Biological Forum – An International Journal **15(3): 640-644(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Bioanalytical Method Development and Validation of Metformin HCl and Repaglinide in Bulk and Combined Dosage Form in Human Plasma by using UV Spectroscopy

K. Bhavya Sri[1*], Samreen Begum[2], D. Suchitra[3], V. Narmada[4] and M. Sumakanth[5]

[1]Associate Professor and Head, Department of Pharmaceutical Analysis,
RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad (Telangana), India.
[2]Research Scholar, Department of Pharmaceutical Analysis,
RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad (Telangana), India.
[3]Assistant Professor,
Vision College of Pharmaceutical sciences and Research, BODUPPAL, Hyderabad (Telangana), India.
[4]Department of Pharmacy,
University College of Technology, Osmania University, Hyderabad (Telangana), India.
[5]Professor and Head, Department of Pharmaceutical Chemistry,
RBVRR Women's College of Pharmacy, Hyderabad (Telangana), India.

(Corresponding author: K. Bhavya Sri)*
(Received: 23 January 2023; Revised: 18 February 2023; Accepted: 23 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: A simple, rapid, accurate, precise, and robust method has been developed and validated for estimating Repaglinide and Metformin HCl in human plasma. Method: The Protein precipitation method was used for obtaining metformin and repaglinide from serum, using ethyl acetate and 0.1N hydrochloric acid. The solvent used was Acetonitrile: water (70:30). The isosbestic point was found to be at 245.5 nm. The challenges in the study were the availability of serum and extraction of drug from the serum. Results: Extraction efficiency for Metformin HCl and Repaglinide was found to be 95.4% and 96.8% respectively. The precision was in terms of %CV which was found to be less than 2%. LLOQ and ULOQ were found to be 1.66:0.06 and 12.5:0.5 respectively. All the validation parameters like linearity, precision, accuracy, LLOQ, ULOQ, LOD, and LOQ were performed according to ICH guideline M 10. % Assay was done by using the simultaneous equation method. All the parameters were found to be within the limits. The proposed UV method can be successfully applied for the estimation of metformin HCl and Repaglinide in human plasma. As diabetic patients are increasing day by day so there is a need to develop a method for simultaneously estimating two drugs i.e., Metformin HCl and Repaglinide in human plasma. The method has been done in UV hence the method is economic and the method can be further extended to HPLC, LC-MS, 2D-LC, and further hyphenated techniques.

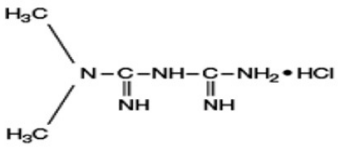
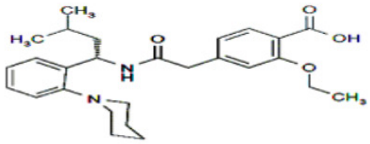
Keywords: Bioanalytical method development, Extraction, Human plasma, Metformin HCl, Repaglinide, UV-Visible Spectrophotometer.

INTRODUCTION

Metformin and Repaglinide are given to diabetic patients suffering from high blood glucose levels. This combination is used to manage high blood sugar in diabetic individuals combined with a diet and exercise program (Domingues *et al.,* 2016; Hiral *et al.,* 2019). By promoting the release of your body's endogenous insulin, repaglinide works (Sharma *et al.,* 2013). As a biguanide, metformin reduces the amount of sugar produced by your liver and absorbed by your stomach and intestines. Both of these drugs work by assisting in the restoration of your body's appropriate reaction to the insulin you naturally make (El-Zaher *et al.,* 2016; Sharma *et al.,* 2012). Blood sugar control can avoid kidney damage, blindness, nerve problems, limb loss, and problems with sexual function. Maintaining proper control of your diabetes may also reduce your risk of having a heart attack or stroke (Ma *et al* 2016; Patel *et al.,* 2021). An innovative, cost-effective Spectrophotometric bioanalytical technique for measuring Metformin HCl and Repaglinide in human plasma has been developed. The validation of bioanalytical methods used for the quantitative measurement of medicines and their metabolites in biological fluids. The study is important in the assessment and interpretation of data from bioavailability, bioequivalence, pharmacokinetic, and toxicokinetic studies. The quantitative measurement of analytes in a specific biological matrix, such as blood, plasma, serum, or urine, is required for bioanalytical technique validation (Ma *et al.,* 2014; Prasanth *et al.*, 2020). The protein precipitation method (PPM) was used to examine the levels of repaglinide and

metformin HCl in human plasma. The PPM concept is based on protein denaturation. Protein denaturation happens when the pH of the medium is changed, organic solvents are used, and salts are used (Rahul *et al.,* 2022; Ramanjireddy *et al.,* 2010). All validation parameters were carried out in accordance with ICH guidelines Q10 M. Table 1 displays the drug profiles for both medications.

Table 1: Drug profile.

Parameters	Metformin HCl	Repaglinide
Colour	white crystalline powder	It is in powder form from white to off-white
Molecular formula	$C_4H_{11}N_5HCl$	$C_{27}H_{36}N_2O_4$
Solubility	It is completely insoluble in acetone and methylene chloride, but very slightly soluble in alcohol and water.	It is a chemical that dissolves poorly in water but readily in methanol, ethanol, and acetonitrile.[11]
Structure		

MATERIALS AND METHODS

Chemicals. Repaglinide and Metformin HCl standards were obtained as a gift sample from a pharmaceutical company, and EUROPA MF 2(Metformin HCl and Repaglinide [500:2]) tablet was purchased from the local pharmacy store. Human plasma was obtained given by the blood bank. All the other chemicals used were of analytical grade.

Instrument and Apparatus. "Elico Sl 210" Double Beam UV-Visible Spectrophotometer, Centrifuge, digital analytical balance, UV chamber, and Ultrasonic water bath were used. Pipettes, beakers, measuring cylinders, volumetric flasks, and centrifuge tubes were used.

Extraction procedure. 20 ml of plasma sample was pipetted out in a 10ml centrifuge tube.0.5ml of working standard solution & 0.5ml of ethyl acetate and 0.1ml of 0.1N HCl were added. Both the compounds were easily soluble in methanol and phosphate buffer pH 4(40:60). Subsequent addition of plasma leads to protein precipitation. Then the mixture was vortexed and 10ml of ACN was added. Mix the solution properly and then vortexed again. Centrifuge for 15 minutes at 10,000 rpm. Then supernatant present after the centrifuge was transferred to a 10ml tube. Then absorbance was checked between 200-400nm.

Method development. Selection of solvent: The diluent was selected by determining the solubility of Repaglinide and Metformin HCl in different solvents like Distilled water, Methanol, Acetonitrile etc. Finally, Acetonitrile: Distilled water (70:30) was selected as a solvent.

Preparation of standard solution. 10mg of the standards of both Repaglinide and Metformin were weighed separately and transferred to a volumetric flask (10 ml). Volume was made up with the respective solvents i.e., Repaglinide in Acetonitrile and Metformin HCl in water to get 1000 ppm of both drugs. From this 0.1ml was pipetted out and transferred to a 10ml volumetric flask to get 10 µg/ml concentration solution. Metformin HCl and Repaglinide standard concentrations were prepared in the ratio of 50:0.2 and were scanned in UV in overlay mode from 200-400 nm to determine the isosbestic point. The isosbestic point was found at 245.5 using Acetonitrile: water (70:30) as blank.

Optimization of parameters. Metformin HCl and Repaglinide (50:0.2) were found to yield a clear colourless solution with ACN: water showing an isosbestic point at 244.5 nm.

Method validation

Specificity. Specificity is performed by scanning the blank and standard from 200-400nm.

Linearity. It is defined as the capacity of analytical methods to yield results directly proportional to the range of analyte concentrations in samples within the necessary concentration level.

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 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 make up to mark with acetonitrile: water (70:30). Then check the absorbance at 245.5 nm. In a similar manner 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 ppm.

LLOQ. The lower Limit of Quantification (LLOQ) is the lowest concentration of the standard curve that can be quantified accurately and precisely. LLOQ is defined as the lowest quantifiable concentration in the calibration curve.

ULOQ. The maximum concentration of the standard curve that can be determined with acceptable accuracy and precision is known as the upper limit of quantitation (ULOQ).

Precision. From the stock of 100µg/ml solution (Repaglinide and Metformin HCl), 6 ppm of Metformin HCl and 10 µg/ml of Repaglinide were made. The absorbances of these solutions were checked 6 times

and noted. For combined drug the precision was performed at 5:0.02 at 245.5. % CV was calculated for all these 3 solutions.

$$\%CV = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

$$\sigma = \sqrt{\frac{\Sigma(x_i - \mu)^2}{N}}$$

Where,

σ = Standard deviation

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

$$\text{Abs\% Bias} = \frac{\text{Measured value} - \text{True value}}{\text{True value}} \times 100$$

Detection limit. The following formula is used to determine LOD.

$$LOD = 3.3 \times SD/Slope$$

Where,

SD=Standard deviation.

Quantitation limit. The following formula is used to determine LOQ.

$$LOQ = 10 \times SD/slope$$

Where,

SD=Standard deviation.

ASSAY. An assay of the combined tablet dosage form was done by using the simultaneous equation method.

Simultaneous equation method. It would be possible to identify both medications using the simultaneous equation approach if a sample contains two absorbing substances (X and Y), each of which absorbs at the λ_{max} of the other (λ_1 and λ_2). 10 tablets of EUROPA MF 2 (Metformin and Repaglinide 500:2) were weighed and taken in a mortar then crushed to get a fine powder. Then the weight equivalents to 10mg was taken in a 100ml volumetric flask then add small amounts of diluent to dissolve the powder. Sonicate in an ultrasonic water bath for 15 mins. Then make up the volume with diluent. Then filter the solution. This filtered solution will be 100µg/ml. From this 1ml was pipetted and taken in a volumetric flask (10 ml) to get a 10 ppm solution. This solution is taken for simultaneous estimation of Metformin HCl and Repaglinide in a combined tablet dosage form. The formula for the simultaneous estimation is given below.

Cx = A1ay2 – A2ay1/AX1ay2 – ax2ay1

Cy = A1ax2 – A2ax1/AX1ax2 – ay2ax1

Where,

A1 and A2 are absorbances of λ1 and λ2 respectively,

Ax1 and ax2 are absorptivities of X at λ1 and λ2 respectively,

Ay1 and ay2 are absorptivities of Y at λ1 and λ2 respectively,

The concentrations of X and Y are Cx and Cy respectively.

RESULTS AND DISCUSSION

Specificity. Specificity is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix. When blank was injected, no peaks were observed at the Rt of Metformin HCl and Repaglinide and when the sample was injected peaks were observed at the respective Rt of the Metformin and Repaglinide. This means that the method is specific to the sample and the instrument is able to distinguish between the analyte and the sample. Spectrums of standard and blank are shown in Fig. 1 and 2 respectively.

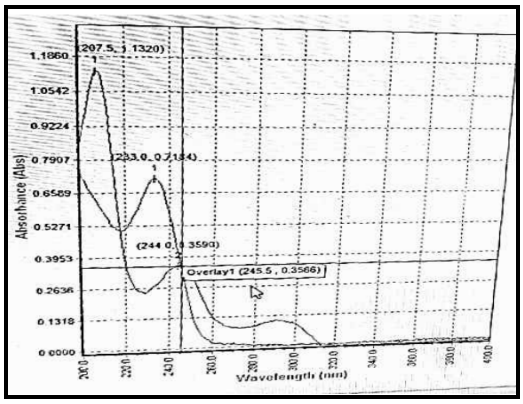


Fig. 1. Spectrum of standard drug.

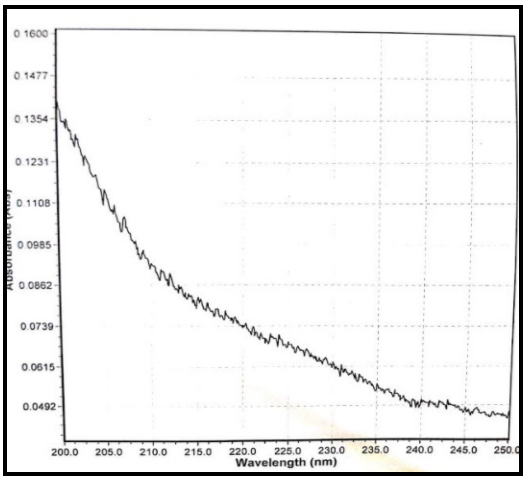


Fig. 2. Spectrum of blank.

Table 2: Results of linearity.

Concentration(ppm)	Absorbance(nm)
1.66:0.006	0.3568
02:008	0.6478
2.5:0.01	0.9345
3.33:0.013	1.254
05:00.0	1.5678
10:00.0	1.876
12.5:0.05	2.1345

Linearity. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity range is found to be 1.66:0.006-12.5:0.05µg/ml. Results of linearity are shown in Table 1. Calibration graph was shown in Fig. 3.

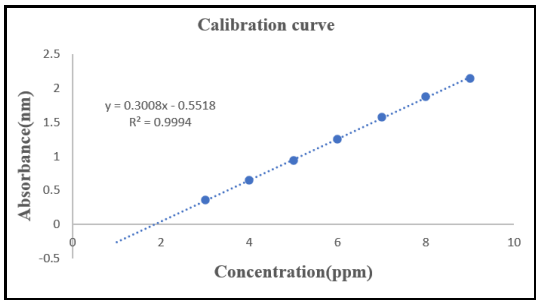


Fig. 3. Calibration curve.

Limits: Correlation Coefficient (r^2) ≥ 0.999.
Result: Correlation Coefficient (r^2) =0.9994

LLOQ

LLOQ (Lowest Limit of Quantification) from the calibration curve was found to be

LLOQ=1.66:0.006

ULOQ

ULOQ (Upper Limit of Quantification) from the calibration curve was found to be

ULOQ=12.5:0.05

Precision. Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The concentrations of the sample selected for the study are 5:0.2. Intraday prescision was carried out within the same day in the same laboratory. Interday precision was carried out by different analyst in different laboratory on different day and on different instrument. Table 3 shows the results of precision of intraday and interday.

Table 3: Results of precision.

Concentration(ppm)	Intraday precision	Inter-day precision
5:0.2	1.4675	1.7121
5:0.2	1.46675	1.7158
5:0.2	1.4672	1.7173
5:0.2	1.4675	1.7202
5:0.2	1.46576	1.7204
5:0.2	1.4676	1.7194
Average	1.467115714	1.717528571
Standard deviation	0.000616021	0.00270962
%CV	0.041988563	0.157762735

Limits: %CV should be less than 2 as per ICH guidelines.
Result: %CV for intraday precision was found to be 0.041988 and %CV for interday precision was found to be 0.157762.%CV is within the limits.

Accuracy. Accuracy is the degree of closeness between the true value of analytes in the sample and the value determined by the method. In this study concentration levels selected were 50%, 100% and 150%. The absorbance and the percentage recovery at these levels were measured. Results of accuracy were shown in Table 4.

Table 4: Results of Accuracy for combined drug.

% Level	Absorbance	% Recovery	Abs %Bias
50%	0.4425 0.4542 0.4498	96.97% 97.5% 98.5%	97.6%
100%	0.6698 0.6659 0.6641	99.11% 98.5% 98.2%	98.6%
%150	0.9810 0.9785 0.9755	99.6% 99.4% 99.11%	99.37%

Limits: Abs% Bias should be 98-102% as per ICH guidelines
Result: Abs% Bias was found to be 97-99%.

LOD and LOQ. Limit of detection (LOD) is the smallest concentration of analyte that can be detected. Limit of quantification (LOQ) is the lowest possible concentration of the analyte that can be quantified. LOD and LOQ were calculated by using the below formula. LOD and LOQ values are given in Table 5.

LOD=3.3x SD/slope
LOQ=10x SD/slope
SD= standard deviation

Table 5: LOD and LOQ Results.

Drug name	LOD	LOQ
Metformin and Repaglinide (500:2)	0.009154µg/mL	0.02786µg/mL

Assay. Assay of the tablet formulation was done by using the Simultaneous equation method

Cx = A1ay2 – A2ay1/AX1ay2 – ax2ay1
Cy = A1ax2 – A2ax1/AX1ax2 – ay2ax1

By substituting the above value for the value in the formula we will get Cx and Cy values. C_x and C_y values are given in Table 6.

C_x=9.660 C_y= 0.0368

95.4% of Metformin HCl was found in combined tablet dosage form

96.8% of Repaglinide was found in combined tablet dosage form

Table 6: **Analysis of formulation by Simultaneous Method.**

Drug name	Concentration(µg/ml)
Metformin HCl (C_x)	9.660
Repaglinide (C_y)	0.0368

CONCLUSIONS

A simple, economic, rapid, precise, and accurate Spectrophotometric method has been developed and validated for quantitative estimation of Metformin HCl and Repaglinide (500:2) in human plasma. Extraction of drugs from plasma has been done by protein precipitation. All the validation parameters like Specificity, LLOQ, ULOQ, precision, accuracy, LOQ, and LOD have been performed according to ICH guidelines Q10 M. All validation parameters were confirmed to be within their respective limitations. Hence the developed method can be successfully used for quantitative estimation of metformin HCl and Repaglinide in combined tablet dosage form in human plasma using UV-spectroscopy.

FUTURE SCOPE

The present method was found to be economic, precise, accurate. Hence it can be used for routine simultaneous analysis of metformin and Repaglinide. The method can be further extended to LC-MS, 2D LC and further hyphenated techniques to make the analysis of these drugs further sensitive and precise.

Acknowledgment. We want to acknowledge our beloved principal and faculty of the Department of pharmaceutical analysis of RBVRR Women's College of Pharmacy for giving us the opportunity to perform research work.
Conflicts of interest. None.

REFERENCES

Domingues, D. S., Pinto, M. A., de Souza, I. D., Hallak, J.E., Crippa, J. A. and Queiroz, M. E. (2016). Determination of Drugs in Plasma Samples by High-Performance Liquid Chromatography-Tandem Mass Spectrometry for Therapeutic Drug Monitoring of Schizophrenic Patients. *J Anal Toxicol.*, *40*(1), 28-36.

Hiral, J., Varachhiya, Rohan, K. Barse, and Suresh, J. (2019). Development and Validation of Spectroscopic Simultaneous Equation Method for Simultaneous Estimation of Itopride Hydrochloride and Omeprazole in Synthetic Mixture. *Asian Journal of Pharmaceutical Research*, *9*(4), 238-242.

El-Zaher, A. A., Elkady, E. F., Elwy, H. M. and Saleh M. A. (2016). Simultaneous Determination of Metformin, Glipizide, Repaglinide, and Glimepiride or Metformin and Pioglitazone by a Validated LC Method: Application in the Presence of Metformin Impurity (1-Cyanoguanidine). *J AOAC Int., 99*(4), 957-963.

Ma, Y. R., Rao, Z., Shi, A. X., Wang, Y. F., Huang, J., Han, M., Wang, X. D., Jin, Y. W., Zhang, G. Q., Zhou, Y., Zhang, F., Qin, H. Y. and Wu, X.A. (2016). Simultaneous Determination of Metformin, Metoprolol and its Metabolites in Rat Plasma by LC-MS-MS: Application to Pharmacokinetic Interaction Study. *J. Chromatogr Sci*., *54*(1),1-9.

Ma, Y. R., Zhou, Y., Zhang, G. Q., Rao, Z., Huang, J., Wei, Y. H. and Wu, X. A. (2014). Simultaneous determination of repaglinide and pravastatin sodium in rat plasma by LC-ms/MS and its application on pharmacokinetic interactions study. *Yao Xue Xue Bao*., *49*(1), 72-7.

Patel, D., Vora, A., Wairkar S. and Yc M. (2021). Design of experiment-based LC-MS/MS method development for simultaneous estimation of nateglinide and metformin hydrochloride in rat plasma. *J Mass Spectrom*., *56*(11), e4789.

Prasanth, V. G., Eapen, S., Velekkat, P., Abdu, S., Saeed, H. and Ragab, H. (2020). Simultaneous Estimation of Repaglinide and Metformin Hydrochloride by using RP-HPLC in Synthetic Mixture and Tablet Dosage form. *American journal of pharmacy and health research, 8*(6), 2321–3647.

Rahul, G., Ingle, Su, Z., Huidi, J. and Wei-Jie F. (2022). Current developments of bioanalytical sample preparation techniques in pharmaceuticals. *Journal of Pharmaceutical Analysis, 12*(4), 517-529.

Ramanjireddy, T., Dhachinamoorthi, D. and Chandrasekhar, B. (2010). Method Development and Validation of Metformin and Repaglinide in Rabbit Plasma by RP-HPLC. *Pharm. Sci., 35*, 69-75.

Sharma, K., Kandaswamy, M., Mithra, C., Meena, A. K., Giri, S., Rajagopal, S. and Mullangi, R. (2012). Highly sensitive LC-MS/MS-ESI method for simultaneous quantitation of albendazole and ricobendazole in rat plasma and its application to a rat pharmacokinetic study. *Biomed Chromatogr., 26*(2), 247-255.

Sharma, K., Pawar, G., Yadam, S., Giri S., Rajagopal S. and Mullangi R. (2013). LC-MS/MS-ESI method for simultaneous quantitation of metformin and repaglinidie in rat plasma and its application to pharmacokinetic study in rats. *Biomed Chromatogr*, *27*(3), 356-364.

How to cite this article: K. Bhavya Sri, Samreen Begum, D. Suchitra, V. Narmada and M. Sumakanth (2023). Bioanalytical Method Development and Validation of Metformin HCl and Repaglinide in Bulk and Combined Dosage Form in Human Plasma by using UV Spectroscopy. *Biological Forum – An International Journal, 15*(3): 640-644.