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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NATEGLINIDE AND METFORMIN

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ABSTRACT:

Objective: In this study, the proposed approach for evaluating nateglinide and metformin is validated. As a result, the approach may be utilised for stability and routine quality control examination.The following describes the planned work's goal and scope:

1. To create an appropriate spectrophotometric approach for drug testing
2. Carry out the method's validation Since this will determine the validation qualities that need to be assessed, the analytical procedure's purpose should be defined explicitly.

Method: The method used a reverse phase column, C18a mobile phase comprising of phosphate buffer Acetonitrile: methanol (30:55:15) 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 4.183 min and Nateglinide at 5.264 min. The calibration curves were linear (r^2=0.999) in the concentration range of 10-120 µg/ml and 1000 µg/ml for Metformin hydrochloride and Nateglinide respectively. The percentage recoveries were found to be 98.59-103. The LOD was found to be 1.25 µg/ml and 0.754 µg/ml for Metformin hydrochloride and Nateglinide respectively. LOQ was found to be 3.81µg/ml and 2.28µg/ml for Metformin hydrochloride and Nateglinide respectively

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

INTRODUCTION

Developing a reliable and precise analytical technique for the medicine is the most crucial phase in any formulation development process. The quantity of the medicine in the formulation must be ascertained using a precise analytical procedure. HPLC and UV spectroscopy are two of the tools that are most often utilised. In the discovery, development, and production of medicines, analytical technique development and validation are crucial processes. To assure the identification, purity, potency, and effectiveness of medicinal goods, quality control laboratories employ the official test procedures that come from these processes. Method validation is the process of demonstrating, by empirical research, that an analytical approach is suitable for the purpose for which it is being used. . A well-developed method should be easy to validate and should be developed with the aim to rapidly test preclinical samples, formulation prototypes and commercial samples.

NATEGLINIDE

An oral antihyperglycemic drug called nateglinide is used to treat non-insulindependent diabetes mellitus (NIDDM). It is a member of the meglitinide class of short-acting insulin secretagogues, which work by attaching to pancreatic beta cells to promote the release of insulin. A derivative of an amino acid called nateglinide causes an early insulin response to meals, which lowers blood glucose levels after meals. Mealtime dosages should be avoided if a meal is missed, and it should only be taken with meals. Before a reduction in fasting blood glucose is noticed, treatment is needed for about a month. Neglitnides may either have no impact on weight or slightly raise it. In comparison to sulfonylureas and insulin, meglitinides tend to produce less weight gain on average. to occur only in those naïve to oral antidiabetic agents. [1]

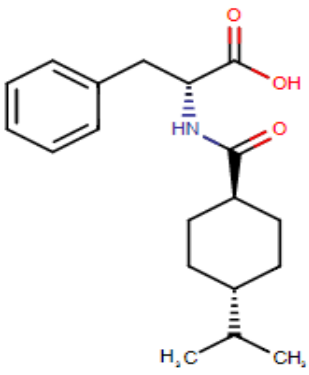

Fig. 1 Nateglinide

METFORMIN

Metformin is an antihyperglycemic drug of the biguanide family, used for the treatment of type II diabetes). Currently, metformin is the primary treatment of choice for the therapy of type II diabetes and is administered to at least 120 million patients globally. Because it decreases type II diabetes patients' blood glucose levels without resulting in hypoglycemia, metformin is regarded as an antihyperglycemic medication. Metformin is frequently defined as an insulin sensitizer that reduces insulin resistance and

plasma fasting insulin levels by a clinically relevant amount. Modest weight reduction is another well-known advantage of this medication. Patients with type II diabetes who are obese typically take metformin. Metformin was first authorised in Canada in 1972, then in the USA in 1995. This drug is available in regular and extended-release forms. [2]

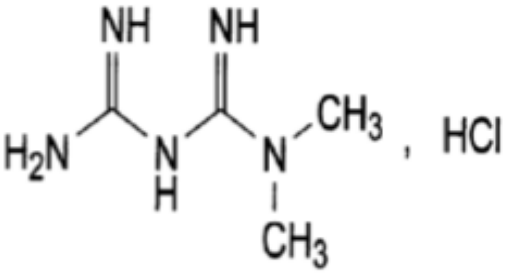

Fig 2. Metformin

MATERIALS AND METHODS:

Chandra laboratories in Hyderabad provided Nateglinide and Metformin hydrochloride as a gift sample, Acetonitrile, Methanol, Orthophosphoric Acid (OPA), and HPLC grade water were all purchased from Merck. In this research, only HPLC-grade solvents were employed. This approach used the RP-HPLC Shimadzu (LC 20ATVP) model with the Spin chrome (LC SOLUTIONS) software. Inertsil ODS C18 (250x4.6 mm, 5) is an analytical column used for the separation of analytes.

Preparation of standard curve of Nateglinide by RP-HPLC

Table 1: Calibration curve of Nateglinide by RP-HPLC

Sr. No.	Concentration µg/ml	Area
1	10	254.00±4.36
2	20	503.33±5.51
3	30	750.33±4.16
4	40	922.33±10.02
5	50	1156.00±9.85
6	60	1365.33±7.64
7	80	1782.67±6.81
8	100	2213.00±7.00

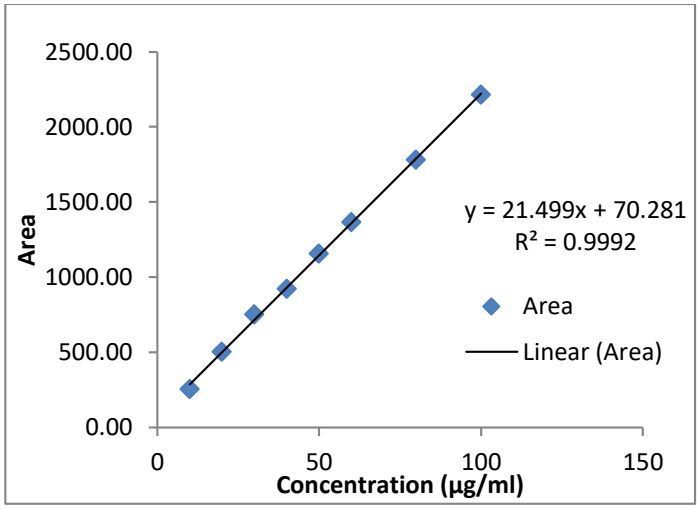

Figure 3: Graph of standard calibration curve of Nateglinide by RP-HPLC

Table 2: Result of Statistical parameters for estimation of Nateglinide

Statistical parameters	**Results**
Regression equation: y=mx+C	Y=21.499x + 70.281
Slope (m)	21.49
Intercept (C)	70.281
Correlation coefficient (r^2)	0.999

Discussion: - The calibration curve for Nateglinide was obtained by using the 10 to 100 µg/ml solution. The area was measured at 221 (Isosbestic point). The calibration curve as shows in graph indicated the regression equation Y = 21.499x + 70.281 and R^2 value 0.999 which shows good linearity.[3]

Preparation of standard curve of Metformin HCL by RP-HPLC

Table 3: Calibration curve of Metformin HCL

Sr. No.	**Concentration µg/ml**	**Area**
1	10	2440.00±44.91
2	20	3438.33±35.16
3	30	4299.33±28.73
4	40	5139.00±15.13
5	50	6051.00±24.64
6	60	6911.67±12.66
7	80	8437.00±14.74
8	100	10188.00±51.73

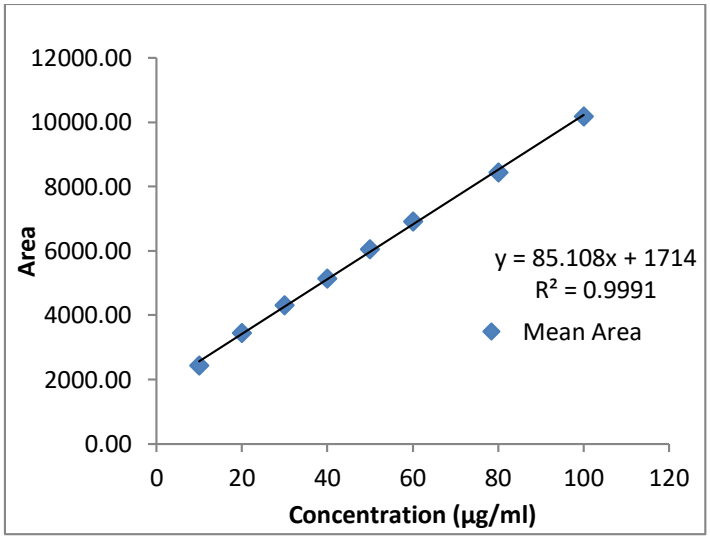

Figure 4: Graph of standard calibration curve of Metformin HCL by RP-HPLC

Table 4: Result of Statistical parameters for estimation of Metformin HCL

Statistical parameters	**Results**
Regression equation: y=mx+C	Y=85.108x + 1714
Slope (m)	85.108
Intercept (C)	1714
Correlation coefficient (r^2)	0.999

Discussion: - The calibration curve for Metformin HCL was obtained by using the 10 to 100 µg/ml solution. The area was measured at 221 (Isosbestic point). The calibration curve as shows in graph indicated the regression equation Y = 85.108x + 1714 and R^2 value 0.999 which shows good linearity as shown in Figure 24.

Fourier transmission Infra-Red Spectroscopy

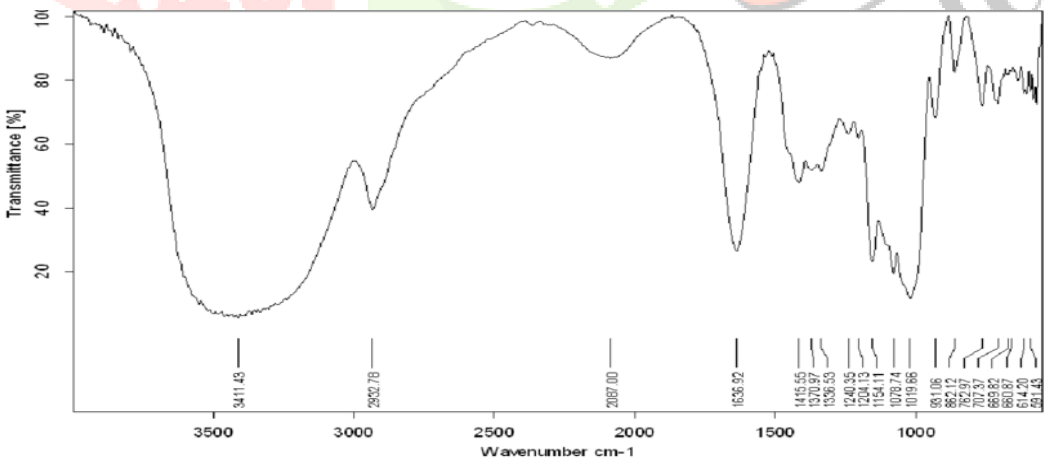

Fig 5. FTIR of Nateglinide

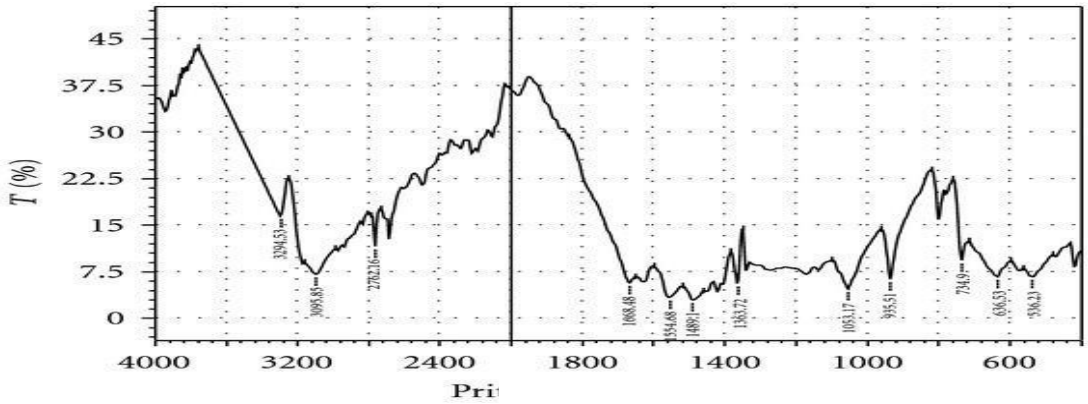

Fig 6. FTIR of Metformin

Table 5: FTIR Study

Characteristics Peaks	Metformin	Nateglinide
C=O stretching	1668.48	1556.92
OH stretching	3294.53	2067.00
CH stretching	3095.85	2983.00
CH bending alkenes, and SP^2-CH stretching	1448.1	3411.43

The FTIR spectra of Metformin HCL and Nateglinide were shown. The principal IR absorption peaks. This observation confirmed the purity and authenticity of the Metformin HCL and nateglinide [4]

Selection of wavelength:

Standard solutions of Metformin hydrochloride and Nateglinide were made at a concentration of 1000 g/ml system scanned at 200-400 nm using a UV/Vis spectrophotometer. [5]

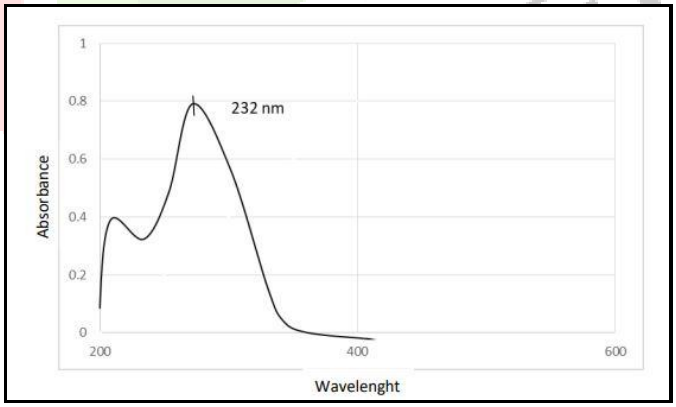

Fig.7 UV spectrum of metformin hydrochloride

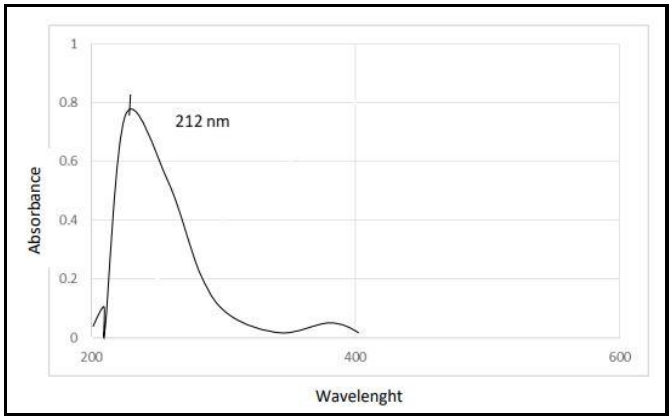

Fig.8 UV spectrum of Nateglinide

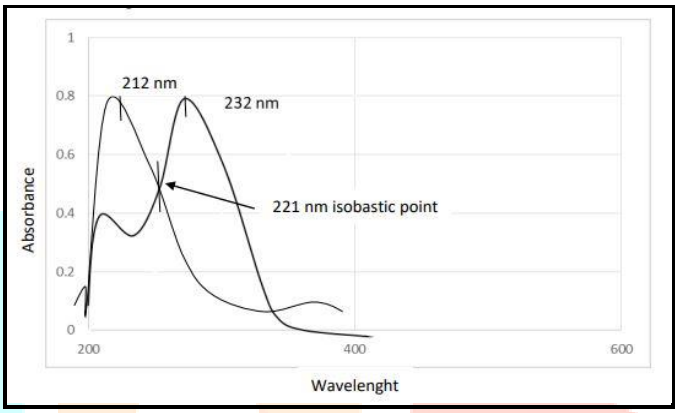

Fig.9 UV overlaps spectrum of Nateglinide and Metformin hydrochloride

Chromatographic conditions:

Stationary Phase	C18, 250 × 4.6 mm, 5µm particle size, Phenomenex
Elution mode	Low pressure gradient mode
Mobile Phase:	Methanol : Water
Detector	UV
Absorption maxima	221 nm
Column Temperature	30 °C
Flow rate:	1 ml/min
Injection volume	20 µl
Diluent	Mobile phase

Preparation of phosphate buffer:

A precise 2.7 g of potassium dihydrogen phosphate (KH2PO4) was weighed and dissolved in water, and a volume of 1000 ml was prepared using water. Orthophosphoric acid was used to modify the PH to 4.0. All fine undissolved particles were filtered out of the buffer.

Preparation of mobile phase:

30 volumes of phosphate buffer, 55 volumes of acetonitrile, and 15 volumes of methanol were mixed together. To eliminate vapours, the mobile phase was sonicated for 10 minutes.

Preparation of standard solutions:

10 mg of pure compound was placed in an eppendorf tube and diluted in 1 ml of diluents (as described above) to make a 1000 g/ml stock solution. 0.01 ml of the stock was obtained and diluted with diluents up to 1 ml to achieve the required dilutions, then filtered through 0.22 millipore membrane filters and injected into the HPLC [6]

HPLC Method Development:

To create optimal separation and resolution, several chromatographic settings were used. A suitable C18-ODS 3V (250 4.6 mm, 5 m) column was discovered. UV detector was used to determine the peak purity of metformin hydrochloride and nateglinide, and 221 nm was found to be sufficient for detecting both medicines with adequate sensitivity. We tested a variety of solvents in various ratios throughout a wide pH range, but either the peak shape was too broad or the resolution wasn't very good. The results of several attempts to generate a nice, crisp peak with an effective resolution between two peaks of metformin hydrochloride and nateglinide using an isocratic HPLC and a C18 column were adequate. The isocratic experiment ran well with phosphate buffer as the mobile phase (pH 4.0): Methanol: Acetonitrile (30:55:15). [7]

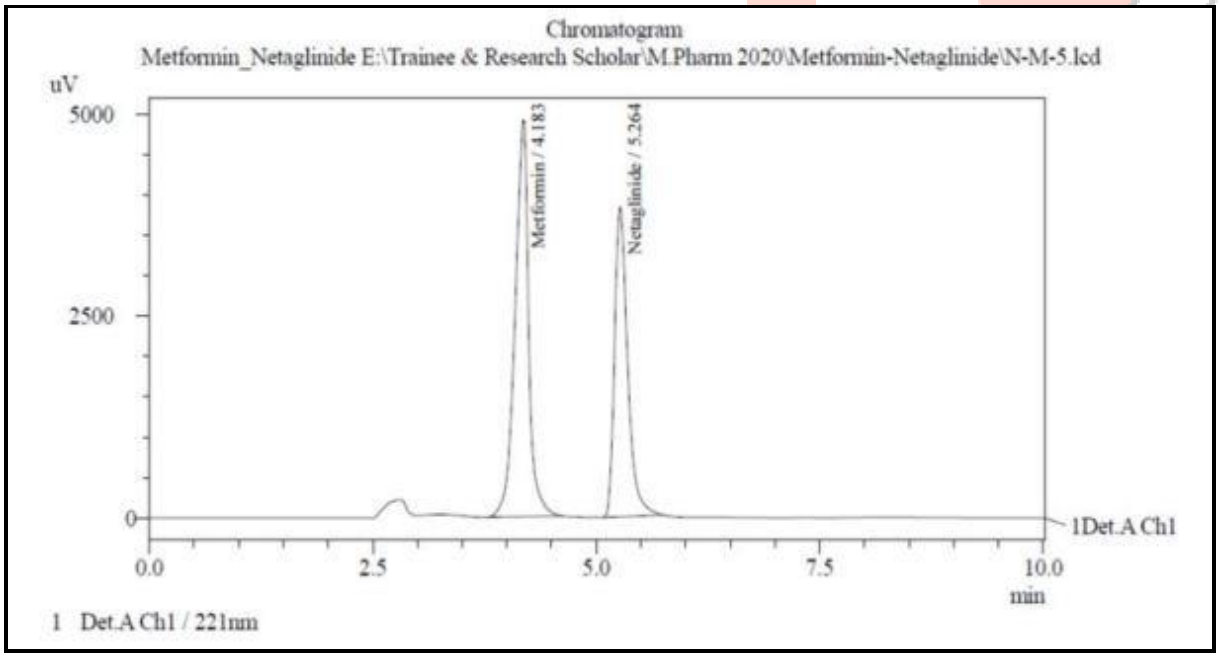

Fig.10 Simultaneous chromatogram of standard metformin hcl and nateglinide

RESULTS AND DISCUSSION:

Method validation:

According to ICH regulations, the developed RP-HPLC technique underwent validation for factors such system appropriateness, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness.

System suitability:

The chromatographic method establishes standard solutions that had been produced in accordance with the procedure. Theoretical plates, resolution, and asymmetry factor were examined as system appropriateness characteristics. Calculations were made for the system appropriateness parameter. The restrictions for each parameter were discovered to be met. Retention time for metformin is 4.183 and for nateglinide is 5.26. 5230 and 5380 on the theoretical plate MET's trailing factor is 1.01, while nateglinide is 1.11.[8]

Table 6: Results of system suitability studies

Parameters	Acceptance limits	Metformin hydrochloride	Nateglinide
Retention time (min)	-	4.183	5.261
Resolution	NLT 2	-	14.232
Theoretical plates	NLT 2000	8489	7583
Tailing factor	NMT 2	1.706	1.556

Linearity:

Metformin hydrochloride and nateglinide standard stock solution were prepared at five concentration levels, ranging from 80% to 120% of the assay concentration, to assess the linearity of the test solutions for the assay technique. Least-squares linear regression technique was used to treat the peak area versus concentration data. The approach was declared to be linear for both pharmaceuticals since the correlation coefficients for both drugs were determined to be 0.999, which meets the method validation acceptance requirements.[9]

Table 7: Linearity of Metformin HCL

Conc. (µg/ml)	Area-1
10	2488
20	3426
30	4332
40	5156
50	6048
60	6898
80	8426
100	10234
120	11900

Table 8: Linearity of Nateglinide

Conc. (µg/ml)	Area-1
10	256
20	498
30	747
40	912
50	1148
60	1357
80	1785
100	2210
120	2590

Figure 11: Linearity 1 graph of Metformin HCL

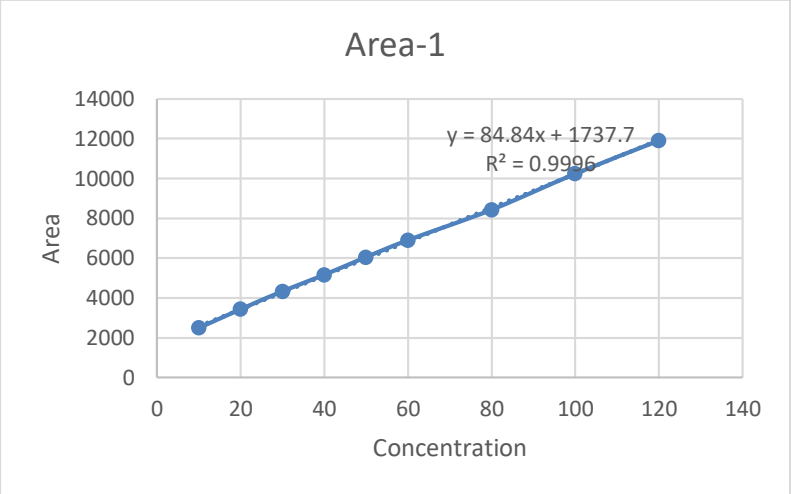

Figure 12: Linearity 1 graph of Nateglinide

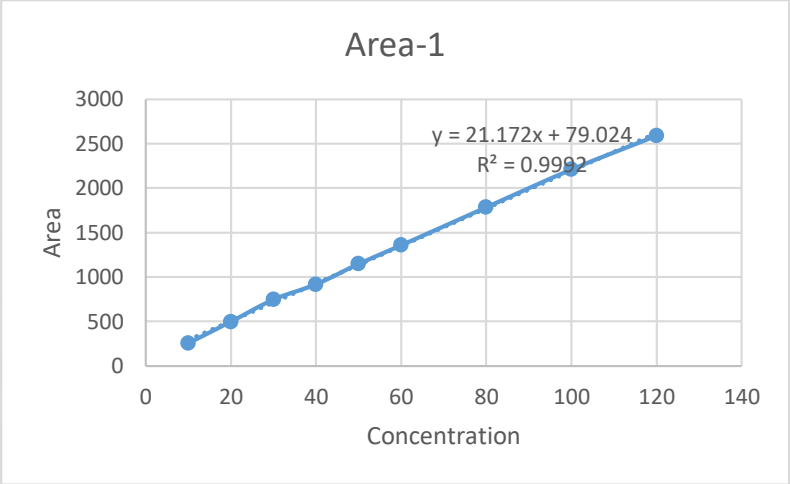

Table 9: Linearity data for Metformin hydrochloride and Nateglinide

Statistical parameters	**Results of Metformin**	**Results of Nateglinide**
Regression equation: y=mx+C	Y=84.84x + 1737.7	21.172x+ 79.024
Slope (m)	84.84	21.172
Intercept (C)	1737.7	79.024
Correlation coefficient (r^2)	0.9996	0.9992

Accuracy:

Accuracy tests that calculated the percent mean recovery of both medications at three distinct levels were used to evaluate the method's accuracy (80 percent , 100 percent and 120 percent). Three decisions were made for each stage. Table 6 and 7 displays the percentage recovery and mean percentage recovery that were determined for the medicine. The fact that the observed data fell within the necessary range shows that the procedure was established accurately and also points to good Accuracy values.[10-11]

Table 10: Accuracy Study of Metformin HCL

			Standard Deviation		Accuracy	Precision
Conc.	Conc.	Area	Mean	SD	%SD	%RSD
	80	8426				
1	80	8454	8437.333333	14.74222959	0.1747262	
	80	8432				
	100	10234				
2	100	10198	10188	51.73006863	0.5077549	0.238184869
	100	10132				
	120	11900				
3	120	11910	11906.33333	5.507570547	0.0462575	
	120	11909				

Table 11: Accuracy Study of Nateglinide

			Standard Deviation		Accuracy	Precision
Conc.	Conc.	Area	Mean	SD	%SD	%RSD
	80	1785				
1	80	1788	1782.666667	6.806859286	0.3818358	
	80	1775				
	100	2210				
2	100	2221	2213	7	0.3163127	0.116307966
	100	2208				
	120	2590				
3	120	2595	2594.333333	4.041451884	0.15578	
	120	2598				

The results indicate that the recoveries are well within the 98 percent to 103 percent acceptability range, demonstrating a high degree of sensitivity for the technique to detect analytes in the sample. As a result, the procedure is reliable and useful for drug estimation.

Method precision:

Presicion method studies were used to verify the approach's precision. The sample solution was made up at a workable concentration, and replicating analysis was done. According to the test technique, sample solutions of metformin hydrochloride and nateglinide were produced and injected into the column six times.Contains a summary of the precision results. The average was determined and the percent RSD was provided. The approach was confirmed to be accurate, and percent RSD values were within the bounds.[12]

Table 12: Method precision data

Sr.no.	Precision	Percentage recovery of Metformin	% RSD	Percentage recovery of Netaglinide	% RSD
1	Repeatability	101.972±0.160	0.157	100.39±0.282	0.281
2	Inter Day	101.882±0.151	0.148	100.641±0.261	0.259
3	Intra Day	101.949±0.128	0.125	100.998±0.415	0.411

Robustness:

Experimental conditions were purposefully changed, and system suitability characteristics were assessed, to ascertain the robustness of the devised procedure. The precision of the test technique was compared to the solutions generated according to the test procedure and injected at various variable circumstances, including flow rate (0.8, 1.2 ml/min.) and wavelength (219, 223 nm). Table 5 is a summary of the findings. A strong peak with high resolution is seen at the flow rate of 1.0 ml/min, while the rest of the flow rates were judged to be unsatisfactory. The technique was resilient since it satisfied all system appropriateness criteria. [13-16]

Table 13: Robustness data of Nateglinide with deliberate change in wavelength

conc.(µg/ml)	Wavelength 216nm (Area)	Wavelength 221nm (Area)	Wavelength 226nm (Area)
50	1147	1148	1143
50	1145	1149	1139
50	1149	1143	1138
50	1151	1151	1141
50	1153	1153	1140
50	1148	1161	1145
Mean	1148.8	1150.8	1141.0
SD	2.9	6.0	2.6
%RSD	0.249	0.523	0.229

Table 14: Robustness data of Metformin HCL with deliberate change in wavelength

conc.(µg/ml)	Wavelength 216nm (Area)	Wavelength 221nm (Area)	Wavelength 226nm (Area)
50	5936	6044	6127
50	5966	6048	6124
50	5929	6055	6143
50	5958	6064	6099
50	5968	6028	6094
50	5947	6043	6111
Mean	5950.7	6047.0	6116.3
SD	16.0	12.2	18.5
%RSD	0.270	0.201	0.302

Table 15: Robustness data of Nateglinide with deliberate change in flow rate (ml/min)

conc.(µg/ml)	Flow rate 0.8ml/min (Area)	Flow rate 1ml/min (Area)	Flow rate 1.2ml/min (Area)
50	1168	1154	1133
50	1171	1155	1129
50	1173	1148	1130
50	1167	1146	1141
50	1166	1153	1138
50	1163	1155	1136
Mean	1168.00	1151.83	1134.50
SD	3.578	3.869	4.680
%RSD	0.306	0.336	0.412

Table 16: Robustness data of Metformin HCL with deliberate change in flow rate (ml/min)

conc.(µg/ml)	Flow rate 0.8ml/min (Area)	Flow rate 1ml/min (Area)	Flow rate 1.2ml/min (Area)
50	6155	6049	5899
50	6148	6048	5898
50	6147	6055	5911
50	6175	6064	5916
50	6162	6028	5921
50	6168	6043	5919
Mean	6159.2	6047.8	5910.7
SD	11.2	12.1	10.0
%RSD	0.2	0.2	0.2

The Percentage RSD should not be more than 2. The %RSD obtained for change of flow rate and wavelength was found to be below 2, which was within the acceptance criteria. Hence the method was robust.

LOD and LOQ

Table 17: LOD and LOQ data

Drug	LOD (µg/ml)	LOQ (µg/ml)
Metformin	1.25	3.81
Netaglinide	0.754	2.28

The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The results obtained were within the limit. [17-19]

Ruggedness

The ruggedness was studied by analyzing the same samples same drug by changing analyst. The change in the responses of drugs was noted in terms of % RSD.

Table 18: Analyst to Analyst variation data

S. No.		Percentage recovery of Metformin	% RSD	Percentage recovery of Nateglinide	% RSD
1	Analyst 1	101.910± 0.098	0.096	100.610± 0.327	0.325
2	Analyst 2	101.784± 0.098	0.096	100.486± 0.186	0.185

The Percentage RSD should not be more than 2. The % RSD obtained for change of analyst was found to be below 2, which was within the acceptance criteria. Hence the method was rugged. [20]

CONCLUSION:

The proposed RP-HPLC technique was discovered to be simple, specific,accurate, precise, robust, rapid and economical. this approach provides high resolution between all two chemicals. The regular analysis of metformin hydrochloride and nateglinide in tablet dosage form may benefit from using the suggested RP-HPLC technique.

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