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# New Validated Method for the Estimation of Metformin HCl and Nateglinide Using RP-HPLC

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**ABSTRACT**

Special, effective high pressure liquid chromatography method has been developed for the simultaneous quantification of Metformin HCl and Nateglinide. By using Waters HPLC e-2695 quaternary pump with a PDA detector of 2998 instrument the chromatographic separation of Metformin HCl and Nateglinide was achieved on the column of Luna Phenyl Hexyl 150X4.6mm, 3.5µ using an isocratic elution with a buffer containing 0.1percent ortho phosphoric acid and acetonitrile at a rate of 70:30 as a mobile phase with a flow rate of 1 ml/min at ambient temperature. A detector wavelength of 221 nm utilizing the PDA detector were given in the instrumental settings. The linearity was studied between the concentration range of 5-75 µg/ml of Metformin HCl and 0.6-9 µg/ml of Nateglinide were injected. The plotted calibration curves were linear with a regression coefficient of $R^2$> 0.999, indicates that the linearity was with in the limit. As a part of method validation the parameters like specificity, linearity, accuracy, ruggedness, robustness were determined and the results were found to be within the allowable limit. The method developed was found to be applicable to routine analysis and to be used for the measurement of both active pharmaceutical ingredients (i.e, Metformin HCl and Nateglinide). Validation of the proposed method was carried out according to an International Conference on Harmonization (ICH) guidelines. Since, there is HPLC method reported in the literature for the estimation of Metformin HCl and Nateglinide, there is a need to develop quantitative methods under different conditions to achieve improvement in specificity, selecivity etc.

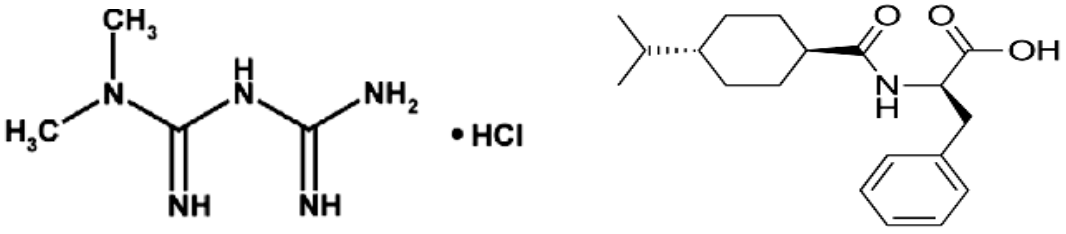
**Key words:** Metformin HCl, Nateglinide, HPLC, Development, Validation.

## INTRODUCTION

**Metformin HCl**, sold under the brand name Glucophage among others, is the first-line medication for the treatment of type 2 diabetes [1, 2], particularly in people who are overweight [3, 4]. It is also used in the treatment of polycystic ovary syndrome [5, 6]. It is not associated with weight gain and is taken by mouth. It is sometimes used as an off-label augment to attenuate the risk of weight gain in people who take antipsychotics [7, 8, 9] as well as phenelzine [10, 11] .

Metformin is generally well tolerated. Common adverse effects include diarrhoea [12], nausea [13, 14], and abdominal pain. It has a low risk of causing low blood sugar. High blood lactic acid level is a concern if the medication is used in overly large doses or prescribed in persons with severe kidney problems. It is not recommended in those with significant liver disease. Metformin is a biguanide antihyperglycemic agent. It works by decreasing glucose production by the liver, by increasing the insulin[15, 16] sensitivity of body tissues, and by increasing GDF15 secretion, which reduces appetite and caloric intake.

**Nateglinide** (INN, trade name Starlix) is a drug for the treatment of type 2 diabetes. Nateglinide was developed by Ajinomoto, a Japanese company and sold by the Swiss pharmaceutical company Novartis. Nateglinide belongs to the meglitinide [17] class of blood [18] glucose-lowering drugs [19, 20]. Figure 1 shows the chemical structures of Metformin HCl and Nateglinide.




**A** **B**

**FIG. 1: CHEMICAL STRUCTURE OF (A) METFORMIN HCl (B) NATEGLINIDE**

## MATERIALS AND METHOD

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**Chemicals:** Acetonitrile, HPLC-grade ortho phosphoric acid, water were purchased from Merck India Ltd, Mumbai, India. APIs of Metformin HCl and Nateglinide standards were procured from Glenmark, Mumbai.

**The Instrumentation:** Waters alliance liquid chromatography (model e-2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

**Preparation of buffer:** 1 ml of ortho phosphoric acid is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

**Chromatographic conditions:** The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% ortho phosphoric acid and Luna Phenyl Hexyl 150X4.6mm, 3.5µ with a flow rate of 1 ml /min.

**Diluent:** 0.1% OPA and Acetonitrile in the ratio (70:30) is used as diluent.

**Preparation of the standard stock solution:** For standard stock solution preparation, add 70ml of diluents to 50mg of Metformin HCl and 6 mg of Nateglinide taken in a 100 ml volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent.

**Preparation of Standard solution:** 5 ml of solution is drawn from the above normal stock solution into a 50ml volumetric flask and diluted up to the level.

**RESULTS AND DISCUSSION**

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance the chromatographic conditions were optimized.

**Method optimization:** To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% Ortho phosphoric acid buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as $C_8$, $C_{18}$ phenyl and amino, luna phenyl columns were tested. From these trials the peak shapes were relatively good with a Luna Phenyl Hexyl 150X4.6mm, 3.5µ with a PDA detector. The mobile phase flow rate has been done at 221 nm in order to obtain enough sensitivity. By using above conditions we get retention times of Metformin HCl and Nateglinide were about 2.770 and 5.118 min with a tailing factor of 1.05 & 0.99. The number of theoretical plates for Metformin HCl and Nateglinide were 3661,9074 which indicate the column's successful output the % RSD for six replicate injections was around 0.14% and 0.41%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the established method was validated.

**Method validation**

The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, accuracy, precision and robustness.

**System suitability:** Device suitability was performed by injecting standard solution containing 50 µg/ml of Metformin HCl and 6 µg/ml of Nateglinide in six replicates. The results show that the machine fitness parameter is within the limit provided by ICH. The results were shown below table 1. Figure 2 represents the chromatogram of standard.

**TABLE 1: RESULTS OF SYSTEM SUITABILITY**

| System suitability parameter | Acceptance criteria | Drug name | |
|---|---|---|---|
| | | Metformin HCl | Nateglinide |
| USP Plate count | NLT 2000 | 3661 | 9074 |
| USP Tailing | NMT 2.0 | 1.05 | 0.99 |
| USP Resolution | NLT 2.0 | - | 11.75 |
| % RSD | NMT 2.0 | 0.14 | 0.41 |
| Retention Time | NLT 2.0 | 2.770 | 5.118 |

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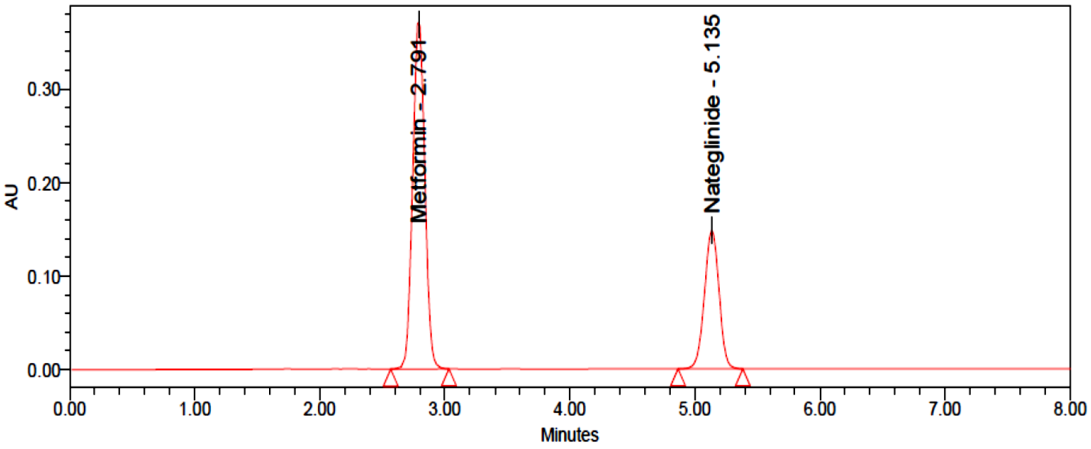


**FIG. 2: CHROMATOGRAM OF STANDARD**

**Specificity:** There was no interference from blank at the retention time of Metformin HCl and Nateglinide. This proves the technique is specific. Figure 3 shows the chromatogram of blank.

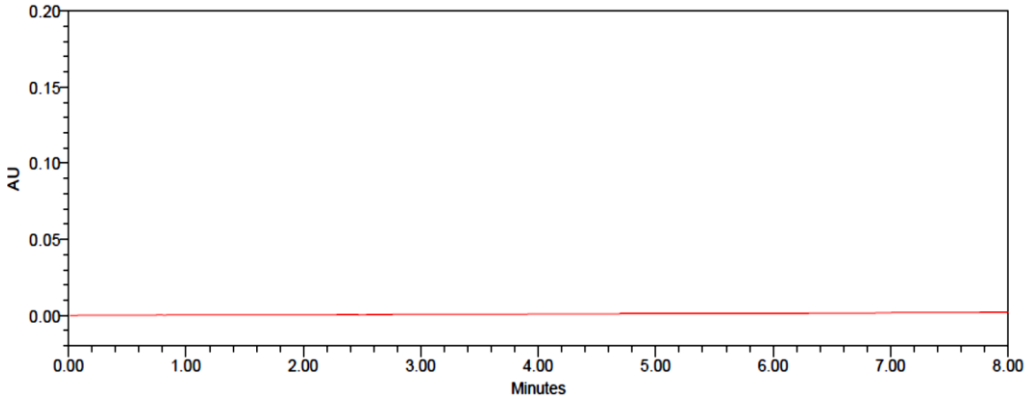


**FIG. 3: CHROMATOGRAM OF BLANK**

**Linearity:** Linearity was calculated by plotting a calibration curve of the peak area against its respective concentration, linearity was determined. From this calibration curve, it was noticed that the curve was linear between the range of 5-75µg/ml of Metformin HCl and 0.6-9µg/ml of Nateglinide. The regression equations for calibration curve was y = 50101.05x +86195.26 ($R^2$=0.9992) for Metformin HCl and y =197881.42x + 757.37 ($R^2$=0.9999) for Nateglinide respectively and the results of linearity were shown in table 2. Calibration plots were shown in figure 4.

**TABLE 2: LINEARITY RESULTS**

| Linearity | Metformin HCl | | Nateglinide | |
|---|---|---|---|---|
| | Conc. (µg/ml) | Area | Conc. (µg/ml) | Area |
| Linearity-1 | 5.01 | 416890 | 0.61 | 121716 |
| Linearity-2 | 12.53 | 761356 | 1.53 | 311818 |
| Linearity-3 | 25.05 | 1305500 | 3.05 | 588822 |
| Linearity-4 | 37.58 | 1951265 | 4.58 | 913374 |
| Linearity-5 | 50.10 | 2586456 | 6.10 | 1205546 |
| Linearity-6 | 62.63 | 3271404 | 7.63 | 1510828 |
| Linearity-7 | 75.15 | 3825525 | 9.15 | 1811815 |
| Slope | 50101.05 | | 197881.42 | |
| Intercept | 86195.26 | | 757.37 | |
| CC | 0.9992 | | 0.9999 | |

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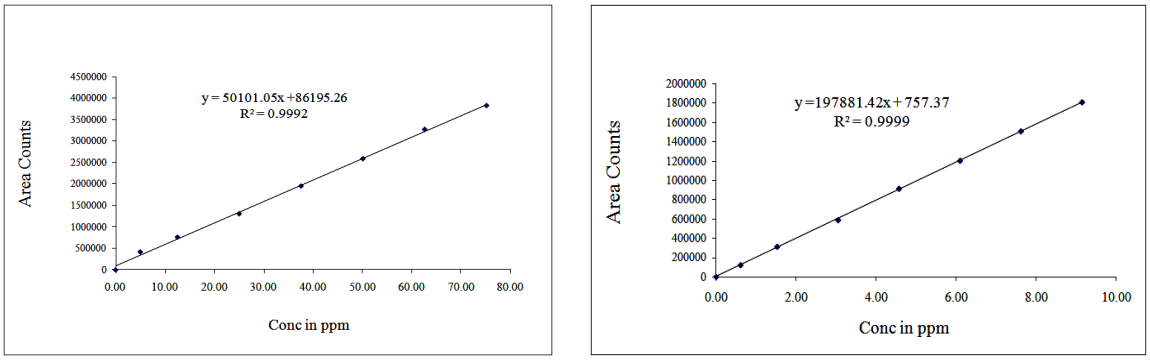


**A** **B**

**FIG. 4: CALIBRATION PLOTS OF (A) METFORMIN HCl (B) NATEGLINIDE**

**Accuracy:** The accuracy of the system was achieved by measuring the recovery experiments at three stages (50 percent, 100 percent and 150 percent). APIs with concentrations of 25, 50 and 75µg/ml of Metformin HCl and 3, 6 and 9µg/ml of Nateglinide were prepared. For each spike stage, the test solution was injected three times and the test was performed according to the test process. The recovery results were similar to 100% and also the RSD values were less than ±2%. The percentage recovery, mean and relative standard deviations were determined. Recovery values shown within the desired range were correct. The results are summarized below. Accuracy findings have been shown in table 3.

**TABLE 3: RESULTS OF ACCURACY**

| S. No. | % Level | Metformin HCl % Recovery | Nateglinide % Recovery |
|---|---|---|---|
| 1 | 50 | 98.4 | 100.6 |
| 2 | 100 | 100.0 | 99.4 |
| 3 | 150 | 98.1 | 100.3 |

**Precision:** The precision of the analytical technique is the degree of proximity of the sequence of measurements obtained from multiple homogeneous mixture samplings. The accuracy of the process of the drugs were calculated by injection of six individual determinations of Metformin HCl (50 µg/ml) and Nateglinide (6µg/ml). Method precision results were shown in table 4 and sample chromatogram was shown in figure 5.

**TABLE 4: RESULTS OF INTRADAY PRECISION**

| **S. No.** | **Metformin HCl** | | | **Nateglinide** | | |
|---|---|---|---|---|---|---|
| | **Conc. (µg/ml)** | **Area** | **% Assay** | **Conc. (µg/ml)** | **Area** | **% Assay** |
| 1 | 50 | 2501286 | 99.6 | 6 | 1165977 | 98.9 |
| 2 | | 2496871 | 99.4 | | 1177574 | 99.8 |
| 3 | | 2512783 | 100 | | 1157354 | 98.1 |
| 4 | | 2506874 | 99.8 | | 1164856 | 98.8 |
| 5 | | 2497136 | 99.4 | | 1175669 | 99.7 |
| 6 | | 2486784 | 99 | | 1167983 | 99 |
| % RSD | 0.35 | | | 0.63 | | |

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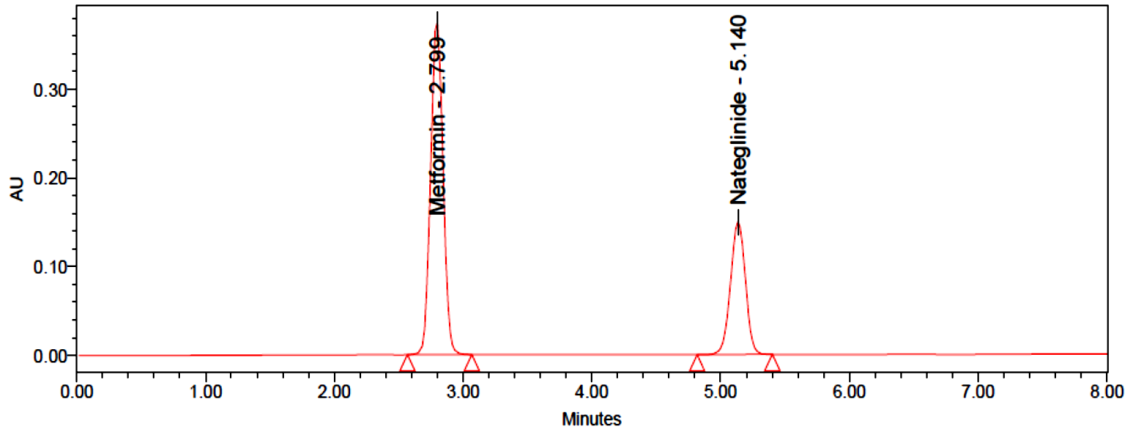


**FIG. 5: CHROMATOGRAM OF SAMPLE**

**Intermediate Precision:** Six replicates of the sample solution were analyzed by different researchers and different tools were checked on separate days. The peak regions used to assess the average percent of RSD values have been determined. The findings are shown in the table 5.

**TABLE 5: INTER-DAY PRECISION RESULTS**

| S. No. | Metformin HCl | | | Nateglinide | | |
|---|---|---|---|---|---|---|
| | Conc. (µg/ml) | Area | % Assay | Conc. (µg/ml) | Area | % Assay |
| 1 | 50 | 2513125 | 100.1 | 6 | 1165379 | 98.9 |
| 2 | | 2515871 | 100.2 | | 1175559 | 99.7 |
| 3 | | 2497154 | 99.4 | | 1174358 | 99.6 |
| 4 | | 2496245 | 99.6 | | 1164652 | 98.8 |
| 5 | | 2496871 | 99.3 | | 1164768 | 98.8 |
| 6 | | 2497652 | 99.5 | | 1169883 | 99.2 |
| %CV | 0.36 | | | 0.42 | | |

**LOD and LOQ:** LOD and LOQ were determined separately using the calibration curve technique. The LOD and LOQ of the compound were measured using the developed RP-HPLC method by injecting lower and lower concentrations of the standard solution. The LOD and LOQ concentrations and their s/n values of Metformin HCl and Nateglinide were represented in the following table 6 and the chromatograms of LOD and LOQ were shown in figure 6.

**TABLE 6: LOD AND LOQ RESULTS**

| Metformin HCl | | | | Nateglinide | | | |
|---|---|---|---|---|---|---|---|
| LOD | | LOQ | | LOD | | LOQ | |
| Conc. (µg/ml) | s/n | Conc. (µg/ml) | s/n | Conc. (µg/ml) | s/n | Conc. (µg/ml) | s/n |
| 0.0501 | 7 | 0.501 | 27 | 0.0061 | 4 | 0.061 | 23 |

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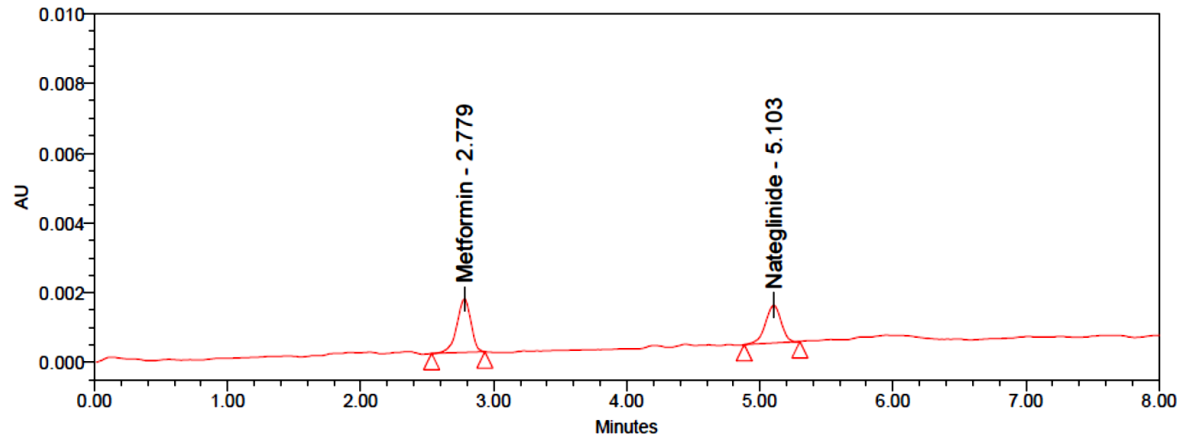
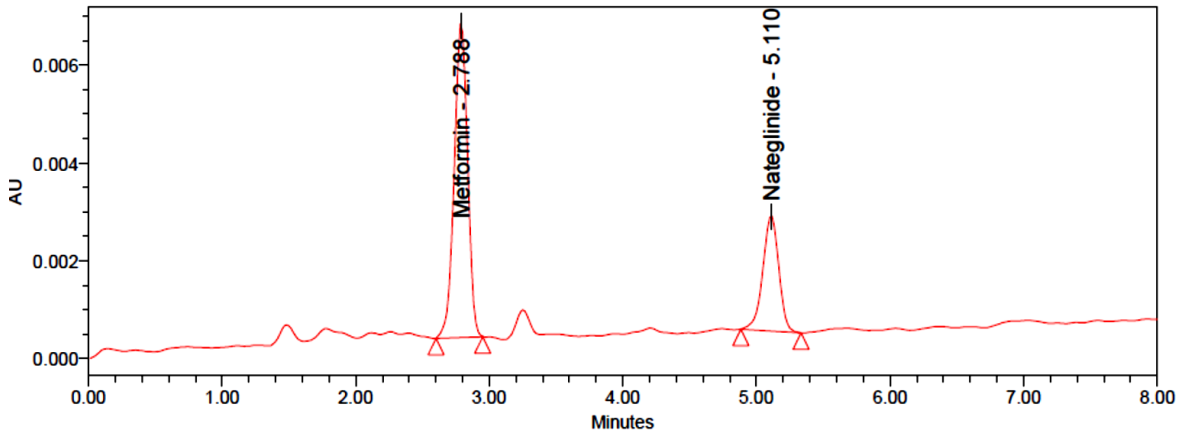



**A**




**B**

**FIG. 6: CHROMATOGRAM OF (A) LOD (B) LOQ**

**Robustness:** The conditions of the experiment was designed to measure the robustness of the intentionally changed conditions such as flow rate, organic percentage in mobile phase. Results of robustness were shown in table 7.

**TABLE 7: ROBUSTNESS RESULTS**

| Parameter name | % RSD | |
|---|---|---|
| | Metformin HCl | Nateglinide |
| Flow rate (0.8 ml/min) | 0.29 | 0.50 |
| Flow rate (1.2 ml/min) | 0.88 | 1.32 |
| Org Plus (37:63) | 0.71 | 0.35 |
| Org Minus (23:77) | 0.75 | 0.83 |

**Degradation studies:** Metformin HCl and Nateglinide standard was subjected to various conditions of forced degradation in order to induce partial degradation of the compound. Forced degradation experiments have been performed to establish that the process is acceptable for degradation materials. In addition the studies include information on the condition under which the drug is unstable, such that the steps are also taken during formulation to prevent possible instabilities. Forced degradation results were shown in table 8.

**Acid degradation:** 5 ml of standard stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 1N HCl and left it for 15 min. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark.

**Alkali degradation:** 5 ml of standard stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 1N NaOH and left it for 15 min. After 15 min add 1 ml of 1N HCl and make up to the mark.

**Peroxide degradation:** 5 ml of standard stock solution was moved to a volumetric flask of 50 ml, add 1 ml of 30% hydrogen peroxide solution and make upto the mark with diluents.

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**Reduction degradation:** 5 ml of standard stock solution was moved to a volumetric flask of 50 ml and add 1 ml of 30% sodium bi sulphate solution and make upto the mark with diluents.

**Thermal degradation:** The standard solution was set in an oven at 110°C for 24 hrs. The resultant solution was injected into HPLC system.

**Photolytic degradation:** The standard solution was placed in sun light for 24 hrs. The resultant solution was injected into HPLC system.

**TABLE 8: FORCED DEGRADATION RESULTS**

| Degradation condition | Metformin HCl | | Nateglinide | |
|---|---|---|---|---|
| | % Assay | % deg | % Assay | % deg |
| Control | 100.1 | -0.1 | 100.1 | -0.1 |
| Acid deg | 85.3 | 14.8 | 85.3 | 14.8 |
| Alkali deg | 84.9 | 15.2 | 82.9 | 17.2 |
| Peroxide deg | 84.5 | 15.6 | 88.1 | 12 |
| Reduction deg | 84 | 16.1 | 84.8 | 15.3 |
| Thermal deg | 83 | 17.1 | 82.9 | 17.2 |
| Photolytic deg | 82.6 | 17.5 | 82.4 | 17.7 |

## CONCLUSION

This method described the quantification of Metformin HCl and Nateglinide in bulk and pharmaceutical formulation as per ICH guidelines. The evolved technique was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of sample preparation and reproducibility data are satisfactory. The evolved chromatographic method can be effectively applied for regular investigation in drug research.

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## CONFLICTS OF INTEREST

Author declares that there have been no conflicts of interest.

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None

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