VALIDATION OF A DEVELOPED ANALYTICAL METHOD FOR DETERMINATION OF NATEGLINIDE AND METFORMIN HCL IN PURE AND PHARMACEUTICAL DOSAGE FORM BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ITS DEGRADATION STUDIES

K.MD ISMAIL1, A LAKSHMANA RAO2*
1Department of Pharmaceutical Analysis, Nizam Institute of Pharmacy, Deshmukhi Village, Nalgonda, Telangana, India. 2Department of Pharmaceutical Analysis, V.V. Institute of Pharmaceutical Sciences, Gudlavalleru, Krishna, Andhra Pradesh, India. Email: dralrao@gmail.com

ABSTRACT

Objective: The objective of the study was to develop a versatile analytical method and validate according to International Council for Harmonization guidelines for simultaneous estimation of nateglinide and metformin HCl by reversed-phase high-performance liquid chromatography (RP-HPLC) in active pharmaceutical ingredient and in tablet dosage form.

Methods: Analytes, metformin and nateglinide, are separated and eluted from stationary phase luna phenyl hexyl column (150 mm ×4.6 mm, 3.5 µm) (micrometer) using polar mobile phase composed of acetonitrile:1% orthophosphoric acid 30:70 v/v, with flow rate of 1 ml/min for 8 min at ambient column temperature, at 221 nm (nanometer) detection. Acid, base, peroxide, thermal, and photolytic-induced degradation studies were performed on nateglinide and metformin.

Results: Through isocratic flow, both metformin and nateglinide are detected at retention times of 2.79 min and 5.13 min, respectively, at 221 nm. The linearity and range of analytical method for nateglinide and metformin were 0.61-9.15 µg/ml and 7.5-75.15 µg/ml, respectively. The R² value for nateglinide was 0.9998 and for metformin HCl was 0.9991. The limit of detection and limit of quantification for nateglinide were 0.21 µg/ml and 0.63 µg/ml and for metformin were 4.8 µg/ml and 14.6 µg/ml, respectively. The % relative standard deviation for method precision was found to be 0.22% and 0.64% for both nateglinide and metformin, respectively. The mean %recovery for nateglinide and metformin was 99.88% and 99.21%, respectively. The %thermal degradation was identified as 17.7% and 17.5% for nateglinide and metformin, respectively.

Conclusion: The developed chromatographic (RP-HPLC) method was selective, specific, economic, precise, and accurate. Hence, it can be one of the preferred analytical methods of choice for the estimation of nateglinide and metformin by RP-HPLC in pure and in tablet dosage form.

Key words: Nateglinide, Metformin, Reversed-phase high-performance liquid chromatography, Isocratic, Acetonitrile.

INTRODUCTION

Nateglinide is chemically 3-phenyl-2-[(4-propan-2-yl cyclohexane carbonyl) amino] propanoic acid (Fig. 1) with molecular formula C₁₇H₁₉NO₂. It acts by blocking adenosine triphosphate sensitive potassium channels of beta cells of pancreas, causes membrane depolarization results in calcium influx and their by stimulation of insulin secretion. Metformin HCl is chemically N, N-Dimethyl imidodicarbonimidic diamide hydrochloride (Fig. 2) with molecular formula C₇H₉N₃HCl. The main mechanism of metformin HCl was lowering glucose intestinal absorption, inhibition of hepatic glucose production, and improving glucose uptake and utilization [1-6].

It was found that very few articles are available in detailed literature survey on simultaneous estimation of nateglinide and metformin HCl by reversed-phase high-performance liquid chromatography (RP-HPLC) in pure and dosage form [7-9]. The resting literature was found on analytical and bioanalytical methods by HPLC, LC-MS/MS, RP-LC, high-performance thin-layer chromatographic, and ultraviolet (UV) spectrophotometric estimations, in combination with glinides (nateglinide, repaglinide, and mitiglinide) and metformin HCl [10-21].

The comprehensive literature survey disclosed diverse analytical techniques of estimating nateglinide and metformin HCl in single and in combination with other drugs. The present study was taken up to develop a sensitive, accurate, precise, and simple method of analysis for the estimation of both drugs in combined dosage forms.

METHODS

Chemicals and reagents
The active pharmaceutical ingredients (APIs), nateglinide and metformin hydrochloride, were supplied as a gift sample by Care Labs, L.B Nagar, Hyderabad, and marketed formulation was purchased from the local market. HPLC grade orthophosphoric acid, acetonitrile, and water were of Merck grade. Waters auto sampler RP-HPLC, e2695 pump, and 2998 photodiode array (PDA) detector with Empower2 software were employed in this method.

Selection and preparation of mobile phase and diluent
In RP-HPLC, pure API mixture containing nateglinide and metformin HCl at lower concentration levels were prepared, injected, and run with different solvent systems. Different combination of solvents using acetonitrile, triethylamine, and orthophosphoric acid at different compositions, flow rates, and ratios were tried to optimize the mobile phase. Finally from the trials, mobile phase and diluent (acetonitrile and 0.1% orthophosphoric acid in a ratio of 30:70 v/v) are selected since they were fulfilling the requirements and the results obtained were within the acceptable limits.

Preparation of standard stock solution
Powder analytes equivalent to 6 mg and 50 mg of nateglinide and metformin HCl, respectively, were accurately weighed and transferred...
nateglinide and 50 mg of metformin HCl of tablet powder was weighed. The tablets were triturated into fine powder and the weight equivalent to 6 mg of nateglinide and 50 mg of metformin HCl was calculated. The average weight of each tablet was calculated from 20 tablets weighted. The analysis of nateglinide and metformin HCl from marketed tablets was performed. The intercept, slope, and R² were taken into consideration to plot a calibration curve. The linearity, range, S/N (signal to noise) ratio, peak tailing, and United States Pharmacopeia (USP) plate count were considered. The peak areas obtained from chromatograms were tabulated for amount of nateglinide and metformin HCl present in each tablet.

System suitability
To ensure that the system was working perfectly and exploring the feasibility of the proposed method, system suitability test was performed. To evaluate system suitability, six working standard solutions were injected and the parameters such as resolution (Rs), retention time (Rt), tailing factor (Tf), USP plate count (N), and %RSD of peak areas were calculated from the chromatograms of analytes of interest.

Validation of analytical method [22-25]

Selectivity and specificity
The developed method was said to be selective, when nateglinide and metformin HCl are completely separated from each other with fixed resolution and retention time at optimized chromatographic conditions. Selectivity of the recommended method was evaluated by repeated injections of working standard solutions.

Accuracy
According to the guidelines to estimate the accuracy of the method, triplicates of three different concentration levels of standard solutions (50%, 100%, and 150%) containing both nateglinide and metformin HCl were injected into RP-HPLC system. Before standard injections, a blank solution (mobile phase) was injected. From each chromatogram obtained, percentage of drug recovery, mean % of drug recovery, and %RSD are calculated.

Precision
System precision
System precision or chromatographic system performance was estimated by injecting six replicate freshly prepared working standard solutions containing 6 µg/ml of nateglinide and 50 µg/ml of metformin HCl (100% test concentration) into HPLC system. The percentage relative standard deviation (%RSD) was computed from the peak areas of nateglinide and metformin HCl chromatograms.

Method precision
To determine method precision of proposed analytical method, six replicates of working standard solutions and six replicates of sample solutions containing 6 µg/ml of nateglinide and 50 µg/ml of metformin HCl were injected into RP-HPLC system without changing the optimized chromatographic conditions. From the peak areas obtained, calculated the presence of percentage of analytes in each injection. The %RSD for mean peak areas and assay was computed.

Intermediate precision
Six samples of same batch were analyzed to calculate the intermediate precision of the proposed analytical method by different analyst on different day on different instrument. The %RSD for mean peak areas and assay was computed.

Robustness
To ensure the robustness of the proposed method, the flow rate was adjusted to 0.8 ml/min, 1.2 ml/min and organic composition of mobile phase was varied. The parameters such as resolution (Rs), retention time (Rt), tailing factor (Tf), USP plate count (N), and %RSD of peak areas were calculated from the chromatograms of analytes of interest.

Selection of detection wavelength ($\lambda_{\text{max}}$) (maximum absorbance wavelength)
Nateglinide (6 µg/ml) and metformin HCl (50 µg/ml) solutions were prepared separately using diluent and scanned separately in Shimadzu 1800 UV-visible spectrophotometer at a range of 200–400 nm. The %RSD of the absorbance values at respective wavelengths were calculated from the absorbance values at respective wavelengths.

Optimized chromatographic conditions
Many trials have been conducted to optimize all the chromatographic conditions required for simultaneous estimation of nateglinide and metformin HCl. Finally, reverse phase C18 column, luna phenyl hexyl column (150 mm×4.6 mm, 3.5 µ), mobile phase containing acetonitrile:0.1% orthophosphoric acid (30:70 v/v ratio), flow rate 1.0 ml/min, run time 8 min, injection volume – 10 µl and a detection wavelength – 221 nm using a PDA detector are the best possible optimized chromatographic conditions which gave the best resolution, S/N (signal to noise) ratio, peak tailing, and United States Pharmacopeia (USP) plate count for the present estimation.

Preparation of working standard solution
Five milliliters of standard stock solution were transferred into 50 ml calibrated volumetric flask and filled up to the mark with diluent.

Preparation of working standard solution
Eight standard solutions were prepared to construct calibration curve. The %RSD, precision and accuracy of peak areas were calculated from the chromatograms of analytes of interest.

Preparation of calibration curve solutions
Eight standard solutions were prepared to construct calibration curve. Different concentrations of nateglinide (0.61 µg/ml–9.15 µg/ml) and metformin HCl (5.01 µg/ml–75.15 µg/ml) were prepared using diluent and injected into stabilized RP-HPLC system which was in already mentioned optimized chromatographic conditions. The peak areas (on y-axis) obtained from respective concentrations (on x-axis) were taken into consideration to plot a calibration curve. The linearity, range, intercept, slope, and R² were calculated from calibration curve.

Analysis of nateglinide and metformin HCl from marketed tablets
From 20 tablets weight, average weight of each tablet was calculated. The tablets were triturated into fine powder and equivalent to 6 mg of nateglinide and 50 mg of metformin HCl of tablet powder was weighed and transferred to 100 ml volumetric flask, added 50 ml of diluent, sonicated for 10 min, and filled up to the mark with diluent.

Preparation of working standard solution
Five milliliters of standard stock solution were transferred into 50 ml calibrated volumetric flask and filled up to the mark with diluent.

Selection of detection wavelength ($\lambda_{\text{max}}$) (maximum absorbance wavelength)
Nateglinide (6 µg/ml) and metformin HCl (50 µg/ml) solutions were prepared separately using diluent and scanned separately in Shimadzu 1800 UV-visible spectrophotometer at a range of 200–400 nm. The %RSD of the absorbance values at respective wavelengths were calculated from the absorbance values at respective wavelengths.

Optimized chromatographic conditions
Many trials have been conducted to optimize all the chromatographic conditions required for simultaneous estimation of nateglinide and metformin HCl. Finally, reverse phase C18 column, luna phenyl hexyl column (150 mm×4.6 mm, 3.5 µ), mobile phase containing acetonitrile:0.1% orthophosphoric acid (30:70 v/v ratio), flow rate 1.0 ml/min, run time 8 min, injection volume – 10 µl and a detection wavelength – 221 nm using a PDA detector are the best possible optimized chromatographic conditions which gave the best resolution, S/N (signal to noise) ratio, peak tailing, and United States Pharmacopeia (USP) plate count for the present estimation.

Preparation of working standard solution
Five milliliters of standard stock solution were transferred into 50 ml calibrated volumetric flask and filled up to the mark with diluent.

Selection of detection wavelength ($\lambda_{\text{max}}$) (maximum absorbance wavelength)
Nateglinide (6 µg/ml) and metformin HCl (50 µg/ml) solutions were prepared separately using diluent and scanned separately in Shimadzu 1800 UV-visible spectrophotometer at a range of 200–400 nm. The %RSD of the absorbance values at respective wavelengths were calculated from the absorbance values at respective wavelengths.
mobile phase was changed to ±10% and notified the changes occurred in the chromatograms after injecting the working standard solutions and sample solutions containing nateglinide and metformin HCl.

**Limit of detection (LOD) and limit of quantification (LOQ)**

LOD and LOQ are defined as the lowest concentrations to detect and to quantify the analyte(s) respectively. To estimate LOD and LOQ, a series of dilutions of both nateglinide and metformin HCl was injected and plotted a calibration graph between peak areas and concentration. Finally, LOD and LOQ values were finalized using regression analysis.

The preferable formulas to calculate LOD and LOQ are as follows:

\[
\text{LOD} = \frac{3.3}{S} \sigma \\
\text{LOQ} = \frac{10}{S} \sigma
\]

Where \( \sigma \) = Standard deviation of the response and \( S \) = Slope of calibration curve.

**Forced degradation studies**

Sample stock solution for forced degradation studies was prepared by mixing 62.1 mg of sample powder with 70 ml of diluent in 100 ml volumetric flask and sonicated for 30 min and diluted to the mark with the same diluent.

**Acid-induced degradation**

Five milliliters of sample stock solution and 1 ml of 1 N HCl were taken in a 50 ml volumetric flask, placed in water bath for 30 min at 60°C constant temperature. Further after cooling to room temperature, 1 ml of 1 N NaOH was added and diluted to the mark with the same diluent.

**Alkali-induced degradation**

Five milliliters of sample stock solution and 1 ml of 1 N NaOH were taken in a 50 ml volumetric flask, placed in water bath for 30 min at 60°C constant temperature. Further after cooling to room temperature, the resulting solution was injected after 24 h.

**Peroxide-induced degradation**

Five milliliters of sample stock solution and 1 ml of 30% \( H_2O_2 \) were taken in a 50 ml volumetric flask, placed in water bath for 30 min at 60°C constant temperature. Further after cooling to room temperature, the resulting solution was injected after 24 h.

**Thermal-induced degradation**

The sample powder was exposed at 105°C for 72 h. 62.1 mg of this sample was weighed and transferred into 100 ml volumetric flask containing 70 ml of diluent and sonicated for 30 min and diluted to the mark with the same diluent.

**RESULTS AND DISCUSSION**

**Selection of detection wavelength (\( \lambda_{max} \))**

In the UV spectrum, nateglinide has shown \( \lambda_{max} \) at 212.3 nm and metformin HCl has shown \( \lambda_{max} \) at 258.3 nm. In the overlain spectra, both nateglinide and metformin HCl show absorbance at 221 nm as common middle wavelength (Fig. 3).

**Construction of calibration curve**

As shown in Figs. 4 and 5, straight lines are obtained on calibration plots with linearity and range from 0.61 \( \mu g/ml \) to 9.15 \( \mu g/ml \); whereas, the regression equation was \( y=197,864x+458.32 \) for nateglinide and for metformin HCl, the linearity and range from 7.5 \( \mu g/ml \) to 75.15 \( \mu g/ml \); whereas, the regression equation was \( y=50,772x+47,812 \). The \( R^2 \) values for nateglinide and metformin HCl are 0.9998 and 0.9991, respectively (Table 1).

**Analysis of nateglinide and metformin HCl from marketed tablets**

One of the marketed formulations was analyzed with the proposed RP-HPLC method and the mean assay was identified as 100.6% and 99.18% against the label claim of nateglinide (60 mg) and metformin HCl (500 mg), respectively. The %RSD of mean assay of the formulation was within the acceptable limits ±2 (Table 2).

**System suitability**

From the data obtained for six injections, it was observed that the resolution (Rs) was >2, tailing factor (Tt) was <2, USP theoretical plates

![Fig. 3: Selection of isosbestic point for nateglinide and metformin HCl](image-url)
count (N) was >2000, and the %RSD of peak areas of working standard solution was <2. The numerals are represented in Table 3.

Validation of proposed analytical method

Selectivity and Specificity

From the chromatograms Fig. 6 on observation, it was found that the retention times and resolution of nateglinide and metformin HCl were fixed same as such without any changes confirm the selectivity of the analytical method.

The perusal of Fig. 7 reveals that the mobile phase scan and placebo scan did not show any peaks at the retention time of analytes of interest. However, the tablet extracts scan and working standard solutions scan gave characteristic peaks of nateglinide and metformin HCl. These results convey specificity of the method.

Accuracy

The calculated mean % recoveries were 99.21% and 99.88% for metformin HCl and nateglinide, respectively. Hence, the recovery study proves the accuracy of the proposed RP-HPLC method (Table 4).

Precision

The %RSD of peak areas of six replicate injections of working standard solution was estimated as 0.14% and 0.41% for metformin HCl and nateglinide, respectively. This indicates that the system precision was within the limits of acceptability.

The mean assay of six replicate injections of sample solutions was estimated as 99.53% for metformin HCl and 99.05% for nateglinide. Hence, the method was reproducible.

Six replicate injections of sample solutions when analyzed on different day by different analysts with different column and the mean assay was calculated as 99.65% for metformin HCl and 99.17% for nateglinide. Hence, the method was reproducible on different instrument on different day (Table 5).

Robustness

To ensure the robustness of the proposed method, sample solutions were injected for 3 times after each change in optimized chromatographic parameters. From the procured data, mean peak area of sample, %RSD of peak area, and mean assay for sample were calculated. The %RSD of sample peak areas at all the deliberate conditions was identified as ≤2% (Table 6).

Table 3: System suitability results

| Parameter             | Metformin HCl | Nateglinide | %RSD  |
|-----------------------|---------------|-------------|-------|
| Retention time (Rt)   | 2.79±0.00     | 5.11±0.00   | 0.093 |
| Peak areas            | 25.53±2.44    | 12.58±7.5   | 0.362 |
| Theoretical plates (N)| 35.57±34.28   | 881.67±52.27| 0.964 |
| Tailing factor (TF)   | 0.87±0.11     | 0.95±0.024  | -     |
| Resolution (Rs)       | 11.28±1.15    | -           | 1.38  |

*Average of six determinations, Rt: Retention time, Rs: Resolution, USP: United States Pharmacopeia, TF: Tailing factor; RSD: Relative standard deviation
Ismail and Rao

Table 7: LOD and LOQ results

| S. No. | Parameter | Nateglinide | Metformin HCl |
|--------|-----------|-------------|---------------|
| 1      | LOD       | 0.21 µg/ml  | 4.8 µg/ml     |
| 2      | LOQ       | 0.63 µg/ml  | 14.6 µg/ml    |

LOD: Limit of detection, LOQ: Limit of quantification

Flow rate 1.2 ml
The sample assay at 1.2 ml/min was calculated as 99.87% for nateglinide, and for metformin HCl, it was 99.83%.

Flow rate 0.8 ml
The sample assay at 0.8 ml/min was calculated as 99.53% for nateglinide, and for metformin HCl, it was 99.83%.

More organic mobile phase (+10%)
The mobile phase organic composition when changed to +10% the calculated assay was 98.8% for nateglinide, and for metformin HCl, it was 99.07%.

Less organic mobile phase (−10%)
The mobile phase organic composition when changed to +10% the calculated assay was 99.67% for nateglinide, and for metformin HCl, it was 99.73%.

LOD and LOQ
SD of intercept was calculated by regression analysis. Using slope of calibration plot and SD of intercept, LOQ and LOD are calculated.

The estimated LOD and LOQ values for nateglinide are 0.21 µg/ml and 0.63 µg/ml, respectively; for metformin HCl, the LOD and LOQ are 4.8 µg/ml and 14.6 µg/ml, respectively (Table 7).

---

Table 4: Accuracy results

| Drugs        | Level % | Amount added (µg/ml) | Amount found (µg/ml) | %Recovery±SD (n=3) | %RSD  |
|--------------|---------|----------------------|----------------------|--------------------|-------|
| Metformin HCl| 50      | 25.1                 | 24.78                | 98.73±0.3          | 0.30  |
|              | 100     | 50.1                 | 49.99                | 99.73±0.25         | 0.25  |
|              | 150     | 75.1                 | 74.50                | 99.16±0.94         | 0.94  |
|              | 50      | 3.1                  | 3.13                 | 100±0.79           | 0.79  |
| Nateglinide  | 100     | 6.2                  | 6.17                 | 99.53±0.15         | 0.15  |
|              | 150     | 9.2                  | 9.24                 | 100.1±0.43         | 0.429 |

STD: Standard, SD: Standard deviation RSD: Relative standard deviation

Table 5: Precision results

| Parameter             | Drug        | Mean peak area* | %RSD  |
|-----------------------|-------------|-----------------|-------|
| System precision      | Metformin HCl| 2,517±3624.25   | 0.14  |
|                       | Nateglinide | 1,199±4929.91   | 0.41  |
| Method precision      | Metformin HCl| 99.53±0.35      | 0.35  |
|                       | Nateglinide | 99.05±0.63      | 0.63  |
| Intermediate precision| Metformin HCl| Mean % recovery*|       |
|                       | Analyst-1   | 99.53±0.35      | 0.35  |
|                       | Analyst 2   | 99.65±0.39      | 0.39  |
|                       | Nateglinide | Mean % recovery*|       |
|                       | Analyst-1   | 99.05±0.63      | 0.39  |
|                       | Analyst 2   | 99.17±0.4       | 0.41  |

Mean % recovery*: Average of six determinations, %RSD: Percentage relative standard deviation

Table 6: Robustness results

| Parameter                              | Metformin HCl | Nateglinide |
|----------------------------------------|---------------|-------------|
| Flow plus (1.2 ml/min)                 | Area*         | 2,266±340   | 1,046±318   |
|                                        | Rt*           | 2.31        | 4.23        |
|                                        | Tt*           | 1.04        | 1.01        |
| Flow minus (0.8 ml/min)                | Area*         | 2,853±697   | 1,569±444   |
|                                        | Rt*           | 3.44        | 6.35        |
|                                        | Tt*           | 1.06        | 1.00        |
| Organic solvent ratio (+10%)           | Area*         | 2,626±110   | 1,153±106   |
|                                        | Rt*           | 2.45        | 3.84        |
|                                        | Tt*           | 1.04        | 1.01        |
| Organic solvent ratio (−10%)           | Area*         | 2,753±249   | 1,348±965   |
|                                        | Rt*           | 3.25        | 7.36        |
|                                        | Tt*           | 1.05        | 0.98        |

Area*: Average of three determinations, Rt: Retention time, Tt: Tailing factor

---

Fig. 7: Chromatogram of placebo

The sample assay at 1.2 ml/min was calculated as 99.87% for nateglinide, and for metformin HCl, it was 99.83%.

The sample assay at 0.8 ml/min was calculated as 99.53% for nateglinide, and for metformin HCl, it was 99.83%.

The mobile phase organic composition when changed to +10% the calculated assay was 98.8% for nateglinide, and for metformin HCl, it was 99.07%.

The mobile phase organic composition when changed to +10% the calculated assay was 99.67% for nateglinide, and for metformin HCl, it was 99.73%.

The estimated LOD and LOQ values for nateglinide are 0.21 µg/ml and 0.63 µg/ml, respectively; for metformin HCl, the LOD and LOQ are 4.8 µg/ml and 14.6 µg/ml, respectively (Table 7).

---

Table 7: LOD and LOQ results

SD of intercept was calculated by regression analysis. Using slope of calibration plot and SD of intercept, LOQ and LOD are calculated.

The estimated LOD and LOQ values for nateglinide are 0.21 µg/ml and 0.63 µg/ml, respectively; for metformin HCl, the LOD and LOQ are 4.8 µg/ml and 14.6 µg/ml, respectively (Table 7).
Table 8: Stability indicating method data for nateglinide and metformin HCl

| Degradation condition | Nateglinide Peak area | % degradation | Metformin HCl Peak area | % degradation |
|-----------------------|-----------------------|---------------|-------------------------|--------------|
| Control               | 1,202,125             | –0.1          | 2,523,178               | –0.1         |
| 1 N HCl (acid)        | 102,347               | 14.8          | 2,151,437               | 14.8         |
| 1 N NaOH (alkaline)   | 994,732               | 17.2          | 2,139,878               | 15.2         |
| 30% H2O2 (peroxide)   | 105,7824              | 12            | 2,128,941               | 15.6         |
| Thermal (105°C)       | 994,712               | 17.2          | 2,091,462               | 17.1         |
| Photolytic            | 980,793               | 17.7          | 2,082,987               | 17.5         |

N: Normality, HCl: Hydrochloric acid, NaOH: Sodium hydroxide, H2O2: Hydrogen peroxide

Forced degradation studies
Estimated assay after forced degradation with 1 N HCl was 85.3% and 85.3% of nateglinide and metformin HCl, respectively, it was 82.9% and 84.3% of nateglinide and metformin HCl, respectively, with 1 N NaOH, and with peroxide, it was 88.1% and 84.5% of nateglinide and metformin HCl, respectively. When the sample was exposed at 105°C, the estimated assay was 82.9% and 83.0% of nateglinide and metformin HCl, respectively, and sample on exposure with UV radiation the assay was 82.4% and 82.6% of nateglinide and metformin HCl respectively (Table 8).

CONCLUSION
From the available literature, it was observed and notified that only few articles are reported the stability indicating simultaneous estimations of nateglinide and metformin HCl by RP-HPLC. A new stability indicating method was developed and fully validated with mobile phase (acetoni-trile:0.1% orthophosphoric acid 30:70 v/v), column (Luna phenyl hexyl 150 mm × 4.6 mm, 3.5 µ), detection wavelength at 221 nm, and at other optimized chromatographic conditions. From the validation report, it was found that the developed RP-HPLC method was suitable, simple, economic, specific, and precise for the estimation of nateglinide and metformin HCl in tablet dosage forms. Stability studies indicated that the nateglinide and metformin HCl could be evaluated simultaneously by RP-HPLC in the presence of their degradation products. Hence, this method can be applied and implemented to study stability samples in the industry.

ACKNOWLEDGMENT
The authors are thankful to Care Labs for their technical support in finishing this research work.

AUTHORS’ CONTRIBUTIONS
K Md Ismail: Concept and design of work, data collection, data analysis, drafting and revision of article, and final approval of the revision to be published.

Dr. A. Lakshmana Rao: Design of work, drafting and revision of article, and final approval of the revision to be published.

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest.

AUTHORS’ FUNDING
Nil.

REFERENCES
1. Hirschberg Y, Karara AH, Pietri AO, McLeod JF. Improved control of mealtime glucose excursions with coadministration of nateglinide and metformin. Diabetes Care 2000;23:349-53.
2. Horton ES, Clinkingbeard C, Gatlin M, Foley J, Mallows S, Shen S. Nateglinide alone and in combination with metformin improves glycemic control by reducing mealtime glucose levels in Type 2 diabetes. Diabetes Care 2000;23:1660-5.
3. Geetha P, Sundharam PS. Drug utilization evaluation of antidiabetic drugs among Type 2 diabetes patients of Tamil Nadu. Asian J Pharm Clin Res 2017;10:202-5.
4. Muhas C, Naseef PP. A review article-gestational diabetes mellitus. Int J Curr Pharm Res 2017;9:1-5.
5. Natsya A, Andrajati R, Sauriasari R. Cross-sectional study of association between glyceremic control and quality of life among diabetic patients. Int J App Pharm 2018;10:92-6.
6. Dhodi JB, Mistry SN, Juvekar AR. Diabetic nephropathy-genesis, prevention, and treatment. Int J Pharm Sci 2014;6:42-7.
7. Thomas A, Patil S, Nanda R, Kothapalli L, Deshpande A. Stability-indicating RP-HPLC method for determination of metformin hydrochloride and nateglinide in bulk and tablet formulations. Curr Pharm Anal 2012;8:381-8.
8. Chengalva P, Parameswari SA, Aruna G. Development and validation of RP-HPLC method for metformin hydrochloride and nateglinide in bulk and combined dosage form. Int J Pharm Pharm Sci 2016;8:267-71.
9. El-Zaheer AA, Elkady EF, Elwy HM, Saleh MA. A new rapid and economic liquid chromatographic method for simultaneous determination of meglitinides with metformin: Application in the presence of metformin and repaglinide impurities and related compounds. J Iran Chem Soc 2018;15:61-74.
10. Haranadha R, Chunduri B, Danna BS. Development and validation of LC-MS/MS method for simultaneous quantification of metformin HCL and nateglinide in human plasma and its application to a pharmacokinetic study. World J Pharm Pharm Sci 2016;5:651-67.
11. Ramanjireddy T, Duraiswamy D, Kothapalli C. Method development and validation of Metformin and repaglinide in rabbit plasma by RP-HPLC. Fabad J Pharm Sci 2012;35:69-75.
12. Patel JR, Shahana BN, Patel BH. Simultaneous spectrophotometric estimation of metformin and repaglinide in a synthetic mixture. Indian J Pharm Sci 2007;69:844-6.
13. Thomas AB, Patil SD, Kothapalli LP, Nanda RK, Bhosle SS, Deshpande AD. Estimation of nateglinide and metformin hydrochloride in tablet dosage form by spectrophotometric methods. J Pharm Res 2011;10:102-5.
14. Soni LK, Narasinghani T, Jain M. Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and repaglinide in tablet dosage form. J Liq Chromatogr Relat Technol 2012;35:385-92.
15. Aslan SS, Yilmaz B. Derivative spectrophotometric and isocratic high performance liquid chromatographic methods for simultaneous determination of repaglinide and metformin hydrochloride in pharmaceutical preparations. Am J Anal Chem 2017;8:541-52.
16. Foadid M, Rashed NS. Development and validation of chromatographic and spectroscopic methods for estimation of repaglinide and metformin HCL in combined dosage form. J Glob Trends Pharm Sci J 2014;5:1844-8.
17. Sheth KB, Sagar GV. Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and mitiglinide calcium dihydrate in combined dosage form. Pharm Sci Monit 2012;3:2681-93.
18. Elkady EF, El-Zaheer AA, Elwy HH, Saleh MA. Validated liquid chromatographic method for simultaneous determination of Metformin, pioglitazone, sitagliptin, repaglinide, glibenclamide and gliclazide-application for counterfeit drug analysis. J Anal Bioanal Tech 2015;5:13-18.
19. El-Wasseef DR. Simultaneous determination of metformin, nateglinide and gliclazide in pharmaceutical preparations using micellar liquid chromatography. Int J Biomed Sci 2012;8:144-51.
20. Thomas AB, Patil SD, Nanda RK, Kothapalli LP, Bhosle SS, Deshpande AD. Stability-indicating HPTLC method for simultaneous determination of nateglinide and metformin hydrochloride in
21. Ahir KB, Patelia EM, Shah A. Simultaneous estimation of metformin hydrochloride and repaglinide in pharmaceutical formulation by HPTLC-densitometry method. J Chromatogr Sep Tech 2013;4:1-5.
22. Skoog DA, West DM, Holler FJ, Crouch S. Fundamentals of Analytical Chemistry. 8th ed. London: Thomson Asia Pvt Ltd.; 2004. p. 160-7, 971-95.
23. Ahuja S, Dong M, editors. Handbook of Pharmaceutical Analysis by HPLC. Vol. 6. Amsterdam, Boston: Elsevier Academic Press; 2005. p. 19-44, 47-69, 197-210.
24. Snyder L, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. Hoboken, New Jersey: John Wiley and Sons Inc.; 1997. p. 1-542.
25. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures- Text and Methodology; ICH Q2 (R1); 2005.