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METHOD DEVELOPMENT AND VALIDATION OF METFORMIN HYDROCHLORIDE AND NATEGLINIDE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract: This study includes development of RP-HPLC method for simultaneous estimation of Metformin hydrochloride and Nateglinide. The developed method was validated as per ICH guidelines in terms of specificity, Accuracy, Precision, Linearity, LOD, LOQ, Ruggedness and Robustness. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of Metformin HCI and Nateglinide estimated in the tablet dosage form were free from the interference of excipients, which indicate that developed method was specific. Recovery of standard drugs added was found to be 100.07-100.82% for Metformin HCI and 101.0-102.22% for Nateglinide indicating that the proposed method was accurate. A good linear relationship was observed for Metformin HCI and Nateglinide in the concentration ranges of 10-80µg/ml and 10-80µg/ml respectively. The correlation coefficient for Metformin HCI was found to be 0.9991 and for Nateglinide was 0.9997. The LOD were 0.710 and 0.380µg/ml for Metformin HCI and Nateglinide, respectively. For Metformin HCI and Nateglinide LOQ were found to be 2.15 and 1.16µg/ml respectively. This demonstrated that developed RP-HPLC method was simple, linear, precise, accurate, robust and rugged could be conveniently adopted for the routine quality control analysis of Metformin HCI and Nateglinide simultaneously, from its pharmaceutical formulation and bulk drug.

Index Terms **- Metformin hydrochloride, Nateglinide, RP-HPLC, Method development, Validation**

I. INTRODUCTION

Metformin hydrochloride (fig. 1) is 1, 1-dimethylbiguanide hydrochloride having formula $C_4H_{11}N_5$, HCI. It is use in treatment of type 2 diabetes mellitus. It improves hyperglycemic primarily by suppressing glucose production by the liver. It activates AMP activated protein kinase, an enzyme is required for metformin's inhibitory effect on the production of glucose by liver cells. Metformin is the only oral anti-hyperglycemic agent that is not associated with weight gain.

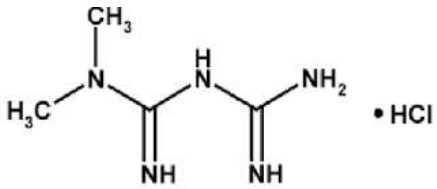

Fig. 1 structure of Metformin hydrochloride

Nateglinide (fig. 2) 3- phenyl-2- [(4-propane-2-yl cyclohexanecarbonyl) amino] propionic acid is an oral antihyperglycemic agent used for the treatment of non-insulin dependent diabetics mellitus (NIDDM). Nateglinide lowers blood glucose by stimulating the release of insulin from pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of 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.

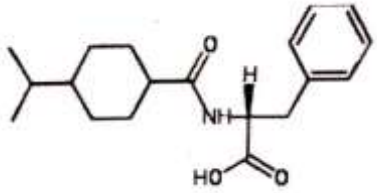

Fig.2 structure of Nateglinide

II) MATERIAL AND METHOD

Pharmaceutical grade Metformin HCI were supplied as gift sample by Emcure pharmaceuticals Ltd, Pune and Nateglinide by Cipla Pharmaceuticals pvt Ltd, Kurkumb, India. The pharmaceutical dosage form used in this study was Glinate-MF, manufactured by Glenmark pharmaceutical limited, Purchased in local market. Acetonitrile and HPLC grade water from Merck, Ortho-phosphoric acid and potassium dihydrogen orthophosphate from Research lab Fine chem Industries, Mumbai. All solvent used in this work are HPLC grade. RP-HPLC System lab corporation, Japan (LC-P-4000) were used. Analytical column used for the separation of analytes is Kromasil C-18 (4.6mm×250mm, 5µm).

METHODS

Selection of wavelength

Standard solution of Metformin hydrochloride and Nateglinide were prepared at the concentration of 10µg/ml scanned by UV spectrophotometer at the range of 200-400 nm. UV spectrum of Metformin Hydrochloride (fig. 3) and Nateglinide (Fig.4) were shown below. The isobestic point selected for simultaneous estimation was 216 nm (fig.5).

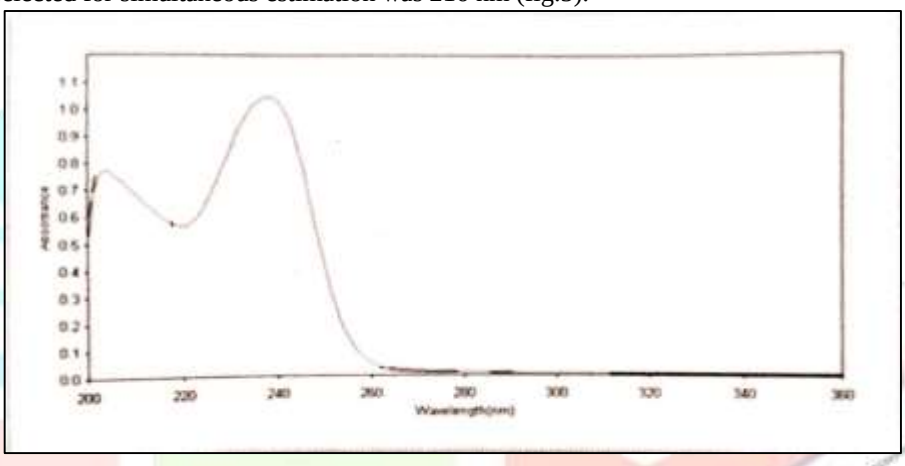

Fig.3 UV spectrum for Metformin hydrochloride

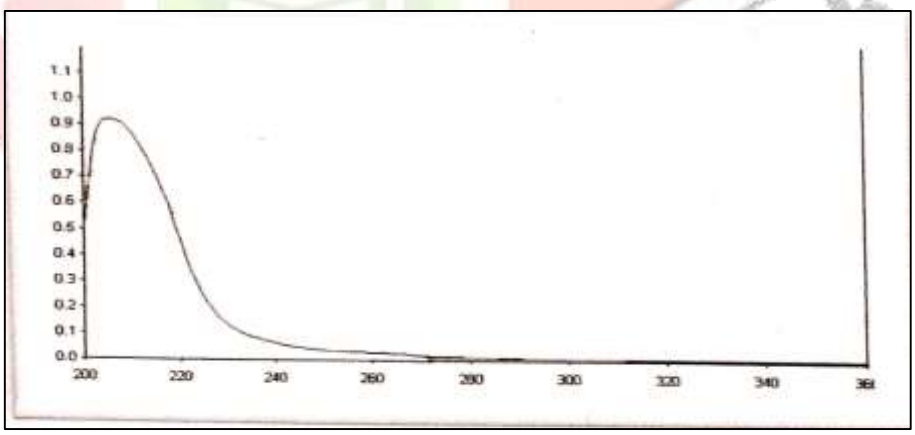

Fig. 4 UV spectrum for Nateglinide

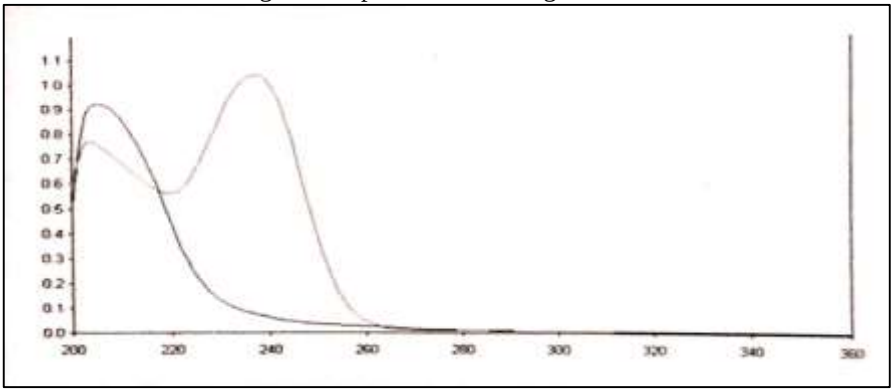

Fig. 5 UV spectrum for combination of Metformin hydrochloride & Nateglinide

Chromatographic conditions

The developed method used reverse phase kromasil C18 column (4.6mm×250mm,5µm), a mobile phase of potassium dihydrogen phosphate: acetonitrile (30:70) with orthophosphoric acid to adjust the pH, flow rate of 1.1ml/min, temperature was ambient, run time 7 min. and detection wavelength of 216 nm using a uv detector.

Preparation of mobile phase

An accurately weighed quantity of about 1.369gm of potassium dihydrogen orthophosphate was taken in 200 ml volumetric flask dissolved in sufficient quantity of water, then sonicated for 15 min and diluted to 200 ml with the HPLC water. Then adjust the pH up to 3 with orthophosphoric acid and filter through 0.45µm filter, gives the formation of buffer. Finally, the optimal composition of the mobile phase contains 30 volume of buffer and 70 volume of Acetonitrile.

Preparation of standard stock solution

Approximately 500 mg of Metformin HCI and 60 mg of Nateglinide respectively were accurately weighed, transferred to separate 100 ml volumetric flack, dissolved in the mobile phase and dilute to a volume with the same solvent mixture to furnish stock solution containing 5000µg/ml of Metformin HCI and 600µl/ml of Nateglinide respectively. 1ml of above solution transferred in 10ml volumetric flask and the volume was made with diluents. The concentration of Metformin HCI and Nateglinide is 500µg/ml and 60µg/ml respectively.

Preparation of sample solution

20 tablets each containing 500mg of Metformin HCI and 60mg of Nateglinide were weighed and taken into a mortar and crushed to fine powder and uniformly mixed, transferred to a100ml volumetric flask, dissolved in 70ml of mobile phase. The solution were sonicated for 30 min then diluted to volume with the mobile phase. Sample solution containing 500µg/ml and 60µg/ml of Metformin HCI and Nateglinide were prepared. Finally the solution were filtered through whatman filter paper.

III) RESULT AND DISCUSSION

Method Development

Different chromatographic conditions were tried for better separation and resolution. kromasil C18 (4.6mm×250mm,5µm) column was found satisfactory. Peak purity of Metformin HCI and Nateglinide was checked using UV detector and 216 nm was consider satisfactory for detecting both the drugs with adequate sensitivity. A number of solvent with different ratio over 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 HCI and Nateglinide done on a C18 column gave satisfactory results. The run time was good in isocratic trial with mobile phase containing phosphate buffer (pH3.0): Acetonitrile (30:70), flow rate 1.1ml/min and detection wavelength 216nm gave the satisfactory results in terms of retention time, resolution and sensitivity. A typical RP-HPLC chromatograph for Metformin HCI and Nateglinide from standard preparation and from pharmaceutical formulation was shown in (fig 6 & 7).

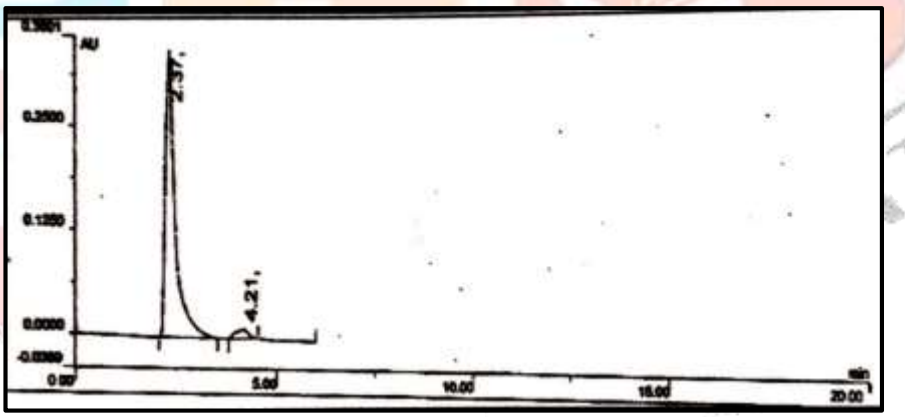

Fig. 6 Chromatograph for standard solution of Metformin HCI and Nateglinide

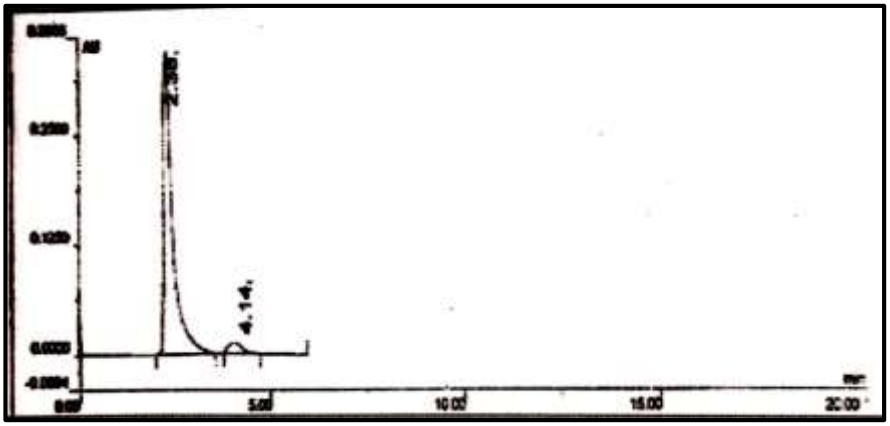

Fig. 7 Chromatograph for sample solution of Metformin HCI and Nateglinide

Method validation

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

System suitability

Standard solutions were prepared as per the method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The system suitability parameter were tabulated in table 1. All the parameters were found to be within the limits.

Table. 1 System suitability parameter

Parameters	Acceptance limit	Metformin Hydrochloride	Nateglinide
Retention time (min)		2.37	4.20
Resolution	NLT 2	2.5	4.1
Theoretical plates	NLT 2000	5850	5380
Tailing factor	NLT 2	1.05	1.11

Precision

Method precision

The intra day and inter day precision study of Metformin HCI and Nateglinide was carried out by estimating the corresponding responses three times and the results are reported in terms of relative standard deviation in table 2

Table. 2 Intra-day & Inter-day variability for Metformin HCI and Nateglinide

Drug	Intra-day precision				Inter-day precision			
	Trial	Area	SD	RSD	Trial	Area	SD	RSD
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		
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

The calibration curves exhibited linear relationship of peak area to concentration in the range 10µg/ml for Metformin HCI and 10-80µg/ml for Nateglinide. The regression coefficient (r2) for Metformin HCI and Nateglinide were 0.998 and 0.996, respectively, maintaining good correlation close to unity. The graph of concentration Vs Average area was plotted which is showing straight line passing through all points. So as per ICH guidelines, the proposed RP-HPLC method for determination of Metformin HCI and Nateglinide was found to be linear. (fig- 8 & 9)

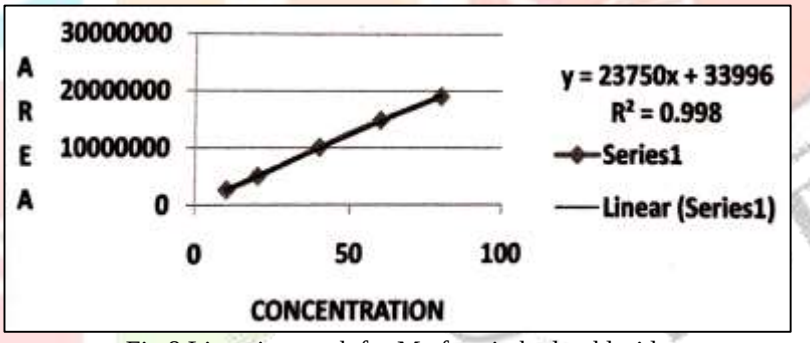

Fig.8 Linearity graph for Metformin hydrochloride

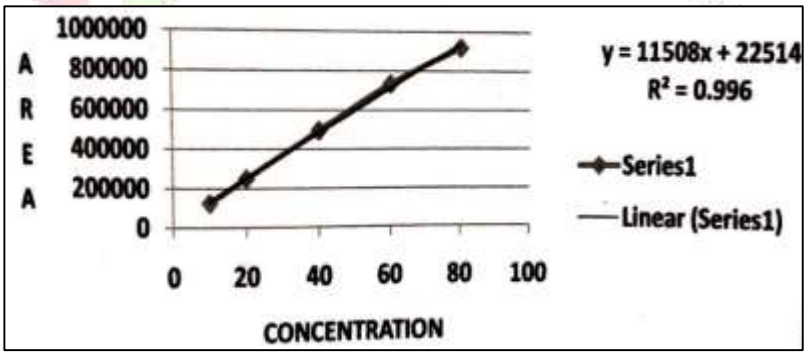

Fig. 9 Linearity graph for Nateglinide

Accuracy

The acceptance criteria was standard deviation should not be more than 2.0%

Accuracy was checked with standard drugs by placebo spiking method at three different concentration levels (multilevel recovery). Recovery of standard drugs added was found to be 100.07-100.86% for Metformin HCI and 101.00-102.22%for Nateglinide with the value of RSD less than 2%(table 3), indicating that the proposed method is accurate for the simultaneous estimation of Metformin HCI and Nateglinide from their combination drug product in presence of their degradation products and excipients.

Table 3. Accuracy data of Metformin hydrochloride and Nateglinide

Drug	Level %	Percent recovery(%)	RSD%
Metformin HCI	115	100.86	1.012
	125	100.56	
	150	100.07	
Nateglinide	115	101.22	1.020
	125	102.66	
	150	101.00	

Ruggedness

To evaluate the ruggedness of the proposed RP-HPLC method, the analysis was perform by different analysts and employing different brands of chemicals and solvents. Overall RSD for results obtained from different analyst are within limits. Therefore, the method for determination of Metformin HCI and Nateglinide was found to be rugged (table.4)

Table 4. Data for Ruggedness

Drug	Area	Percent recovery	SD	RSD
Metformin HCI	2957221.20	100.04	0.1123	0.1122
	2961667.14	100.19		
	2955112.74	99.97		
Nateglinide	122939.03	98.37	0.0680	0.069
	123104.29	98.50		
	122981.71	98.40		

Robustness

Result of the robustness study showed that the elution order and resolution for both components were not significantly affected. RSD of both components were examined and found to be well within the limit of 2%. The plate count and tailing factor was well within the acceptable USP limits, ensuring that the proposed method was capable of providing data of acceptable quality (table 5).

Table 5. Data for Robustness

Drug	Test parameter	%RSD	Plate count	Tailing
Metformin HCI	At low pH	1.50	8456	1.775
	At high pH		8124	1.701
Nateglinide	At low pH	1.36	7566	1.542
	At high pH		7105	1.501

Limit of Detection and Quantification

The limit of detection (LOD) and quantification (LOQ) were determined separately, on the basis of the standard deviation of the y intercept and slop of the calibration plots (table 6)

Table 6. Limit of Detection & Quantification

Drug	LOD(µg/ml)	LOQ(µg/ml)
Metformin HCI	0.710	1.16
Nateglinide	0.380	2.15

IV) CONCLUSION

Based on the results obtained, it can be concluded that the proposed RP-HPLC method for the simultaneous determination of Metformin HCI and Nateglinide is simple, linear, precise, accurate, robust and rugged. The utility of the developed method have been demonstrated by analysis of combined tablet dosage formulation. Hence, the proposed method can be used for quantitative determination of these ingredients in combined tablet dosage formulation.

V) ACKNOWLEDGMENT

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VI) REFERENCE

1. Bhaskar Reddy, P. Raveendra Reddy, Useni Reddy mallu and L. Maheshwara Reddy, Novel RP-HPLC method for Metformin HCI, Glipizide and Repaglinide pharmaceutical drug products, ijrrpas, 2011, volume-1 Issue-3, Page131-139.
2. Asha B.T. and Shrikrushna D.P, Simultaneous spectrophotometric estimation of Nateglinide and Metformin hydrochloride in pharmaceutical dosage formulation, Der Pharma Chemica, 2011, 3(3):271-276.
3. N.R. Shastri, K.V. Surendranath and J. Satish, Validated Stability –Indicating RP_HPLC UV method for simultaneous estimation of Metformin and Repaglinide, Acta Chromatographica 24(2012)3, 419-432.
4. J.Sachin, M. Hatel, B. Sudesh, S. Yogesh, M.Rokade, D.Vinayak, V.Vaidya, Method development and validation of Nateglinide and its related impurities by RP-HPLC, Int.J.Ph.Sci,. Jan-April, 2012;4(1), 1751-1758.
5. Madhavi jain, Ami Sharma and Love kumar Sony, Development and validation of RP-HPLC methods for simultaneous estimation of Metformin hydrochloride and Repaglinide in Tablet dosage form, Int. J. of Pharm. & Research Sci (IJPRS), Vol.1, Issue 4: Oct 2012, 251-258.
6. Jain S., B handari A., Purohit S., Spectrophotometric determination of Nateglinide in bulk and tablet dosage form. Asian Journal Pharm., 2009, 3,218-221.
7. Rastogi A., jha K.k., Verma V., Singh J., The determination of Nateglinide in bulk and pharmaceutical preparation UV spectrophotometric method. The Pharma Research Year, 2009, l, 169-174.
8. Pushpa Latha and D. Ramchndran, method development and Validation for the simultaneous estimation of Vildagliptin and Metformin in tablet dosage form by RP-HPLC, Int J. Pharm Sci, Vol 5, Issue 1, 459-463.
9. Klepser TB and MW Kelly, Metformin hydrochloride; an antihyperglycemic agents, Am J Health System Pharm, 1997, 54, 893-903.
10. T.Raja and A. Lakshmana Rao, validated RP_HPLC method for simultaneous estimation of Metformin hydrochloride and Sitagliptine phosphate in bulk drug and pharmaceutical formulation, Int J Pharm chem Bio Sci, 2012,2(4),696-702.

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