



Novel RP-HPLC Method Development and Validation of Metformin HCl and Repaglinide in Bulk and Tablet Dosage Form

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ABSTRACT

To quantify repaglinide and metformin HCl in large and combination tablet dosage form simultaneously by RP-HPLC, a simple, precise, and accurate approach has been developed. The chromatographic conditions employed were Agilent C18 column (150×4.6mm, 5 μ) column using a Methanol: Water mixture in gradient mode as the mobile phase at a flow rate of 0.6 ml/min, λ_{\max} was found to be 245.5 nm in UV Spectrophotometer. The Retention time (R_t) of the Metformin HCl and Repaglinide was found to be 1.575 and 2.564min respectively. The established technique was confirmed as per ICH Q2 (R1) strategies. The method produces linear responses in the concentration range of 5:0.02-150:0.6 μ g/ml for the RP-HPLC method. For the RP-HPLC procedure, a correlation coefficient value of 0.999 was found. As a result, the recommended method may be employed to successfully quantify Metformin HCl and Repaglinide in bulk and combination tablet dosage form.

Key Words: Metformin HCl, Repaglinide, Simultaneous estimation, RP-HPLC, Validation

eIJPPR 2023; 13(1):7-12

HOW TO CITE THIS ARTICLE: Bhavyasri Kh, Begum S, Sumakanth M. Novel RP-HPLC Method Development and Validation of Metformin HCl and Repaglinide in Bulk and Tablet Dosage Form. Int J Pharm Phytopharmacol Res. 2023;13(1):7-12. <https://doi.org/10.51847/Xc9UhmnyiY>

INTRODUCTION

The antihyperglycemic biguanide metformin is the first-line drug for the treatment of type II diabetes [1]. A hypoglycemic drug named repaglinide is used in diabetes to develop glycaemic treatment [2, 3]. It is seen that this drug decreases the postprandial glucose levels. The appropriate time taking it is with food, and dosages given during meals must be avoided whenever a meal is skipped. Metformin and Repaglinide are given to diabetic individuals who have high blood sugar levels. This combination, combined with a diet and exercise program, is used to control high blood sugar in people with diabetes. Repaglinide function through enhancing the secretion of the body's endogenous insulin [4-6]. A method to measure these drugs in combination tablet form must be developed as the prevalence of diabetes is increasing

daily. Therefore, there is a need to develop a technique to simultaneously measure metformin hydrochloride and repaglinide in bulk and combined tablet dosage forms. An RP-HPLC method was developed for the simultaneous determination of metformin and repaglinide in bulk and composite tablet dosage forms [7-15]. The structures of metformin HCl and repaglinide are shown in **Figures 1 and 2** respectively. In a study, the effects of standard drug used for the management of DM were observed in comparison to Nigella Sativa (Thymoquinone) which is having anti-hyperglycemic and anti-oxidant effects [16]. It was indicated that metformin acts on many organs of the body which include the male reproductive system [17, 18].

Drug profile of Metformin HCl

Color: white crystalline powder

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Received: 18 November 2022; **Revised:** 30 February 2023; **Accepted:** 10 February 2022

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Molecular formula: $C_4H_{11}N_5HCl$

Solubility: It is freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and methylene chloride.

Category: biguanides.

State: solid

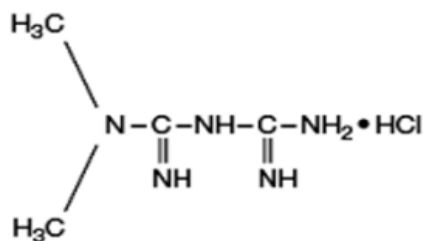


Figure 1. Structure of Metformin HCl

Drug profile of Repaglinide

Color: white to off-white powder

Molecular formula: $C_{27}H_{36}N_{2}O_4$

Solubility: It is a poorly water-soluble compound, freely soluble in methanol, ethanol, and acetonitrile.

Category: Meglitinide

State: solid [12].

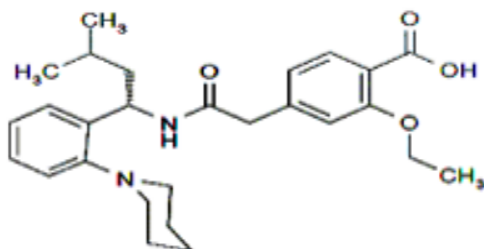


Figure 2. Structure of Repaglinide

MATERIALS AND METHODS

Apparatus & instrument

RP-HPLC “Shimadzu (LC-20AD), digital analytical balance, and Ultrasonic water bath were used. We also used Pipettes, beakers, measuring cylinders, and Volumetric flasks.

Chemicals and reagents

The Pharma company presented Metformin and Repaglinide standard as gift samples. The medication EUROPA MF 2 (Metformin and Repaglinide 500:2) was attained from a nearby pharmacy store. HPLC-grade materials were utilized throughout the experiment.

Preparation of standard solution

Metformin HCl

10mg of Metformin HCl was weighed accurately and acquired in a volumetric flask (10 ml). Add little amount of

water to dissolve the drug. Then make up to the mark with water to obtain 1000 $\mu\text{g/mL}$. Pipette 1 mL of 1000 $\mu\text{g/mL}$ into a 10 mL volumetric flask and dilute to the mark with diluent to give a concentration of 100 $\mu\text{g/mL}$.

Repaglinide

10mg of Repaglinide was weighed accurately and taken in a volumetric flask (10 ml). Add small amounts of water to dissolve the drug. Then mark up to the mark with water to get 1000 ppm. Pipette 1 mL of 1000 $\mu\text{g/mL}$ into a 10 mL volumetric flask and dilute to the mark with diluent to give a concentration of 100 $\mu\text{g/mL}$.

Wavelength selection

Both the standard 10 ppm solutions were scanned from 200-400nm in overlay mode in UV-Spectrophotometer to get the isosbestic point. Figure 3 shows the overlay spectra of Metformin HCl and Repaglinide. Optimized chromatographic conditions are shown in Table 1.

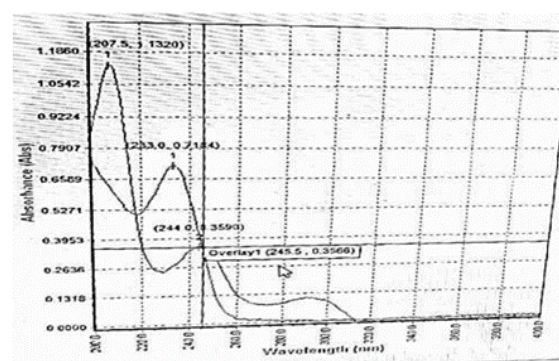


Figure 3. Overlay spectra of Metformin HCl and Repaglinide

Table 1. Optimized chromatographic conditions

| | |
|------------------|---|
| Column | Agilent 150mmx 4.6 mm, 5 μ . |
| Injection Volume | 20.0 μ L |
| Detector | UV 245.5nm |
| Mobile phase | Methanol: Water |
| Flowrate | 0.6ml/min |
| Pump mode | Gradient |
| Rt | 1.575min for Metformin HCl and 2.564min for Repaglinide |
| Runtime | 10min |

Preparation of standard stock solutions

Metformin HCl

10 mg of Metformin HCl was weighed accurately and acquired in a volumetric flask (10 ml). Increase little amounts of water to dissolve the drug. Then make up to the mark with water to get 1000 ppm. Pipette 1 mL of 1000 $\mu\text{g/mL}$ into a 10 mL volumetric flask and

dilute to the mark with diluent to give a concentration of 100 µg/mL.

Repaglinide

10mg Repaglinide was weighed accurately and taken in a 10ml volumetric flask. Add small amounts of water to dissolve the drug. Then mark up to the mark with water to get 1000 ppm. Pipette out 1ml from 1000µg/ml and take in 10ml Volumetric flask, mark up to mark with diluent to get 100µg/ml concentration.

Preparation of combined (Metformin HCl: Repaglinide) standard solution

From 10ppm standard metformin solution, pipette out 2.5ml in 5ml volumetric flask to get 5 ppm, then to this add 0.02ml from 0.5ppm of standard Repaglinide to get 0.02ppm then mark up to mark with methanol: water (70:30) to get metformin: repaglinide in 5:0.02 ppm concentration.

Preparation of sample stock solution (1000µg/ml solution)

5 tablets were weighed accurately and powder the tablets into fine powder. Then take a weight equivalent to 10mg in a 10ml-volumetric flask. Add small amounts of diluent to dissolve it. Then sonicate it for 15 minutes to dissolve the powder. Then mark up with methanol: water (70:30) to get 1000µg/ml.

Preparation of Sample stock solution

Then pipette out 0.1ml from this 1000µg/ml makeup with diluent to get 10µg/ml. From 10ppm, pipette out 5.02ml to get 5.02µg/ml of sample solution.

Validation parameters

Specificity

The interference in the optimized method can be checked to ascertain the specificity. At the retention times of these drugs using this method, we shouldn't observe interference peaks in the placebo or blank samples. This approach was therefore said to be specific. The blank solutions were injected to accomplish the Specificity.

System suitability

Standard solution 5:0.2 ppm was injected in HPLC five times to calculate the area. The %RSD was calculated and was found to be within the limit.

From standard 100 µg/ml metformin HCl, pipette out 2.5 ml in 10 ml volumetric flask, then make up with water to get 25ppm of standard Metformin HCl. From 5µg/ml standard Repaglinide solution, 0.1 ml was pipetted out in a 5 ml volumetric flask to get 0.1 ppm. 2ml of 25 ppm was taken in a test tube then 2ml of 0.1ppm of

Repaglinide was added to get 25:0.1 ppm (Metformin HCl: Repaglinide).

Precision

Intraday precision

It is described as precision over a brief period of time while operating under the same conditions. Six injections of 25:0.1 were injected during the course of the intraday precision.

Inter-day precision

Within the lab variances, such as different days, different instruments, and different analysts, inter-day precision is carried out. Six repeats of 25:0.1 injection were injected during the day with inter-day precision.

Linearity

According to label claim EUROPA MF 2 (500mg:2mg), linearity studies were performed for the combined drug, and absorbance was checked at the isosbestic point i.e., 245.5. The ratios for the combined drug were found to be 1.66:0.006, 02:008, 2.5:0.01, 3.33:0.013, 05:0.02, 10:00.04, 12.5:0.05, 16.6:0.066, 25:00.1, 50:00.2 ppm.

Preparation of 1.66:0.006

From 10ppm standard metformin solution, pipette out 2.5ml in 5ml volumetric flask to get 1.66 ppm, then to this add 0.06ml from 0.5ppm of standard Repaglinide then mark up to mark with acetonitrile: water (70:30). Then check the absorbance at 245.5 nm. Similarly, all the concentrations were prepared to get 02:008, 2.5:0.01, 3.33:0.013, 05:0.02, 10:00.04, 12.5:0.05, 16.6:0.066, 25:00.1, 50:00.2 ppm.

Accuracy

It is performed by spiking the sample with the known concentration of standard solution %recovery was calculated at three different levels (50%, 100%, and 150%).

2ml of 25:0.1 µg/ml was taken in a test tube to add 2ml 50.2µg/ml sample solution and check the absorbance of this solution at 245.5 nm. 2ml of 50.2µg/ml was taken in a test tube to add 2ml 50.2µg/ml sample solution and check the absorbance of this solution at 245.5 nm. 2ml of 75:0.3 µg/ml was taken in a test tube to add 2ml 50.2µg/ml sample solution and check the absorbance of this solution at 245.5 nm.

Robustness

Metformin HCl and Repaglinide (25:0.1) were analyzed by changing the flow rate and keeping all the parameters the same. The absorbance of the solution was checked at 0.6ml/min and 0.8ml/min flow rates.

LOD and LOQ

The detection limit is the smallest amount of analyte that can be detected. The quantitation limit is the smallest quantity of analyte that can be measured. Calculated by using the below formula.

$$LOD = 3.3 \times SD/slope \quad (1)$$

$$LOQ = 10 \times SD/slope \quad (2)$$

Where,
SD=Standard deviation

ESSAY

Preparation of sample solution

The marketed tablet formulation (Europa MF 2 – 2mg Repaglinide + 500 mg of Metformin) weighs 10 Tablets and calculates the average weight of one tablet. Crush the tablets in a motor pestle and make the powder. Weigh the quantity of powder equivalent to 10 mg equivalent to Repaglinide and Metformin.

Weight of 10 tablets = 6260mg

The average weight of 10 tablets = 626mg

Weight to be taken

$$= \frac{\text{Average weight} \times \text{Equivalent weight}}{\text{Label claim}} = \frac{10 \times 626}{502} \quad (3)$$
$$= 12.47 \text{ mg}$$

12.5 mg of powdered sample was taken in 10ml V.F and make up with the diluent to get 1000 ppm. From this 1000 ppm, 0.1 ml was pipetted out in a 10ml volumetric flask to get 10 ppm of sample solution. From this 10ppm, 5.02 ml was taken in a 10 ml volumetric flask and make up with diluent to get 5.02 ppm.

Standard and sample solutions were injected separately into the system and chromatograms were noted and the percentage of drug in the sample was calculated.

RESULTS AND DISCUSSION

Specificity

When blank was injected, no peaks were observed at the Rt of Metformin HCl and Repaglinide.

Linearity

The calibration curve for the determination of metformin HCl and Repaglinide in combined form (5:0.02) in the range of 5.0:0.2: 60 µg/ml-150:0.6µg/ml was found to be linear at $\lambda_{\text{max}}=245.5\text{nm}$. The correlation coefficient value was found to be 0.999. The calibration equation value was found to be $y=304544x-212620$.

Precision

The precision was determined for the concentration of 25:0.1 µg/ml. For intraday precision, the absorbance was measured 5 times. Inter-day precision was done on the next day for the same 25:0.1 µg/ml concentration absorbance was measured 5 times. SD and % RSD was calculated for both inter-day and intraday. %RSD was found to be less than 2.

Accuracy

Accuracy is given in terms of %recovery. The % recovery was found to be 99%. According to USP, it should be 98-102%.

Robustness

Robustness was done by injecting 25:0.1 µg/ml. Robustness was given in terms of %RSD.

LOD and LOQ

By substituting in the LOD and LOQ formula the limit of detection was found to be 0.0143573µg/ml and limit of quantitation was found to be 0.043527 µg/ml.

Assay calculation

The Chromatogram of Standard and sample were shown in **Figures 4 and 5** respectively. **Table 2** shows the summary of all results.

$$\text{Assay} = \frac{\text{Spl area}}{\text{Std area}} \times \frac{\text{Std. Dil. Fac}}{\text{Spl. Dil. Fac}} \times \frac{\text{Avg. Wt of Tab}}{\text{L.C.}} \times \text{Potency of Std} \quad (4)$$

Spl area= Sample Peak area

Std area= Standard Peak area

Std. Dil. Fac= Standard dilution factor

Spl. Dil. Fac= Sample dilution factor

Avg. Wt of Tab= Average weight of the tablet L.C label claim Potency of Std

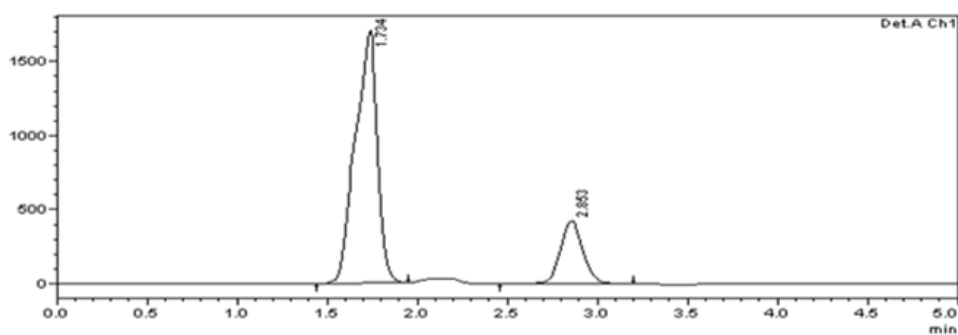


Figure 4. Chromatograms of Standard

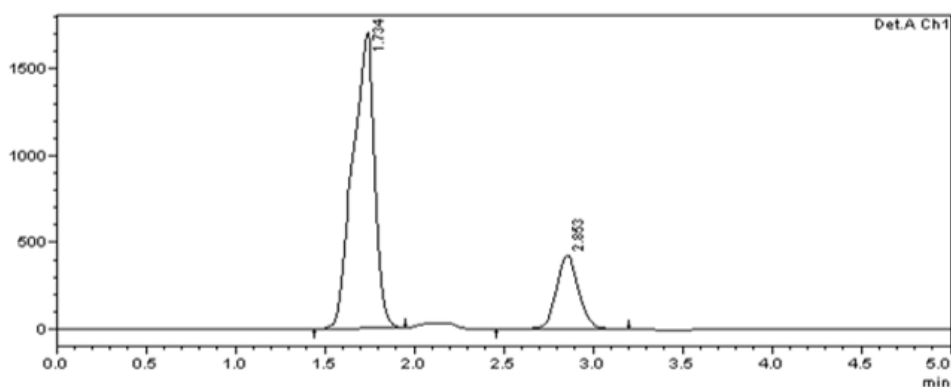


Figure 5. Chromatograms of Sample

Table 2. Summary of results

| Parameters(RP-HPLC) | Combined drug(500:2) |
|---|------------------------|
| Calibration range ($\mu\text{g} / \text{ml}$) | 5:0.02-150:06ppm |
| Optimized wavelength | 246 nm |
| Retention time | Met:1.575 Rep:2.848 |
| Correlation coefficient(r^2) | 0.999 |
| Precision (Intraday) | 0.008% |
| Precision(Inter-day) | 0.0643 |
| % Recovery | 98-99% |
| LOD (ppm) | 0.014357531 ppm |
| LOQ(ppm) | 0.043527372 ppm |

CONCLUSION

The dosage formulation of Metformin and Repaglinide bulk and mixed tablet were studied. The proportion of drugs in concomitant medications was found to be within the limits set by the Indian Pharmacopoeia. All validation parameters were performed and found to be within acceptable limits according to ICH Q2(R1) requirements. Therefore, using the proposed approach, metformin and repaglinide together and in combination with other drugs can be estimated using an RP-HPLC. For the proposed method, HPLC grade Acetonitrile:water(70:30) was used as mobile phase, Agilent (150mmx 4.6 mm, 5 μ) column,

flow rate 0.6ml/min, eluents were scanned with UV detector in the system at 245.5nm. The retention time for Metformin HCl was found to be 1.575 mins and for the Repaglinide is 2.848 mins in gradient mode. Therefore, this proposed method was found to be better than previously reported methods. Hence above method can be used in quality control for routine analysis of tablets of Metformin HCl and Repaglinide.

Acknowledgments: I want to acknowledge our beloved Principal Prof. M. Sumakanth and the Faculty of the Department of Pharmaceutical Analysis of RBVRR women's college of pharmacy for allowing me to perform my research work.

Conflict of interest: None

Financial support: None

Ethics statement: None

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