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Determination of Repaglinide in Pharmaceutical Formulations by RP-HPLC Method

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Abstract: A new Reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of repaglinide in pharmaceutical formulations. Optimum separation was achieved in 12 minutes using C_{18} column (250 mm × 4.6 mm, i.d., particle size 5 mm), and elution was accomplished using a mobile phase (1mL/min). Detection was carried out using a UV detector set at 245 nm. A linear relationship between mean peak area and concentration of repaglinide was observed in the range 0.5-5 µg/mL, with a detection limit of 0.275 µg/mL and a quantification limit of 0.833 µg/mL. Intra-day and Inter-day precision, and accuracy of the method have been established according to the current ICH guidelines. The developed method was successfully applied to the determination of repaglinide in pharmaceutical formulations. The results were statistically compared with the reference method (UV method) by applying Student's t-test and F-test. Accuracy, evaluated by means of the recovery method, was in the range 99.75 ± 0.55 to 100.73 ± 0.34 , with precision (RSD) 0.88%. No interference was observed from the coformulated substances. The proposed method was successfully employed for the determination of repaglinide in various pharmaceutical preparations.

Key words: Repaglinide, HPLC, Limit of detection, Precision.

INTRODUCTION

Repaglinide, chemically, (S)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl] butyl]amino]-2-oxoethyl] benzoic acid (Fig:1), is a new nonsulphonyl urea oral hypoglycemic drug[1]. It is used in the treatment of type-2 diabetes mellitus[2]. A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations and biological samples, which include visible spectrophotometric[3,4], HPLC[5-7] and electrochemical[8] methods.

The purpose of the present study was to develop a simple, sensitive, accurate and precise RP-HPLC method for the determination of repaglinide in pharmaceutical formulations. The developed method has been validated by evaluation of the system suitability, linearity, limits of detection and quantification, precision and accuracy. The validated method was applied to the commercially available pharmaceutical formulations containing repaglinide.

Experimental:

Apparatus: A High pressure liquid chromatographic system (Shimadzu HPLC class VP series) with two LC-10 AT VP pumps, variable wavelength programmable UV-Visible detector SPD-10 A VP, SCL-10A VP system controller (Shimadzu) and C-18 column was used. The HPLC system was equipped with the soft ware Class VP series version 5.03 (Shimadzu).

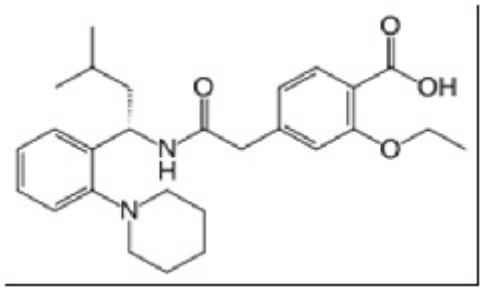

Fig. 1: Structure of Repaglinide

REAGENTS AND STANDARDS

All chemicals used were of analytical reagent grade. Distilled water filtered through 0.45 µm filter (Millipore) was used to prepare solutions.

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Ammonium phosphate buffer (pH 4.0): Prepared by dissolving 1 gm of monobasic ammonium phosphate in 500mL of water. pH was adjusted to 4.0 by addition of orthophosphoric acid. This buffer was used as the diluent for the sample preparations.

Ammonium phosphate buffer (pH 2.5): Prepared by dissolving 1 gm of monobasic ammonium phosphate in 500mL of water. pH was adjusted to 2.5 by addition of orthophosphoric acid. This buffer was used as the mobile phase.

Methanol: HPLC grade methanol was used.

Diluent: The diluent was prepared by mixing methanol and Ammonium phosphate buffer (pH 4.0) in the ratio of 70:30.

Mobile phase: The mobile phase was prepared by mixing methanol and Ammonium phosphate buffer (pH 2.5) in the ratio of 70:30.

Drug sample: Pharmaceutical grade repaglinide, certified to be 99.8% pure was procured from local pharmaceutical industry and was used as received. A stock standard containing 1mg/mL repaglinide solution was prepared by dissolving accurately weighed 100 mg of pure drug in the 25mL of methanol and diluting to 100 mL with the diluent in a calibrated flask.

Procedure:

Chromatographic Conditions: The mobile phase was filtered through a 0.45µm membrane filter, degassed by ultrasonication for 15 min and pumped from the solvent reservoir to the column at a flow rate of 1mL/min, which yielded a column back pressure of 120-140 kg/cm^2. The run time was set at 12 min. The volume of injection loop was 20 µl. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 245nm and the data were acquired, stored and analyzed with the soft ware Class VP series version 5.03 (Shimadzu).

Calibration Graph: Working standard solutions equivalent to 0.5 to 5µg/mL repaglinide were prepared by appropriate dilution of stock standard solution (1mg/mL) with the diluent solution. 20 µl aliquot of each solution was injected on to the column for five times. The peak area and retention time was recorded. Calibration graph was prepared by plotting the mean peak area versus concentration of repaglinide. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the mean peak area-concentration data.

Assay in Dosage Forms: Twenty tablets were weighed and powdered. An accurately weighed portion of the powder equivalent to 100mg of repaglinide was transferred to a 100mL volumetric flask containing about 50mL of methanol. The contents of the flask were sonicated to dissolve repaglinide, made up to volume with diluent and the resulting mixture was filtered through 0.45µm filter. The filtered solution was appropriately diluted with the diluent solution for analysis as described already. The mean value of the peak area was calculated and the drug content in each tablet was quantified using the regression equation.

RESULTS AND DISCUSSION

Method Development: Drug quality control, stability, metabolism, pharmacokinetics, and toxicity studies all necessitate the determination of drugs in pharmaceutical formulations and biological samples. Consequently, efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminarily optimized to develop the present method for the determination of repaglinide. A solution of repaglinide was injected five times on to the column and was monitored by UV detection at 245 nm. The mobile phase consisting of methanol and Ammonium phosphate buffer (pH 2.5) in the ratio of 70:30 was selected after several preliminary experiments. At a flow rate of 1 mL/min the retention time was 2.590 min (Fig. 2). Under the described experimental conditions, the peak was well-defined and free from tailing.

Method Validation: In order to determine the adequate resolution and reproducibility of the proposed method, suitability parameters including retention time, plate number and tailing factor were investigated, and were found to be 2.590 min (Fig.2), 32369.88 and 1.02, respectively, which indicate the method suitability.

Linearity and Range: Calibration curve was constructed by plotting the mean peak area versus concentration which was linear over the concentration range 0.5-5 µg/mL. Using the regression analysis, the linear equation, Y = -15.854 + 1030.20 X, was obtained, where Y is the mean peak area and X concentration in µg/mL. The Linearity co-efficient of mean response ofreplicate determination plotted against respective concentration was found to be 0.9991. The %RSD for peak area response of five replicates was 0.564.

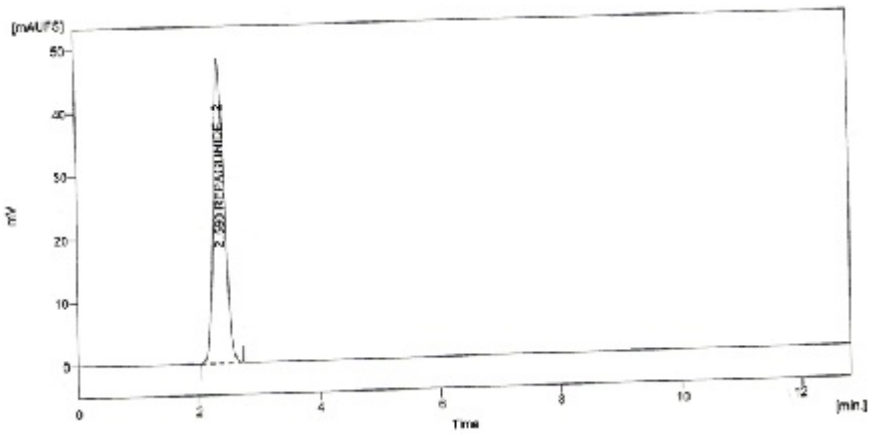

Fig. 2: Chromatogram of Repaglinide

Detection and Quantification Limits: Limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise ratio method[9-11]. LOD is taken as the concentration of analyte where signal to- noise ratio was 3, and it was found to be 0.275µg /mL. LOQ is taken as the concentration of analyte where signal-to-noise ratio was 10, and it was found to be 0.833µg /mL.

Precision: The precision of the method was evaluated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of repaglinide were analyzed in five replicates during the same day (intra-day precision) and three consecutive days (inter-day precision). Within each series, every solution was injected in triplicate. The relative standard deviation (RSD) values were calculated for the peak area. The RSD values of intra-day studies and inter-day showed that the precision of the method was satisfactory. The results of this study are given in Table 1.

Accuracy: The accuracy of an analytical method expresses the closeness between the reference value and found value[9-11]. Accuracy was evaluated as percentage relative error between the measured mean concentrations and taken concentrations. The results obtained for three concentrations are shown in Table-1, from which it is clear that the accuracy is excellent. The accuracy was also assessed by analyzing the pharmaceutical formulation containing the repaglinide and calculated the percent recovery of the active ingredient which was found to be in the range of 99.75 ± 0.55 to 100.73 ± 0.34, indicating that the co-formulated substances such as talc, starch, gum acacia, lactose, dextrose, hydroxyl methyl cellulose, sodium alginate and magnesium stearate did not interfere in the assay.

Application of the Method for the Analysis of Commercial Formulation: The developed and validated method was applied to the determination of repaglinide in two brands of tablets containing repaglinide (0.5 and 1.0 mg repaglinide per tablet) which are available in the local market using the procedure described earlier. Evaluation was performed using the calibration curve method since no significance difference between the slopes of the calibration curves for standards and tablet extracts was observed. The results obtained by the proposed method were statistically compared with the reference method by applying the Student's t-test for accuracy and *F*-test for precision. As shown by the results compiled in Table 2, the calculated *t*- and *F*-values did not exceed the tabulated values, $t = 2.77$ and $F = 6.39$ at the 95% confidence level for four degrees of freedom suggesting that the proposed method and the reference method (UV method) do not differ significantly with respect to accuracy and precision. The accuracy and validity of the proposed method were further ascertained by performing recovery experiments. The recovery studies were carried out by mixing a known quantity of drug with preanalyzed sample and the contents were reanalyzed by the proposed method. The recovery of pure drug from the pharmaceutical formulation revealed that co-formulated substances did not interfere in the determination of the drug (Table -3). There was a high recovery of repaglinide indicating that the proposed procedure for the determination of repaglinide in the tablet dosage forms is highly accurate.

Conclusion: The proposed method was found to be simple, precise, accurate and rapid for the determination of repaglinide in pure form and its dosage form. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims. Hence, this can be conveniently adopted for routine analysis of repaglinide in pure form and its dosage forms.

Table 1: Intra-day and Inter-day precision

Concentration of Repaglinide(µg/mL)	Observed concentration of Repaglinide(µg/mL) Intra-day Mean*	% RE	%RSD	Inter-day Mean*	% RE	%RSD
4	3.98	0.50	0.88	4.05	1.25	1.39
8	8.03	0.37	0.35	7.96	0.50	0.98
12	11.97	0.25	0.56	12.06	0.50	0.84

RE- Relative error, RSD- relative standard deviation
*Mean value for five determinants

Table 2: Determination of Repaglinide in tablets and statistical comparison with reference method

Brand name	Labelled amount(mg)	% Found** (± s.d) Reference method*	Proposed method	t-value	F-value
Eurepa	0.5	101.45 ± 0.69	99.75 ± 0.55	1.86	4.43
Eurepa	1.0	98.29 ± 0.84	100.73 ± 0.34	2.17	5.16

* Reference method was UV method developed in the laboratory.
** Recovery amount was the average of five determinants
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39

Table 3: Recovery of Repaglinide

Amount of Drug added (mg)	Mean (±s.d) amount(mg) found (n=5)	Mean (±s.d)% of recovery (n=5)
2	1.99 ± 0.11	99.50 ± 1.07
4	4.06 ± 0.09	101.50 ± 0.57

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