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Liquid Chromatographic Method for the Determination of Metformin and Nateglinide in Pharmaceutical Formulations

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A simple and sensitive reverse phase high performance liquid chromatographic method for the determination of metformin and nateglinide was developed on a Shimadzu class vp series HPLC system on a reverse phase Gemini C_{18} column (150 mm × 4.6 mm, i.d., 5 μ) using a mobile phase mixture containing methanol and phosphate buffer (pH 3) in the ratio of 60:40. The flow rate was 0.8 mL/min and effluents were monitored at 235 nm and eluted at 2.63 (metformin) and 4.51 min (nateglinide). Calibration curve was plotted with a range from 0.5-50.0 μg/mL for metformin and 0.06-6.00 μg/mL for nateglinide. The assay was validated for the parameters like accuracy, precision, robustness, detection and quantification limits and system suitability parameters. The method was applied for the assay of dosage forms. The results were found to be satisfactory and the method can be adapted for the routine quality control of the drugs in pure and pharmaceutical dosage forms.

Key Words: Metformin, Nateglinide, RP-HPLC, Validation, Pharmaceutical Formulations.

INTRODUCTION

Metformin (Fig. 1a) (I,N,N-dimethyldiguanide) where as nateglinide (Fig. 1b) is [N(*trans*-4-isopropylcyclohexylcarbonyl)-d-phenylalanine][1] and are used in the treatment of type 2 diabetes. Metformin improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis, where as nateglinide, a d-phenylalanine derivative lacking either a sulfonylurea or benzamido moiety, is a novel oral meal-time glucose regulator and was approved for the treatment of type II diabetes mellitus recently[2,3] works by stimulating the pancreas to release insulin by closing the ATP-dependent potassium channels in the β-cell membrane, which leads to an opening of the calcium channels. The resulting influx of calcium induces insulin secretion.

The literature reveals that there are some of the methods have been reported for metformin and nateglinide in single dosage forms[4-9].

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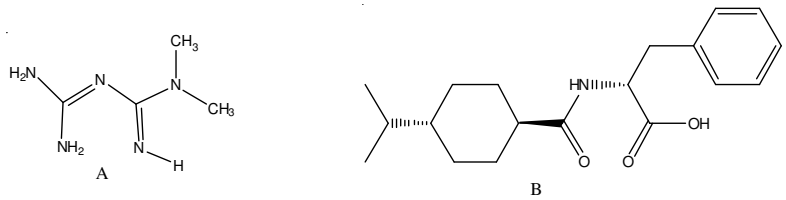

Fig. 1. Chemical structure of (A) metformin and (B) nateglinide

The present paper describes a simple, sensitive, validated and economic method for the determination of metformin and nateglinide.

EXPERIMENTAL

Metformin was obtained from Macleoids Pharmaceuticals Ltd., Mumbai, India and nateglinide was obtained from Divis laboratories, Hyderabad, India. Methanol (HPLC grade, Qualigens, Mumbai), MilliQ water was used through out the analysis. All the other reagents were of analytical reagent grade.

The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-visible detector. The data acquisition was performed by Spincotech 1.7 software. The method was developed on a reverse phase column Gemini C_{18} (150 mm × 4.6 mm i.d., 5 µm) (Phenomenex, Torrance, USA) with a guard column maintained at ambient temperature. The mobile consisted of a mixture of methanol and phosphate buffer (pH 3) fixed in the ratio of 60:40 at a flow rate of 0.8 mL/min.

Preparation of stock and sample solutions: The standard stock solutions were prepared with methanol to give the final concentration of 1000 mg/mL. The working standard solutions of metformin and nateglinide were prepared by taking suitable aliquots of drug solution from the standard solutions and the volume was made up to 10 mL with mobile phase to get concentrations of 0.5-50.0 µg/mL for metformin and 0.06-6.00 µg/mL for nateglinide.

For the analysis of pharmaceutical formulations, ten tablets were weighed and powdered. A quantity equivalent to labeled amount was weighed and transferred into extraction flask. To this suitable amount of methanol was added and the mixture was subjected to vigorous shaking for 0.5 h for complete extraction of drugs and the solution filtered and diluted with mobile phase and injected to HPLC system for the analysis.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: Since metformin is a highly polar and strongly basic compound (pK_a = 12.4). It is poorly retained in reversed-phase LC mode. Preliminary experiments indicated that using different concentrations of acetonitrile or methanol with different pHs of the buffers, did not produce a suitable retention and peak shape of metformin on a C_{18} column. A reverse-phase

column procedure was proposed as a suitable method for the simultaneous determination of metformin and nateglinide in combined dosage forms. The chromatographic conditions were optimized by changing the mobile phase composition, pH and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of methanol and phosphate buffer (pH 3) in the ratio of 60:40 fixed at flow rate of 0.8 mL/min was used.

A typical chromatogram obtained by using the aforementioned mobile phase from 20 µL of the assay preparation is illustrated in Fig. 2 (a). The retention factors of metformin and nateglinide were 2.63 and 4.51 min, respectively.

Validation of the method: The proposed method was validated with respect to stability, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness according to the ICH guidelines[10].

Stability: Stability of the standard solutions of metformin and nateglinide was evaluated under different storage conditions. For short-term stability, working standard solutions were kept at room temperature for 24 h. The long-term stability was assessed after storage of stock solutions at 4 ºC for 2 months. The stability results were evaluated by comparing peak area ratios of metformin and nateglinide with those of freshly prepared standard solutions. The results found within 97.24-102.13 % of initial values indicate that metformin and nateglinide can be considered stable under the conditions investigated.

Specificity: Specificity, described as the ability of a method to discriminate the analyte from all potential interfering substances, was evaluated by preparing the analytical placebo and it was confirmed that the signals measured were caused only by the analytes. A solution of an analytical placebo (containing all the ingredients of the formulation except the analyte) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of the inactive ingredients (placebo), before and after being spiked with standards, standard solutions (Fig. 2a) and the commercial pharmaceutical preparations including metformin and nateglinide (Fig. 2b) were analyzed by the proposed method. The representative chromatograms show no other peaks which confirm the specificity of the method.

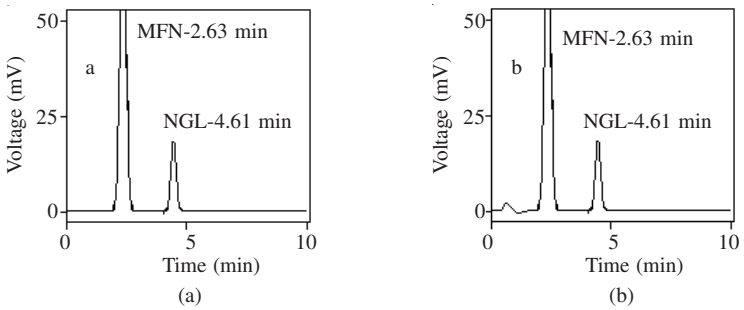

Fig. 2. A typical chromatogram showing the peaks of 5 µg/mL metformin (2.63 min) and 0.6 µg/mL nateglinide (4.61 min) (a) in pure and (b) formulations

Linearity: The linearity of the method was tested with the concentrations of 0.5, 1.0, 5.0, 10.0, 25.0 and 50.0 µg/mL for metformin and 0.06, 0.12, 0.60, 1.20, 3.00 and 6.00 µg/mL for nateglinide. Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/mL. In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations and the correlation coefficients for the regression line were 0.9996 and 0.9991 for metformin and nateglinide, respectively.

LOD and LOQ: The limits of detection (LOD) defined as signal-to-noise ratio of 3:1 and limit of quantification (LOQ) defined as signal-to-noise ratio of 10:1 were about 0.140, 0.480, 0.016 and 0.056 µg/mL, respectively for metformin and nateglinide with acceptable precision and accuracy under the stated conditions.

Precision: The assay was investigated with respect to repeatability and intermediate precision. In order to measure repeatability of the system (injection repeatability), ten consecutive injections were made with a standard solution containing 2 µg/mL metformin and nateglinide. The results were evaluated by considering retention time, peak area, capacity factor, peak asymmetry and resolution values of metformin and nateglinide. The data show good precision of the system with a RSD = 1 % (Table-1). Three different concentrations of metformin and nateglinide (in the linear range) were analyzed in six independent series in the same day (intra-day precision) and six consecutive days (inter-day precision) within each series every sample was injected three times. The RSD values of intra- and inter-day studies varied from 0.87-7.64 % showed that the precision of the method was satisfactory (Table-2).

TABLE-1
SYSTEM PRECISION DATA FOR METFORMIN AND NATEGLINIDE

	Metformin	Nateglinide
Retention time (min)	2.63 ± 0.002	4.51 ± 0.001
Peak area	207652.21 ± 421.67	129453.13 ± 124.21
Capacity factor[a]	0.71 ± 0.001	1.19 ± 0.001
Peak asymmetry	1.26 ± 0.007	1.38 ± 0.005
Resolution	–	2.56 ± 0.02

Mean ± standard error, methanol was used for the determination of dead volume.

Accuracy and recovery studies: The accuracy of the proposed method is determined by calculating the per cent difference (bias %) between the measured mean concentrations and the corresponding nominal concentrations. Table-2 shows the results obtained for intra and inter-day accuracy. The accuracy of the proposed method was also tested by recovery experiments. Recovery experiments were performed by adding known amounts of metformin and nateglinide to the analytical placebo solution. metformin and nateglinide were spiked at three different concentrations (60 mg nateglinide/500 mg nateglinide, 120 mg nateglinide/500 mg metformin) according to label claim in the pharmaceutical preparations. Six samples were prepared

TABLE-2
INTRA-DAY AND INTER-DAY ACCURACY AND PRECISION DATA OF METFORMIN AND NATEGLINIDE (n = 6)

	Added (µg/mL)	Intra-day			Inter-day		
		Found[a] (ng/mL)	Precision (RSD %)	Accuracy[b] (Bias %)	Found[a] (ng/mL)	Precision (RSD %)	Accuracy[b] (Bias %)
Metformin	10.0	9.92 ± 0.02	1.64	-0.80	9.87 ± 0.03	3.64	-1.30
	20.0	9.85 ± 0.03	0.54	-0.75	19.82 ± 0.05	2.17	-0.90
	50.0	0.37 ± 0.05	1.28	0.74	49.89 ± 0.06	1.31	-0.22
Nateglinide	1.2	1.19 ± 0.003	1.09	-0.83	1.18 ± 0.002	2.76	-1.00
	3.0	2.89 ± 0.005	2.13	-3.67	3.01 ± 0.006	0.89	0.33
	6.0	6.03 ± 0.008	0.69	0.50	5.93 ± 0.004	1.47	-1.67

(RSD %: Relative standard deviation). a: Mean ± standard error, b: Bias%: [(measured value - added value)/added value] × 100.

for each recovery level. Samples were treated as described in the procedure for sample preparation. The obtained recoveries were between 100.61-98.64 % with RSD between 0.75-1.88 % (n = 3).

Robustness: Robustness relates to the capacity of the method to remain unaffected by small but deliberate variations introduced into the method parameters. Several experimental parameters, like mobile phase methanol ratio (58-62 %), buffer pH (2.8-3.2) and flow rate (0.7-0.9 mL/min), were varied around the value set in the method to reflect changes likely to arise in different test environments. Analyses were carried out in triplicate and only one parameter was changed in the experiments at a time. The determination of 0.6 µg/mL nateglinide and 5 µg/mL metformin under the various conditions was performed. Each mean value was compared with the mean value obtained by optimum conditions. The statistically comparison was done with t test[11] and no difference was found between results (p = 0.05). Therefore, the method is robust to the small changes in experimental conditions (Table-3).

Application of the method for the analysis of pharmaceutical formulations: The method developed was found to be specific for the quantitative determination of metformin and nateglinide in bulk drugs and also subjected to validation for different parameters. This has been applied for the estimation of drugs in pharmaceutical. Each sample was analyzed in triplicate after extracting the drug as mentioned above in experimental section. The amount of metformin and nateglinide were found to be within the range of 97.56-101.21 %. None of the tablet excipients were found to interfere with the analyte peak and the results were shown in Table-4.

Conclusion

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of metformin and nateglinide from pure and pharmaceutical dosage forms. The mobile phase is simple to prepare and the run time was less than 6 min which consumes less than 5 mL of mobile phase that shows the method

TABLE-3
ROBUSTNESS DATA FOR 0.6 µg/mL OF NATEGLINIDE AND 5 µg/mL OF METFORMIN

Condition		RSD (%)	
		Metformin	Nateglinide
Standard		0.92	0.89
Methanol variants (%)	58	1.71	1.45
	62	1.56	0.91
Flow rate variants (mL/min)	0.7	1.29	1.67
	0.9	0.87	1.28
pH Variants	2.8	1.35	1.73
	3.2	1.43	1.54

TABLE-4
DATA FROM ANALYSIS OF PHARMACEUTICAL FORMULATIONS BY THE DEVELOPED LC METHOD

Brand		Label claim (mg)	Found (mg)	RSD (%)	Amount recovered (%)
Glinate MF	Metformin	500	496.27	2.78	99.25
	Nateglinide	60	59.08	3.01	98.47
Glinate MF	Metformin	500	495.01	1.86	99.00
	Nateglinide	120	120.45	2.34	100.37

was economical. The sample recoveries in all dosage forms were in good agreement with their respective label claims and none of excipients interfered in the estimation. Hence, the method can be easily and conveniently adopted for routine quality control of metformin and nateglinide in combined dosage forms and can also be used for dissolution or similar studies.

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