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

Khagga Bhavyasri[1]*, Samreen Begum[1], Mogili Sumakanth[2]

[1]Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Barkatpura, Hyderabad, Telangana-500027, India.
[2]Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Barkatpura, Hyderabad, Telangana-500027, India.

ABSTRACT

To quantify repaglinide and metformin HCl in large and combination tablet dosage forms simultaneously by RP-HPLC, a simple, precise, and accurate approach has been developed. The Chromatographic conditions employed were Agilent C18 column (150 × 4.6 mm, 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 a UV spectrophotometer. The retention time (R_t) of the metformin HCl and repaglinide was found to be 1.575 and 2.564 min, 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 µg/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 forms.

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. Appropriate time taking 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 functions through enhancing the secretion 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 a standard drug used for the management of DM were observed in comparison to *Nigella sativa* (Thymoquinone) which has anti-hyperglycemic and anti-oxidant effects [16]. It was indicated that metformin acts on many organs of the body including the male reproductive system [7, 17].

The present study aimed to investigate the development and validation of a new RP-HPLC method for the determination of metformin HCl and

Corresponding author: Khagga Bhavyasri
Address: Department of Pharmaceutical Analysis, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Barkatpura, Hyderabad, Telangana-500027, India.
E-mail: ✉ bhavya.khagga@gmail.com
Received: 18 November 2022; **Revised:** 03 February 2023; **Accepted:** 10 February 2023

This is an **open access** journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

repaglinide in bulk and tablet form.

Drug profile of metformin HCl
Color: white crystalline powder
Molecular formula: $C_4H_{11}N_5HCl$
Solubility: It is freely soluble in water; slightly soluble in alcohol; and practically insoluble in acetone and methylene chloride.
Category: biguanides.
State: solid

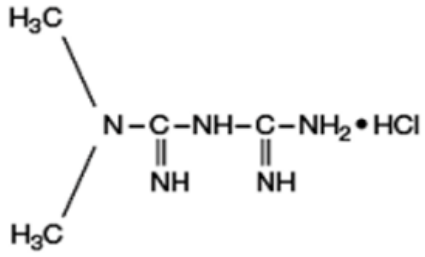

Figure 1. Structure of metformin HCl

Drug profile of repaglinide
Color: white to off-white powder
Molecular formula: C_{27} H_{36} N_{2O4}
Solubility: It is a poorly water-soluble compound, freely soluble in methanol, ethanol, and acetonitrile.
Category: Meglitinide
State: solid [12].

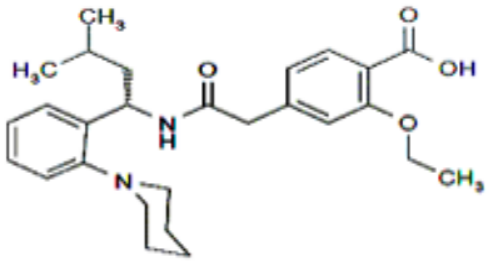

Figure 2. Structure of repaglinide

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 obtained from a nearby pharmacy store. HPLC-grade materials were utilized throughout the experiment.

Preparation of standard solution
Metformin HCl
10 mg of metformin HCl was weighed accurately and acquired in a volumetric flask (10 ml). Add a small amount of water to dissolve the drug and makeup to the mark with water to obtain 1000 µg/mL. Pipette 1 mL of 1000 µg/mL into a 10 mL volumetric flask and dilute to the mark with diluent to give a concentration of 100 µg/mL.

Repaglinide
10 mg 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 µg/mL into a 10 mL volumetric flask and dilute to the mark with diluent to give a concentration of 100 µg/mL.

Wavelength selection
Both the standard 10 ppm solutions were scanned from 200-400 nm in overlay mode in a 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**.

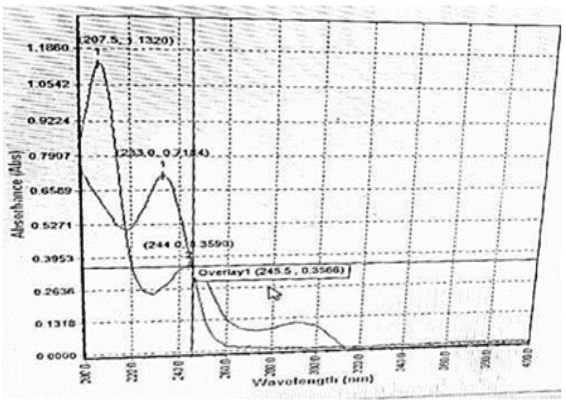

Figure 3. Overlay spectra of metformin HCl and repaglinide

Table 1. Optimized chromatographic conditions

Column	Agilent 150 mmx 4.6 mm, 5µ
Injection volume	20.0 µL
Detector	UV 245.5 nm
Mobile phase	Methanol: Water
Flowrate	0.6 ml/min
Pumpmode	Gradient
Rt	1.575 min for metformin HCl and 2.564 min for repaglinide
Runtime	10 min

Preparation of standard stock solutions

Metformin HCl

10 mg of metformin HCl was weighed accurately and acquired in a volumetric flask (10 ml). Increases salt amounts of water to dissolve the drug. Then make up to the mark with water to get 1000 ppm. Pipette 1 mL of 1000 µg/mL into a 10 mL volumetric flask and dilute to the mark with diluent to give a concentration of 100 µg/mL.

Repaglinide

10 mg repaglinide was weighed accurately and taken in a 10 ml 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 1000 µg/ml and take in 10 ml volumetric flask, mark up to mark with diluent to get 100 µg/ml concentration.

Preparation of combined (metformin HCl: repaglinide) standard solution

From 10 ppm standard metformin solution, pipette out 2.5 ml in 5 ml volumetric flask to get 5 ppm, then to this add 0.02 ml from 0.5 ppm of standard repaglinide to get 0.02 ppm 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 (1000 µg/ml solution)

5 tablets were weighed accurately and the tablets were 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 1000 µg/ml.

Preparation of Sample stock solution

Then pipette out 0.1 ml from this 1000 µg/ml makeup with diluent to get 10 µg/ml. From 10 ppm, pipette out 5.02 ml to get 5.02 µg/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 a 10 ml volumetric flask, then make up with water to get 25 ppm of standard metformin HCl. From 5 µg/ml standard repaglinide solution, 0.1 ml was pipetted out in a 5 ml volumetric flask to get 0.1 ppm. 2 ml of 25 ppm was taken in a test tube then 2 ml of 0.1 ppm 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 while operating under the same conditions. Six injections of 25:0.1 were injected during 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 (500 mg:2 mg), 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, and 50:00.2 ppm.

Preparation of 1.66:0.006

From 10 ppm standard metformin solution, pipette out 2.5 ml in 5 ml volumetric flask to get 1.66 ppm, then to this add 0.06 ml from 0.5 ppm 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, and 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%).

2 ml of 25:0.1 µg/ml was taken in a test tube to add 2 ml 50.2 µg/ml sample solution and check the absorbance of this solution at 245.5 nm. 2 ml of 50.2 µg/ml was taken in a test tube to add 2 ml of 50.2 µg/ml sample solution and check the absorbance of this solution at 245.5 nm. 2 ml of 75:0.3 µg/ml was taken in a test tube to add 2 ml of 50.2 µg/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. These parameters are calculated by using the Eqs. (1) and (2).

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

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

Where,

SD = Standard deviation

ESSAY

Preparation of sample solution

The marketed tablet formulation (Europa MF 2 – 2 mg 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 = 6260 mg

The average weight of 10 tablets = 626 mg

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

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

Standard and sample solutions were injected separately into the system, 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 5.0.02: 60 µg/ml-150:0.6 µg/ml was found to be linear at λ_{max} = 245.5 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 were 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 0.0143573 µg/ml and the limit of quantitation was found to be 0.043527 µg/ml.

Assay calculation

The chromatogram of the standard and sample are shown in **Figures 4 and 5**, respectively. **Table 2** shows the summary of all results.

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

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 claims Potency of Std.

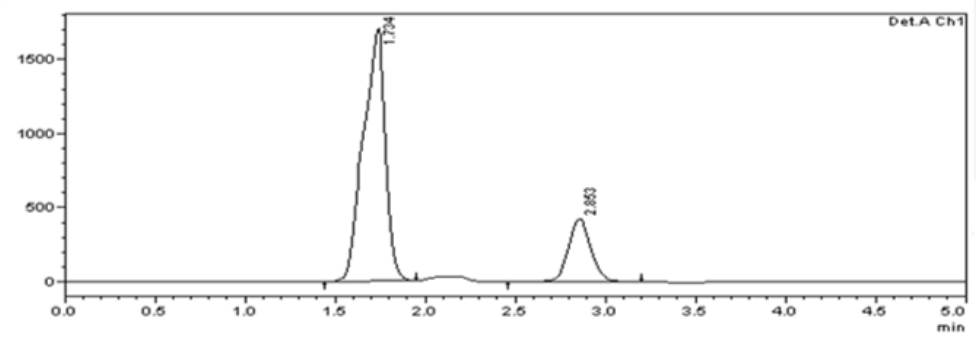

Figure 4. Chromatograms of standard

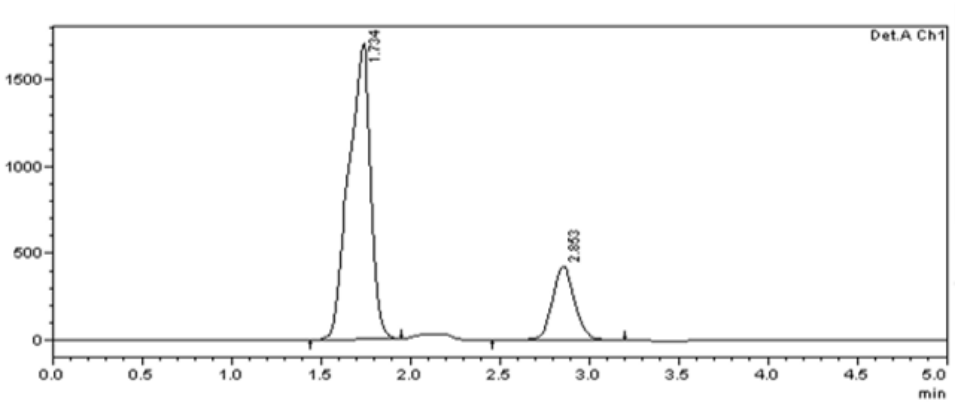

Figure 5. Chromatograms of sample

Table 2. Summary of results

Parameters (RP-HPLC)	Combined drug (500:2)
Calibration range (µg / ml)	5:0.02-150:06 ppm
Optimized wavelength	246 nm
Retention time	Met:1.575 Rep:2.848
Correlation coefficient (r2)	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 IHQ2 (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, HPLCgradeAcetonitrile: water (70:30) was used as mobile phase, Agilent (150 mmx 4.6 mm, 5 µ) column, flow rate 0.6 ml/min, eluents were scanned with UV detector in the system at 245.5 nm. 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

REFERENCES

[1] Inzucchi SE, Bergenstal RM, Buse JB. Management of hyperglycemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European association for the study of diabetes. Diabetes Care. 2012;38(1):140-9.

[2] Patan A, Basha SR, Ketha RK, Cheriyan BV, Muthukumar VA. Development and validation of a new RP-HPLC method for the simultaneous estimation of metformin hydrochloride and repaglinide in pure and pharmaceutical formulations. Res J Pharm Technol. 2021;14(3):1323-8.

[3] Shokouhi S, Sohrabi MR. Net analyte signal and radial basis function neural network for development spectrophotometry method for the simultaneous determination of metformin and sitagliptin in the anti-diabetic commercial tablet. Optik. 2021;243:167518.

[4] Godge RK, Shinde GS, Joshi S. Simultaneous estimation and validation of dapagliflozin and saxagliptin in bulk drug and dosage form by RP-HPLC. Res J Sci Technol. 2019;11(1):59-63.

[5] Shah P, Pandya T, Gohel M, Thakkar V. Development and validation of HPLC method for simultaneous estimation of rifampicin and ofloxacin using experimental design. J Taibah Univ Sci. 2019;13(1):146-54.

[6] Bonde S, Bonde CG, Prabhakar B. Quality by design-based development and validation of HPLC method for simultaneous estimation of paclitaxel and vinorelbine tartrate in dual drug-loaded liposomes. Microchem J. 2019;149:103982.

[7] Mahrouse MA, Lamie NT. Experimental design methodology for optimization and robustness determination in ion pair RP-HPLC method development: application for the simultaneous determination of metformin hydrochloride, alogliptin benzoate, and repaglinide in tablets. Microchem J. 2019;147:691-706.

[8] Wadhwa G, Krishna KV, Dubey SK, Taliyan R. Development and validation of RP-HPLC method for quantification of repaglinide in mPEG-PCL polymeric nanoparticles: QbD-driven optimization, force degradation study, and assessment of in vitro release mathematic modeling. Microchem J. 2021;168:106491.

[9] Tejashree KR, Dashetwar AR, Kardile DP, Jadhav AP, Bhagat VC, Shete RV. Q-absorbance ratio spectrophotometric method for the simultaneous estimation of metformin hydrochloride and voglibose in the tablet dosage form. Res J Pharm Technol. 2021;14(6):3179-83.

[10] Kamal AH, Hammad MA, Kannouma RE, Mansour FR. Response surface optimization of a vortex-assisted dispersive liquid-liquid microextraction method for highly sensitive determination of repaglinide in environmental water by HPLC/UV. BMC Chem. 2022;16(1):1-10.

[11] Marie AA, Salim MM, Kamal AH, Hammad SF, Elkhoudary MM. Analytical quality by design based on design space in reversed-phase-high performance liquid chromatography analysis for simultaneous estimation of metformin, linagliptin, and empagliflozin. Royal Soc Open Sci. 2022;9(6):220215.

[12] Soni LK, Narsinghani T, Jain M. Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and repaglinide in the tablet dosage form. J Liq Chromatogr Relat Technol. 2012;35(3):385-92.

[13] Karaderi S, Mazı C, Yılmazbilek S. Determination of the stability constants complexes of Metformin Hydrochloride with Cu (II), Cd (II) and Zn (II). Int J Pharm Res Allied Sci. 2020;9(1).

[14] Sangi SM, Bawadekji A, Alotaibi NM, Aljalaud NA. Preventive and curative effects of Metformin, *Nigella sativa*, Punica granatum and Zingiber officinale on male reproductive dysfunction in diabetic rats. Int J Pharm Res Allied Sci. 2019;8(2):48-57.

[15] Nguyen SN, Le TT, Nga HT, Tho NT. Lifestyle interventions reduce the risk of type II diabetes and cardiovascular diseases development among pre-diabetic adults. Int J Pharm Res Allied Sci. 2021;10(2):94-102.

[16] Sangi H, Alotaibi NM, Ahmedani EI, Muhammad S, Sangi A. The effect of *Nigella sativa*, and Metformin on the Changes in liver, heart, and kidney caused by DM in experimental animals. Int J Pharm Res Allied Sci. 2020;9(2):15-24.

[17] Alzain SD, Mudawi MM, Mohamed AW. Review on metformin effect on male reproductive system. Int J Pharm Res Allied Sci. 2020;9(2):158-67.