful scientific device.

The strategy shows great reproducibility and great recuperation. From the explicitness contemplates, it was discovered that the created techniques were explicit for Metformin and Nateglinide.

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