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Stability Indicating Simultaneous RP-HPLC Method for the estimation of Metformin Hydrochloride and Nateglinide in Bulk

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ABSTRACT: A new stability-indicating RP-HPLC method for the determination of metformin hydrochloride and nateglinidein bulk was developed. The separation wasperformed on a Hypersil BDS-C_{18}column (250 X 4.6 mm ID, 5 µm) by using 0.05 M potassium dihydrogen orthophosphate (pH 3.5) acetonitrile (30:70 v/v) as the mobile phase with UV detection at 226 nm. Under these chromatographic conditions,favorable retention parameters were obtained with good symmetry of peaks for the studied compounds. The validation studies performed as per ICH guidelines.The proposed method showed high degree of accuracy, and precision, with a good degree of sensitivity and robustness. The drugs were subjected to forced degradation using acidic, alkaline, oxidation, wet heat, dry heat, and photodegradationconditions. The results confirmed that the method could effectively separate the drugs in the presence of their degradation products, hence it can be regarded as stability indicating.

Keywords: Metformin hydrochloride, Nateglinide, HPLC, Stability-indicating method.

INTRODUCTION

Metformin hydrochloride (MET), or N, N-dimethylimidodicarbonimidicdiamide hydrochloride, is a hypoglycemic medication of the biguanide family used to treat type 2 diabetes in people who do not produce insulin. Glycemic management is enhanced because insulin sensitivity is increased, leading to less glucose being absorbed in the intestines [1,2].

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The meglitinideclass of nateglinide (NAT; chemically N-(trans-4-isopropyl cyclo hexyl carbonyl)-D-phenylalanine) lowers blood sugar via increasing insulin production in the pancreas. The beta-cells in the pancreatic islets must be active for this to happen [3].

Metformin hydrochloride and Repaglinide can be analyzed simultaneously using the HPTLC method [4], and a spectrophotometric method for simultaneous estimation of Metformin with other combinations and Nateglinide in tablet dosage form has been established. Several different RP-HPLC techniques for the detection of Metformin hydrochloride in combination with other medications have been developed [5-12]. However, there was few 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 method's validity was confirmed in accordance with ICH regulations [13].

MATERIALS AND METHODS

All solvents utilized in this study are HPLC grade. Methanol, Orthophosphoric acid (OPA), Acetonitrile and HPLC grade water were acquired from Merck. RP-HPLC Shimadzu (LC 20) model with LC SOLUTIONS software was applied in this procedure. Analytical column utilized for the separation of analytes is Hypersil BDS-C18 column (250 X 4.6 mm ID, 5 µm).

METHOD

Selection of wavelength for Metformin hydrochloride and Nateglinide

Metformin hydrochloride and Nateglinide 10 µg/ml standard solutions were produced and scanned using a UV/Vis spectrophotometer between 200 and 400 nm. UV spectrums of Metformin hydrochloride and Nateglinide were overlapped as shown below. For the purpose of simultaneous estimate, the isosbestic point of 226 nm was chosen (figure 1).

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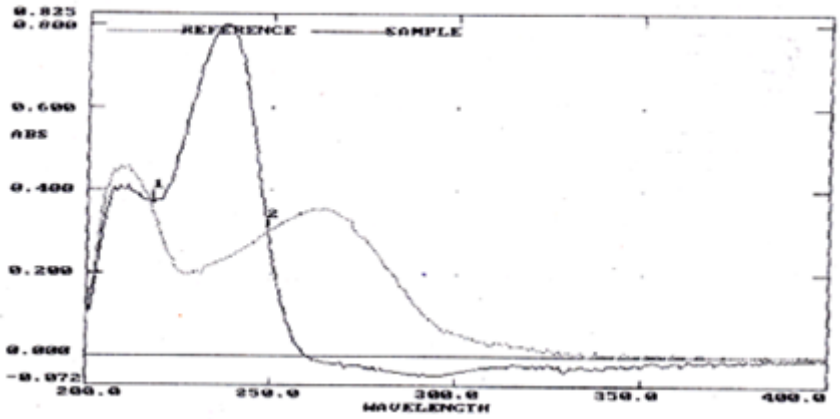

Fig. 1: overlapped UV spectrum of Nateglinide and Metformin hydrochloride

Chromatographic conditions

The developed method used a reverse phase C18 column, Hypersil BDS-C_{18}column (250 X 4.6 mm ID, 5 µm), a mobile phase of phosphate buffer (pH 3.5): Acetonitrile(30:70), flow rate of 1.0 ml/min and a detection wavelength of 226 nm using a UV detector.

Preparation of phosphate buffer

Accurately 2.7 g of potassium dihydrogen phosphate (KH_2PO_4) was weighed and dissolved in water and volume was made up to 1000 ml with water. The pH was adjusted to 3.5 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 and 70 volumes of Acetonitrilewas prepared. The mobile phase was sonicated for 10 min to remove gasses.

Diluent

The mobile phase was used as diluent.

Preparation of standard solutions

Metformin hydrochloride standard stock solutions were made by dissolving 100 mg of Metformin and 24 mg of Nateglinide in an appropriate volume of mobile phase to achieve the

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desired concentrations. Then, after being sonicated for 5 minutes and diluted to 100 ml with mobile phase, the solution was filtered. Additional dilutions of 100 g/ml Metformin and 24 g/ml Nateglinide were generated in 5 replicates by adding 1 ml of stock solution to 10 ml of mobile phase. All concentration goals have been assumed to have been met.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

To improve separation and resolution, several chromatographic settings were used. The performance of the Hypersil BDS-C18 column (250 X 4.6 mm ID, 5 m) was evaluated and determined to be suitable. The UV detector was used to verify the peak purity of Metformin hydrochloride and Nateglinide, and the wavelength of 226 nm was found to be suitable for detecting both drugs with sufficient sensitivity. Many various solvent and pH ratios were explored, but all of them resulted in poor peak form or resolution. The use of a C18 column in isocratic HPLC yielded excellent results in repeated attempts to get a nice, sharp peak with efficient resolution between the two peaks of Metformin hydrochloride and Nateglinide. With a phosphate buffer (pH 3.5): acetonitrile (30:70) mobile phase and a C18-ODS column, running at a flow rate of 1.0 ml/min with a detection wavelength of 226 nm, we obtained excellent retention time, resolution, symmetry, and sensitivity in an isocratic experiment. Metformin hydrochloride and nateglinide were both determined from a standard preparation, and their RP-HPLC chromatograms are shown in (fig. 2).

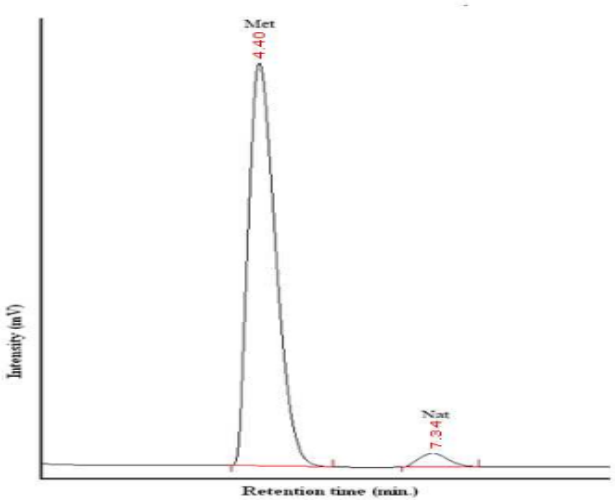

Fig. 2: Typical chromatogram of MET and NAT in Bulk

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SYSTEM SUITABILITY

The chromatographic apparatus was then injected with standard solutions that had been produced in accordance with the testing procedure. Parameters such as theoretical plate count, resolution, and asymmetry factor were examined to determine the system's viability. Table 1 lists the parameters that should be considered while designing a system. It was determined that all of the criteria were suitable.

Table 1: System suitability study Results of MET and NAT

Parameters	Acceptance limits	MET	NAT
Retention time	-	4.40	7.34
Resolution	NLT 2	-	8.232
Theoretical plates	NLT 3000	9489	9583
Tailing factor	NMT 2.0	0.706	0.556

SPECIFICITY

Acid hydrolysis: Upon performance of acid degradation studies 25.3%of MET and 26.8%of NAT was degraded. Alkali hydrolysis: Upon performance of alkali degradation studies 18 % of MET and 19.4%of NAT was degraded. Peroxide hydrolysis: Upon performance of peroxide degradation studies 22.7% of MET and 22.3 % of NAT was degraded. Thermal degradation: Upon performance of Thermal degradation studies 22.23 %of MET and 20.52 %of NAT was degraded. Photolytic degradation: Upon performance of Photolytic degradation studies 26.51 % of MET and 20.22 % of NAT was degraded. Degradation studies results are tabulated in table no.2

Table 2: Degradation study Results of MET and NAT

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Degradation Study	Metformin		Nateglinide	
	Mean Area (n=5)	% Degradation	Mean Area (n=5)	% Degradation
Acid	2988	25.3	293	26.8
Alkali	3267	18	322	19.4
Peroxide	3080	22.7	311	22.3
Thermal	3100	22.23	318	20.52
Photo	2928	26.51	320	20.22

PRECISION

Accuracy of Procedure Studies using the precision approach confirmed the method's accuracy. Working concentration sample solutions were prepared for examination at replicating concentration. Following the test protocol, six injections of sample solutions of MET and NAT were made into the column. Table 3 displays the accuracy findings. The average was determined, and the percent RSD was given. The results for % RSD were determined to be within the allowed range, demonstrating the method's accuracy.

Table 3: Precision study Results of MET and NAT

Preparation	Retention Time	Peak area	Retention Time	Peak area
Preparation 01	4.42	3985.428	7.34	400.124
Preparation 02	4.43	3972.254	7.25	399.159
Preparation 03	4.43	3948.254	7.38	398.568
Preparation 04	4.42	3958.256	7.29	399.587
Preparation 05	4.4	3955.124	7.3	395.235
Preparation 06	4.41	3978.456	7.29	399.567
Average	4.42	3966.30	7.31	398.71
SD	0.01	14.59	0.05	1.78
% RSD	0.2646	0.3679	0.6205	0.4459

LINEARITY

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The linearity of the assay method was assessed by preparing test solutions using standard stock solutions of MET and NAT at five different concentration levels ranging from 50% to 150% of the assay concentration. The relationship between peak area and concentration was analyzed using least-squares linear regression analysis, as depicted in figures 2 and 3. The obtained results demonstrated a strong correlation between peak areas and concentration within the concentration range of 50–150 µg/ml for MET and 10–50 µg/ml for NAT, as presented in tables 4 and 5. The correlation coefficients for both drugs were determined to be 0.999, which satisfies the acceptance criteria for method validation. Consequently, it can be concluded that the assay method exhibits linearity for both Metformin hydrochloride and Nateglinide.

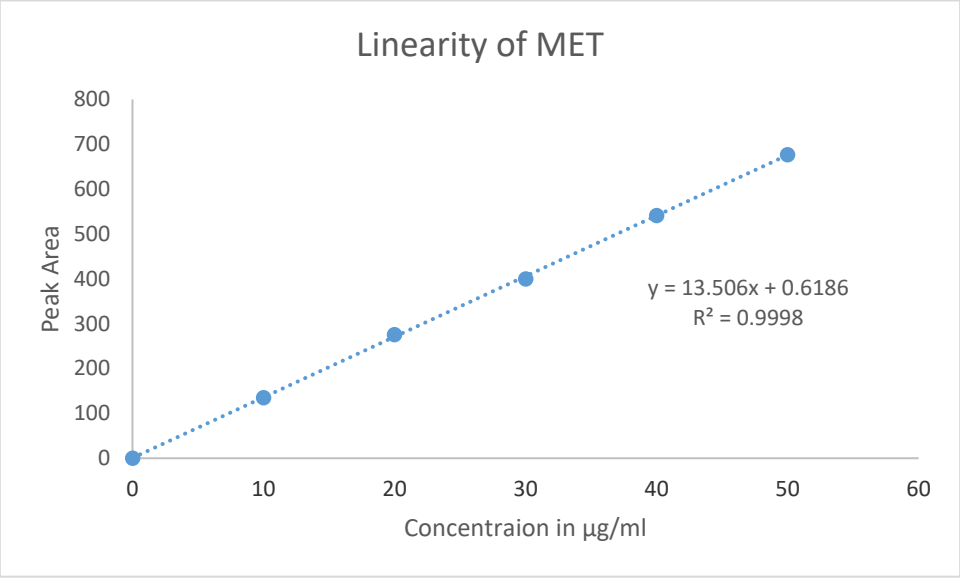

Fig. 2: Linearity chart for MET

Table 4: Linearity study Results of MET

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	1328.46
3	20	2657.45
4	30	3985.13

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5	40	5353.12
6	50	6741.88

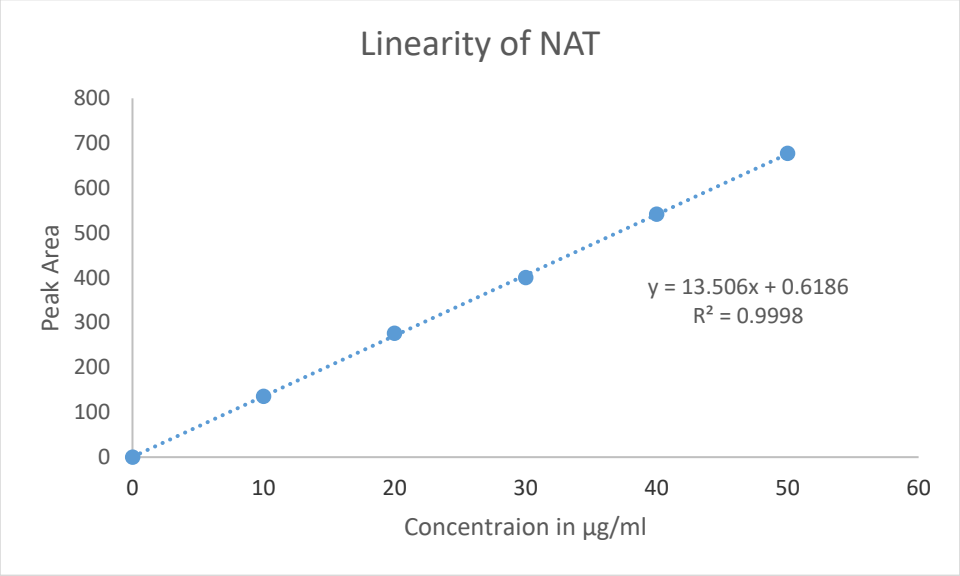

Fig. 3: Linearity chart for NAT

Table 5: Linearity study Results of NAT

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	135.375
3	20	275.751
4	30	400.125
5	40	541.514
6	50	676.875

ACCURACY

The method's accuracy was assessed by recovery trials, which included determining the percentage mean recovery of both medicines at three distinct levels (75%, 100%, and 125%) Three determinations were conducted at each level. The calculation of the percentage recovery and mean percentage recovery for the medicine is shown in Table 6. The data that

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was observed fell within the specified range, suggesting that the approach established is accurate, as seen by the excellent recovery values.

Table 5: Accuracy study Results of MET and NAT

Level (%)	MET		NAT	
	% Recovery	% Mean	% Recovery	% Mean
75	99.89	99.96	100.12	100.30
75	99.87		100.23	
75	100.12		100.56	
100	100.35	100.28	100.56	100.54
100	100.59		100.48	
100	99.89		100.59	
125	100.45	100.26	99.89	100.11
125	100.23		99.87	
125	100.10		100.56	

ROBUSTNESS

In order to assess the robustness of the established methodology, purposeful modifications were made to the experimental settings, and an evaluation of the system suitability characteristics was conducted. The solutions were made in accordance with the prescribed test procedure and then injected under several variable settings, including varying flow rates (0.8 and 1.2 ml/min) and wavelengths (221 nm and 231 nm). The system suitability parameters obtained from these injections were then compared to the technique precision. The findings were recorded and organized in Table 6. The flow rate of 1.0 ml/min exhibits a distinct peak with high resolution, whereas the other flow rates were deemed unsatisfactory. The approach successfully satisfied all system appropriateness characteristics, suggesting its robustness.

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Condition	MET		NAT	
	Plate Count	Tailing Factor	Plate Count	Tailing Factor
1) Change in Flow rate				
Normal Condition (1.0 ml per minute)	9548	1.09	9687	1.02
Flow rate (0.8ml per minute)	9578	1.08	9568	1.12
Flow rate (1.2 ml per minute)	9512	1.08	9521	1.05
2) Change in Wave Length				
Normal: Wave Length 226nm	9545	1.09	9545	1.12
Wave Length 221nm	9565	1.05	9785	1.05
Wave Length 231nm	9556	1.07	9632	1.23

CONCLUSION

The RP-HPLC technique that was suggested demonstrated characteristics of simplicity, specificity, accuracy, precision, robustness, rapidity, and cost-effectiveness. This approach demonstrates high precision in distinguishing between the two drugs, while also offering a rapid analytical time. The RP-HPLC approach that has been presented demonstrates potential use for the routine analysis of Metformin hydrochloride and Nateglinide in bulk forms.

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