K. Bhavyasri et al /J. Pharm. Sci. & Res. Vol. 15(1), 2023, 985-990

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Application of Simultaneous Equation Method for Determination of Metformin HCl And Repaglinide in Bulk, Combined Dosage Form, and Dissolution Samples by UV-Spectrophotometer

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Abstract:

Metformin and Repaglinide both are anti-diabetic drugs used to treat patients suffering from type-2 diabetes mellitus. Both the drugs Repaglinide and Metformin HCl are given in combination to people who have high blood sugar levels and cannot be treated by a single medication. A simple, precise, accurate method has been developed for simultaneous estimation of Repaglinide and Metformin HCl in bulk and Combined tablet dosage form by Simultaneous equation method by using a UV-Visible spectrophotometer. All the validation parameters have been performed according to ICHQ2(R1) guidelines. Dissolution of the Combined tablet dosage form was performed to know the release of drug per unit time. ACN: Water (60:40) has been used as a diluent. The protein precipitation method is used for knowing the %Recovery in bio-samples. λ_{max} for Metformin and repaglinide was found to be 232nm and 244nm respectively. The slope value for metformin and Repaglinide was found to be 0.1561 & 0.034 respectively. R^2 for Metformin and repaglinide was found to be 0.999. %Recovery for Metformin and repaglinide was found to be 95-98%. %RSD for Metformin and repaglinide was found to be 0.3655 & 0.3401 respectively. Drug release for the combined tablet was found to be linear. By using the Simultaneous equation method, the %Repaglinide and Metformin present in Combined tablet dosage, and in bulk has been estimated along with their dissolution studies.

Keywords: UV-Visible Spectrophotometer, Metformin HCl, Repaglinide, Simultaneous estimation, Dissolution.

INTRODUCTION:

Biguanide antihyperglycemic medication metformin is the first-line drug used to treat type II diabetes[1].Because it lowers type II diabetes patients' blood glucose levels without resulting in hypoglycemia, metformin is regarded as an antihyperglycemic medication. An antihyperglycemic drug called repaglinide is employed in diabetes to enhance glycaemic management. Repaglinide is used to treat non-insulin-dependent diabetic Mellitus (NIDDM)[2,3,4].Repaglinide lowers postprandial glucose levels. It is best taken with food, and dosages administered at mealtimes should be omitted if a meal is missed. 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. Repaglinide works by promoting the release of your body's endogenous insulin[5]. 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. 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. A method for measuring these medications in the combined tablet dosage form needs to be developed because the prevalence of diabetes is rising daily. Therefore, a technique for simultaneous estimation of metformin HCl and repaglinide in bulk and combined tablet dosage form needs to be developed. The simultaneous equation approach using a UV-Visible spectrophotometer has been devised for the simultaneous estimation of Metformin & Repaglinide in bulk and Combined tablet dose form[6]. The structures of metformin HCl and repaglinide are depicted in Figures 1 and 2, respectively.Dissolution is the process in which a substance forms a solution. Dissolution testing measures the extent and rate of solution formation from a dosage form, such as a tablet, capsule, ointment, etc. The dissolution of a drug is important for its bioavailability and therapeutic effectiveness. Drug release and dissolution are both used in the same sentences[7,8].

Procedures must be standardized to accurately assess the solubility of drug products. This standardization aids in demonstrating consistent production quality and could be used as an indicator of efficacy. An apparatus with particular test circumstances is used in conjunction with acceptance criteria in a dissolving test to assess the product's performance. Chapter 711, General Four standardized devices are used in the dissolution process: a flow-through cell, a paddle, a reciprocating cylinder, and a basket[9].

Drug profile of Metformin HCl:

IUPAC Name: 3-(diaminomethylidene)-1,1-dimethylguanidine hydrochloride
Colour: white crystalline powder
Molecular formula: $C_4H_{11}N_5HCl$

pH: pH of a 1% aqueous solution of metformin hydrochloride is 6.68.
Solubility: It is freely soluble in water; slightly soluble in alcohol; practically insoluble in acetone and in methylene chloride.
Category: Belongs to a class of drugs called biguanides. Metformin helps to control the amount of glucose (sugar) in your blood.
State:solid

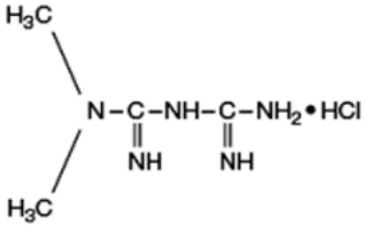

Figure 1:Structure of Metformin HCl

Drug profile of Repaglinide:
IUPAC Name: 2-ethoxy-4-[2-[[(1S)-3-methyl-1-(2-piperidin-1-ylphenyl)butyl]amino]-2-oxoethyl]benzoic acid
Colour: white to off-white powder
Molecular formula: C_{27} H_{36} N_{2O4}
pH: Being a weakly acidic compound, the drug is ionized at higher pH values, owing to its higher aqueous solubility at higher pH values.
Solubility: It is a poorly water-soluble compound, freely soluble in methanol, ethanol, and acetonitrile.
Category: It belongs to a class of ant hyperglycaemic agents known as meglitinides. Meglitinides work to reduce blood glucose levels by stimulating endogenous insulin production
State: solid[10,11].

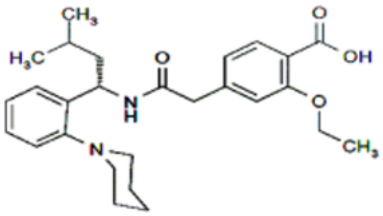

Figure 2: Structure of Repaglinide

MATERIALS AND METHODS:

Apparatus & Instrument:
Double Beam UV-Visible Spectrophotometer "Elico Sl 210", Dissolution test apparatus, digital analytical balance, and Ultrasonic water bath were used. Pipettes, beakers, measuring cylinders, and Volumetric flasks were used.

Chemicals and reagents:
The Pharma company offered Metformin and Repaglinide drug samples as gift samples. The medication EUROPA MF 2 (Metformin and Repaglinide 500:2) was bought from a neighboring pharmacy. Analytical-grade materials were utilized throughout the experiment.

Solvent selection:
ACN, methanol, ethanol, and Acetonitrile (ACN): Water (70:30) was selected as the solvent after considering the solubility and stability factor of Repaglinide. Metformin is freely soluble in water so the water was selected as the solvent for metformin.

Standard Solution Preparation:(Repaglinide):
Standard solution of Repaglinide was prepared by taking 10mg in 10ml volumetric flask make up to 10ml with diluents like ACN, methanol, ethanol, Acetonitrile(ACN): Water (70:30) to get 1000µg/ml make up to the mark with suitable diluents. Pipette out 1ml from 1000µg/ml and take in 10ml Volumetric flask, make up to mark with diluent to get 100µg/ml concentration (Working Standard). 1ml was taken from a working standard solution in a 10ml volumetric flask make up to mark with diluent to get 10µg/ml.

Standard Solution Preparation:(Metformin):
Standard solution of Metformin was prepared by taking 10mg in 10ml volumetric flask making up to 10ml with diluents like ACN, methanol, ethanol, and Acetonitrile(ACN): Water (70:30) to get 1000µg/ml make up to the mark with suitable diluents. Pipette out 1ml from 1000µg/ml and take in 10ml Volumetric flask, makeup to mark with diluent to get 100µg/ml concentration (Working Standard). 1ml was taken from a working standard solution in a 10ml volumetric flask make up to mark with diluent to get 10µg/ml.

Wavelength Determination:
To determine the wavelength, the standard solution containing 10 g/ml of repaglinide and metformin was scanned between 200 and 400 nm. The λ_{max} of repaglinide and metformin HCl are depicted in Figures 3 and 4.

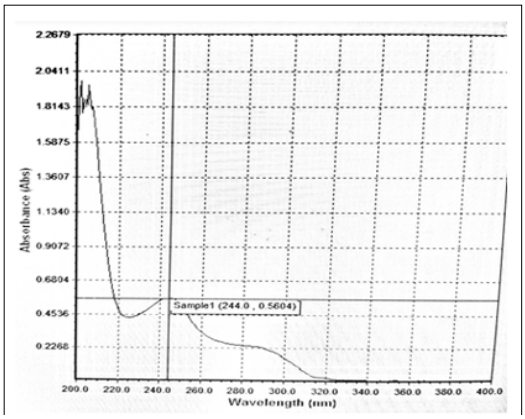

Figure 3. λ_{max} of Repaglinide

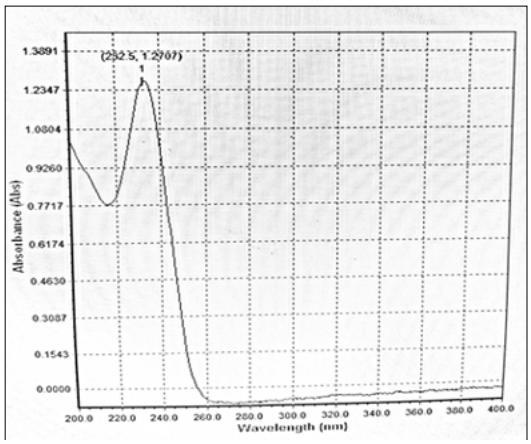

Figure 4: λ_{max} of Metformin HCl

Validation Parameters:

1. Linearity

Linearity refers to the ability of analytical procedures to produce results in direct proportion to the concentration range of analyte in samples within the required concentration level

2. Precision:

A homogenous sample of a sufficient number of aliquots is assayed to produce statistically accurate values of SD or %RSD.The formula for calculating %RSD is shown in equation 1. The formula for calculating standard deviation is shown in equation 2.

%RSD =(SD of measurement/mean value of measurement) X 100 (1)

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \quad (2)$$

where,

s=standard deviation

x=each value in the data set

$\bar{x}$=mean of all values in the data set

n=number of values in the data set

Limit: %RSD should be less than 2%. Results of precison

3. Accuracy:

The accuracy was determined by spiking standard solution to sample solution at three concentrations i.e., 50,100,150. %RSD was computed. %Recovery formula is shown in equation 3.

%Recovery= (Absorbance of Sample/Absorbance of Standard) x 100 (3)

4. Robustness

The ±nm from the fixed wavelength.Three aliquots of a standard solution containing 300 g/ml were made, and they were scanned at fixed wavelengths of 1 nm and 2 nm.

Limit:%RSD was found to be within the limits i.e., less than 2%

5.Ruggedness

The absorbance was examined in a variety of ways, including by several analyzers and using various instruments. In this method absorbance of the same solution is checked by two different analyst and %RSD was calculated.

Limit:%RSD was found to be within the limits i.e., less than 2%.

6.LOD

The lowest amount of analyte in a sample that can be identified but not necessarily quantitated under the specified experimental setting is referred to as the LOD of an analytical process. The formula for calculating LOD is given in equation 4.

LOD= 3.3 x SD/slope. (4)

SD = Standard deviation

7. LOQ

It is the smallest amount of analyte in the sample that can be quantitatively determined under the specified experimental circumstances with acceptable precision and accuracy. The formula for calculating LOD is given in equation 5.

LOQ= 10x SD/slope. (5)

SD = Standard deviation

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 equivalent 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 15mins. 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 10ml volumetric flask to get a 10µg/ml solution. This solution is taken for simultaneous estimation of Metformin HCl and Repaglinide in a Combined tablet dosage form.

The formula for the simultaneous equation is given in equation 6.

$$Cx = \frac{A1\ ay2 - A2\ ay1}{Ax1ay2 - ax2\ ay1}$$

$$Cy = \frac{A1\ ax2 - A2\ a\ x1}{ay1ax2 - ay2\ ax1} \quad (6)$$

Dissolution:

Dissolution is the in vivo process to know the drug release. The dissolution process for Metformin HCl and Repaglinide given in USP has been followed in this experiment.The medium prepared for dissolution should have the pH 5.0 buffer. 10.2g of citric acid was accurately weighed and taken in a 1000ml volumetric flask. Then weigh 18.6g of dibasic sodium phosphate dihydrate then add small amounts of water to dissolve these ingredients. After all the ingredients have been thoroughly dissolved makeup to the mark with water. From this 900ml was taken in a dissolution basket. USP apparatus 2 is used for dissolution.The process was done at 75rpm for 30 minutes. The samples were aliquoted every 5 minutes. Dissolution studies graph is shown in Figure 6.

RESULTS AND DISCUSSION:

Linearity:

Standard solution Preparation (Metformin)

Pipette out 0.2,0.4,0.6,.8,1.01.2,1.4,1.6,1.8,2.0ml from 100ppm stock solution and transfer to separate10ml volumetric flasks and make up to the mark with diluent to yield 2,4,6,8,10,12ppm solution respectively.

Standard solution Preparation (Repaglinide)

Pipette out 0.2,0.4,0.6,.8,1.01.2,1.4,1.6,1.8,2.0ml from 100ppm stock solution and transfer to separate10ml volumetric flasks and make up to the mark with diluentto yield 2,4,6,8,10,12ppmsolution respectively.

Linearity results of Metformin and Repaglinide are shown in table 1 and 2 respectively.

The calibration curve of Metformin HCl and Repaglinide is shown in figure 5.

Table 1: Results of Linearity (Metformin)

Concentration(µg/ml)	Absorbance(nm)
2	0.0764
4	0.1784
6	0.2679
8	0.3576
10	0.456
12	0.5502

Table 2: Results of Linearity (Repaglinide)

Concentration(µg/ml)	Absorbance(nm)
2	0.2924
4	0.5999
6	0.9543
8	1.2766
10	1.5708
12	1.8365

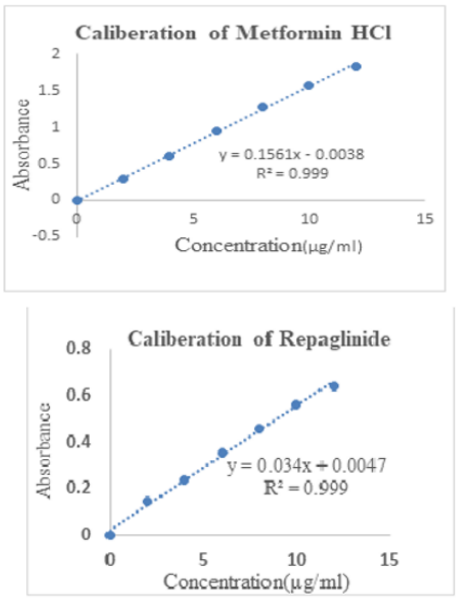

Figure 5: Calibration of Metformin HCl and Repaglinide

Precision:

From the stock of 100µg/ml solution(Repaglinide and Metformin HCl),6µg/ml of Metformin HCl and 10µg/ml of Repaglinide were made. The absorbances of these solutions were checked 6 times and noted. %RSD was calculated.Precision results of Metformin and Repaglinide are shown in table 3 and 4 respectively.

Table 3: Results of Precision (Metformin)

Concentration(ppm)	Absorbance(nm)
6	0.9543
6	0.9532
6	0.9462
6	0.9526
6	0.9543
6	0.9578
Average	0.953066667
Standard deviation	0.003484091
%RSD	0.365566329

Table 4: Results of Precision (Repaglinide)

Concentration(ppm)	Absorbance(nm)
10	0.456
10	0.4552
10	0.4562
10	0.4601
10	0.4566
10	0.4568
Average	0.456816667
Standard deviation	0.001553938
RSD%	0.340166584

Accuracy:

%Recovery was calculated by spiking the standard solutions with sample solutions at 50%,100% and 150%. 2ml 0f 2ppm standard solution was added to 2ml of 4ppm of sample to get 50%. 2ml 0f 4ppm standard solution was added to 2ml of 4ppm of sample to get 100%.2ml 0f 6ppm standard solution was added to 2ml of 4ppm of sample to get 150%. The absorbance of these solutions were checked and %Recovery was calculated.Accuracy results of Metformin and Repaglinide are shown in Table5 and 6 respectively.

Table 5: Results of Accuracy (Metformin)

% Level	Absorbance	% Recovery	Mean % Recovery
50% (2ppm+4ppm)	0.8197 0.8191 0.8199	97.58% 97.73% 97.82%	97.71%
100% (4ppm+4ppm)	0.9832 0.9821 0.9830	98.64% 98.53% 98.62%	98.59%
%150 (6ppm+4ppm)	1.1398 1.1368 1.1378	99.49% 99.23% 99.31%	99.34%

Table 6: Results of Accuracy (Repaglinide)

% Level	Absorbance	% Recovery	Mean % Recovery
50% (2ppm+4ppm)	0.7654 0.7632 0.7243	97.58% 97.73% 97.82%	97.71%
100% (4ppm+4ppm)	1.2322 1.3463 1.2453	98.64% 98.53% 98.62%	98.59%
%150 (6ppm+4ppm)	1.5432 1.5427 1.5723	99.49% 99.23% 99.31%	99.34%

Robustness:

From the stock of 100µg/ml solution(Repaglinide and Metformin HCl),6 µg/ml of Metformin HCl and 10 µg/ml of Repaglinide were made. The absorbances of these solutions were checked ± λ_{max} at least 6 times and noted. For Metformin HCl absorbance was checked at 232nm,233nm, and 234nm. For Repaglinide absorbance

was checked at 243nm,244nm, and 245nm. %RSD was calculated. The robustness results of Metformin and Repaglinide are shown in Table7 and 8 respectively.

Table 7: Results of Robustness (Metformin)

Concentration (ppm)	Absorbance at 231nm	Absorbance at 232nm	Absorbance at 233nm
6	0.7438	0.9543	0.6544
6	0.7432	0.9532	0.6543
6	0.7433	0.9462	0.6547
6	0.7438	0.9526	0.6542
6	0.7439	0.9543	0.6544
6	0.7433	0.9578	0.6543
Average	0.74355	0.953066667	0.654383333
Standard deviation	0.000287228	0.003484091	0.000157233
%RSD	0.038629296	0.365566329	0.024027663

Table 8: Results of Robustness (Repaglinide)

Concentration (ppm)	Absorbance at 243nm	Absorbance at 244nm	Absorbance at 243nm
10	0.3877	0.456	0.4266
10	0.3877	0.4552	0.4377
10	0.3829	0.4562	0.4367
10	0.3867	0.4601	0.43212
10	0.3866	0.4566	0.4353
10	0.3809	0.4568	0.4352
Average	0.385416667	0.456816667	0.433936667
Standard deviation	0.002588704	0.001553938	0.003705409
RSD%	0.671663765	0.340166584	0.853905476

Ruggedness:

6ppm and 10ppm of Metformin HCl and Repaglinide were taken for ruggedness. The absorbance of these solutions was checked by 2 analysts to know whether the method is rugged or not. %RSD was calculated. Ruggedness results of Metformin and Repaglinide are shown in table 9 and 10 respectively.

Table 9: Results of Ruggedness (Metformin)

Concentration (ppm)	Analyst-1 (nm)	Analyst-2 (nm)
6	0.9543	0.7438
6	0.9532	0.7432
6	0.9462	0.7433
6	0.9526	0.7438
6	0.9543	0.7439
6	0.9578	0.7433
Average	0.953066667	0.74355
Standard deviation	0.003484091	0.000287228
%RSD	0.365566329	0.038629296

Table 10: Results of Ruggedness (Repaglinide)

Concentration(ppm)	Analyst-1(nm)	Analyst-2(nm)
10	0.3877	0.456
10	0.3877	0.4552
10	0.3829	0.4562
10	0.3867	0.4601
10	0.3866	0.4566
10	0.3809	0.4568
Average	0.385416667	0.456816667
Standard deviation	0.002588704	0.001553938
RSD%	0.671663765	0.340166584

LOD & LOQ:

LOD:(Metformin)

LOD= 3.3 x SD/slope

= 3.3x0.003484/0.1561

= 0.07365µg/ml

LOD:(Repaglinide)

LOD= 3.3 x SD/slope

=3.3x0.001553/0.0462

=0.1109 µg/ml

LOQ:(Metformin)

LOQ= 10 x SD/slope

= 10x0.003484/0.1561

= 0.2231µg/ml

LOQ: (Repaglinide)

LOQ= 10 x SD/slope

= 10x0.001553/0.0462

= 0.33614µg/ml

Simultaneous Equation Method

$$Cx = \frac{A1\ ay2 - A2\ ay1}{Ax1ay2 - ax2\ ay1}$$

$$Cy = \frac{A1\ ax2 - A2\ a\ x1}{ay1ax2 - ay2\ ax1}$$

Where

A1=1.288 ax1=0.1668 ay1=0.00132

A2=0.1668 ax2=0.0024 ay2=0.045

By substituting the above value for the value in the formula we will get Cx and Cy values.

C_x=9.960 C_y= 0.0398

% of Metformin HCl in combined tablet dosage form was found to be 99.9%

% of Repaglinide in combined tablet dosage form was found to be 99.5%.

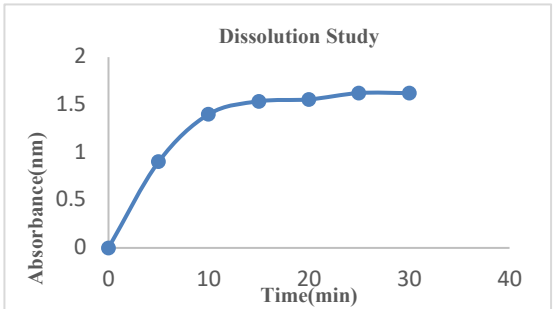

Figure 6: Dissolution of Combined tablet dosage form

Table 11: Summary of Results

Parameters	Metformin HCl	Repaglinide
Linearity range	0-40µg/ml	0-70µg/ml
Slope	0.1561	0.034
Standard Deviation	0.00348	0.001553
%RSD	0.3655	0.3401
LOD	0.07365	0.1109
LOQ	0.2231	0.3361
%Assay	98.89	98.24
Simultaneous Equation method	**99.9%**	**99.5%**

CONCLUSION:

Both bulk and mixed tablet dosage formulations of Metformin and Repaglinide were analyzed. The percentage of drugs in combined drugs was found to be within the limits according to Indian Pharmacopeia. According to ICHQ2(R1) requirements, all validation parameters were carried out, and it was determined that every parameter was within acceptable limits. Therefore, the suggested approach can be used to estimate Metformin and Repaglinide in bulk and in combination with other medications using a UV-Visible Spectrophotometer by the Simultaneous equation method. The percentage of Metformin and Repaglinide in the Combined tablet was found to be 99.9% and 99.5% which is within the limits according to IP. The dissolution process was also carried out according to USP<77> and the drug release was found to be linear the graph of drug release was depicted in the results section.

Acknowledgement:

I want to acknowledge my beloved Principal Professor M. Sumakanth and the Faculty of the Department of Pharmaceutical Analysis for giving us the opportunity to perform research work in our college.

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