

Stability Indicating RP-HPLC Method for the Estimation of Metformin Hydrochloride and Repaglinide as API and Estimation in Tablet Dosage Form

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ABSTRACT

A new simple, precise, sensitive, economical RP-HPLC method was developed for the estimation of Metformin hydrochloride and Repaglinide in bulk and tablet dosage form. The λ_{max} for Metformin hydrochloride and Repaglinide was found to be nm and nm respectively. The linearity in the concentration range of 25 $\mu\text{g/ml}$ to 150 $\mu\text{g/ml}$ ($r^2=0.999$) and 0.1 to 0.6 $\mu\text{g/ml}$ ($r^2=0.999$) for Metformin hydrochloride and Repaglinide respectively. The chromatographic values for Metformin hydrochloride and Repaglinide were found to be satisfactory on BDS Hypersil 18, 250 x 4.6mm, 5 μm , column using mobile phase of ACN: Buffer in the ratio of 58: 42 v/v with the flow rate of 1.0ml/min. The developed method was validated according to ICH guidelines and found to be accurate, precise, rugged and robust and found to be in good accordance with the prescribed values. The newly developed method can be used for routine analysis estimation of Metformin hydrochloride and Repaglinide in bulk and tablet dosage form in pharmaceutical industry.

Keywords: Metformin hydrochloride, Repaglinide, Stability studies, method development and validation.

INTRODUCTION

Glucose is the obligatory source of energy for the brain and physiological control of blood glucose reflects the need to maintain adequate fuel supplies in the face of intermittent food intake and variable metabolic demands. Increased blood sugar causes increased insulin secretion, whereas reduced blood sugar reduces insulin secretion. Insulin is the main hormone controlling intermediary metabolism. It's most obvious acute effect is to lower blood glucose¹⁻². Metformin hydrochloride is the drug of choice in Biguanide classification. It is chemically 1, 1-dimethyl biguanide hydrochloride³ (Figure 1). Metformin lowers blood glucose additionally reduces low density and very low density lipoprotein LDL and VLDL respectively⁴. Repaglinide is chemically (S)-(+)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl) phenyl] butylamino)-2-oxoethyl] benzoic acid⁵ (Figure 2). It acts by blocking the sulfonyl urea receptor on K_{ATP} channels in pancreatic B-cell membranes⁶. From literature review shows that there is developed method including UV⁷⁻⁸, Fluorometric⁹, HPTLC¹⁰, LC-MS¹¹⁻¹² methods. The review of the literature reveals that the present study is to develop and validate a stability indicating HPLC method for metformin hydrochloride and Repaglinide according to ICH guidelines¹³.

MATERIALS AND METHODS

The reagents were used are of Analytical grade. HPLC Model Shimadzu equipped with BDS Hypersil C¹⁸ (4.6 x

250mm, 5 μm) Column and pump of LC-20 AT VP series with injector of Rheodyne.

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized to Acetonitrile: NaH_2PO_4 ratio 58:42, Flow rate: 1.0 ml/min and Wave length: 231 nm.

Preparation of standard solution of Metformin Hydrochloride and Repaglinide

250 mg Metformin hydrochloride and 1 mg Repaglinide was taken and made up the volume to 100 ml.

Preparation of standard solution of Repaglinide

Accurately weighed 10mg Repaglinide of transferred to

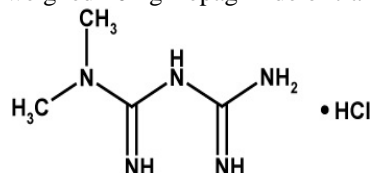


Figure 1: Metformin hydrochloride

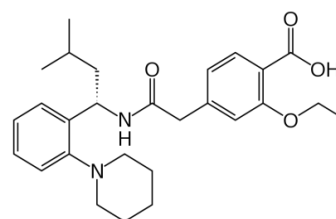


Figure 2: Repaglinide

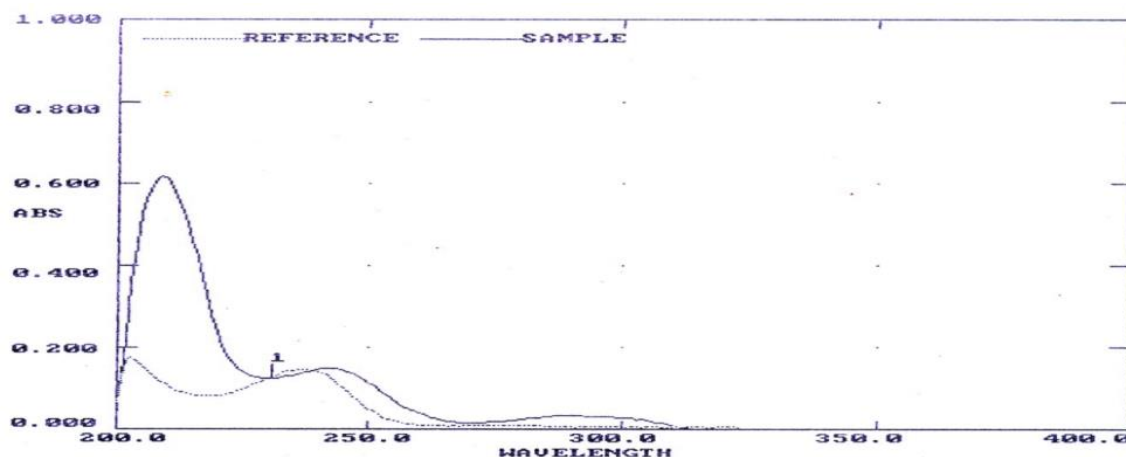


Figure 3: the chromatogram of both the standard and sample.

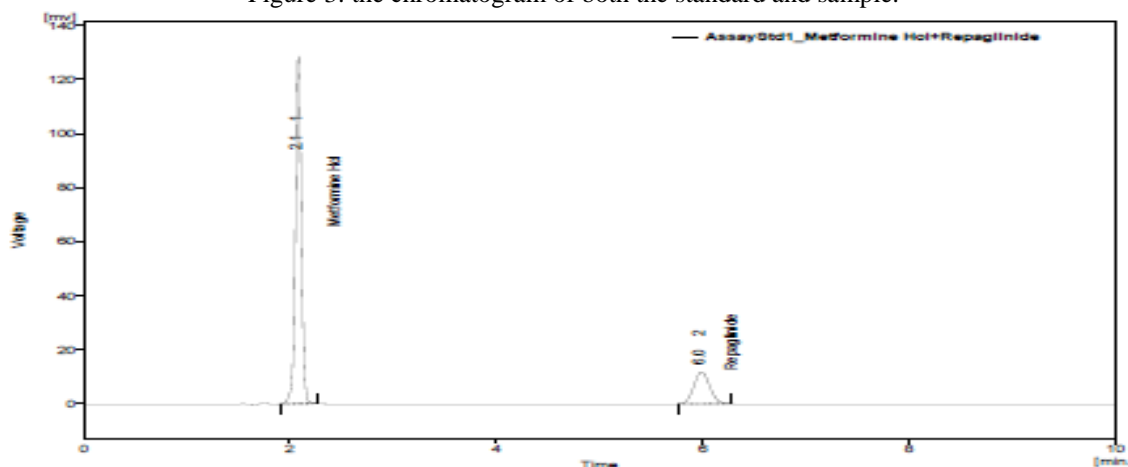


Figure 4: the chromatogram of both the standard and sample.

Table 1: Standard stock solution of Repaglinide / Metformin Hydrochloride

S. No	Solvent	Solubility	
		Metformin Hydrochloride	Repaglinide
1.	Water	+	+
2.	Acetonitrile	+	+
3.	0.1N NaOH	+	+
4.	0.1N HCl	+	+
5.	Methanol	+	+

10ml volumetric flask volume made up to 10ml using mobile phase. Pipetted 0.1ml and made up to the mark using mobile phase.

Solubility

Solubility of drugs was observed by dissolving it in different solvents and it was found that drugs having good solubility in following solvents. The table 1 shows the different solubility of Metformin hydrochloride and Repaglinide.

Standard stock solution of Repaglinide / Metformin Hydrochloride

Accurately 10 mg of Repaglinide / Metformin HCl was weighed into a clean and dry 10 ml volumetric flask, dissolved with sufficient volume of mobile phase and then made up to the volume with mobile phase. The maximum

wavelength was found to be 231 nm. The chromatogram of both the standard and sample in Figure 3.

Assay

Weigh about 20 tablets and powdered. Powder equivalent to 500mg and 2mg of Metformin HCl and Repaglinide were taken into 50 ml volumetric flask. Add about 10 ml of mobile phase and sonicated until the contents were dissolved. Filter the contents by using 0.45µ membrane filter under vacuum. Make up to the mark with mobile phase. Inject 20µl of sample solution into the chromatographic system. Measure the area of Metformin HCl and Repaglinide and calculate the percentage of assay.

Standard

Market sample analysis

Linearity

To 250 mg Metformin hydrochloride and 1 mg Repaglinide was taken and made up the volume to 100 ml. pipette out 1, 2, 3, 4, 5, and 6ml from the stock solution and made up the volume using 100 ml respectively. The results were tabulated in Table 2 and Chart showing the linearity of Repaglinide and Metformin hydrochloride in Figure

Accuracy

From the Standard stock solution pipette out 5 ml and make upto 100 ml (Spiking Standard Solution). From the standard stock solution pipette out 1, 2, 3, 4 and 5ml

Table 2:

Sample No.	Label Claim (mg)		Amount present (mg)		Standard Deviation	
	Metformin HCl	Repaglinide	Metformin HCl	Repaglinide	Metformin HCl	Repaglinide
1.	500	2	499.85	1.98	0.018663	0.012235
2.	500	2	499.87	1.99	0.018659	0.012239
3.	500	2	499.89	1.99	0.018661	0.012240
4.	500	2	499.86	1.97	0.018665	0.012230
5.	500	2	499.87	1.99	0.018672	0.012237
6.	500	2	499.83	1.99	0.018669	0.012231
Mean	500	2	499.87	1.99	0.018663	0.012235

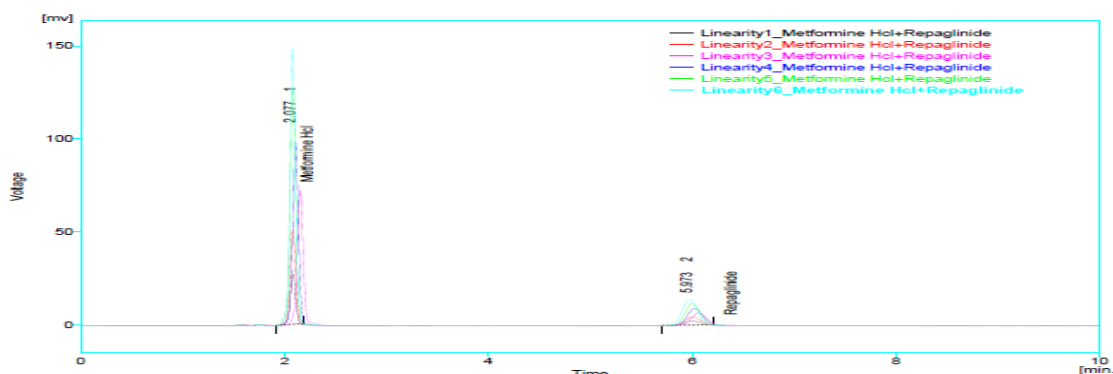
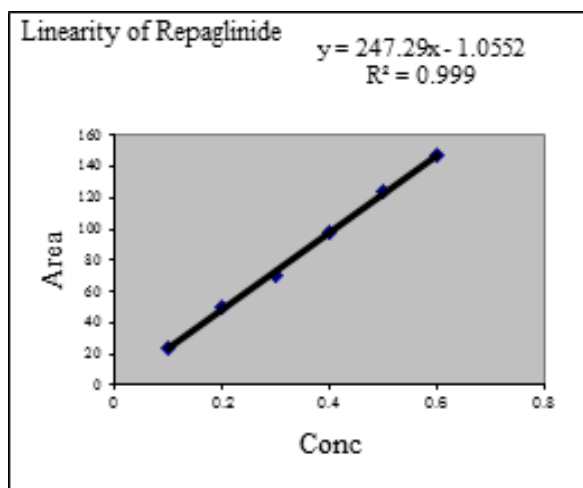
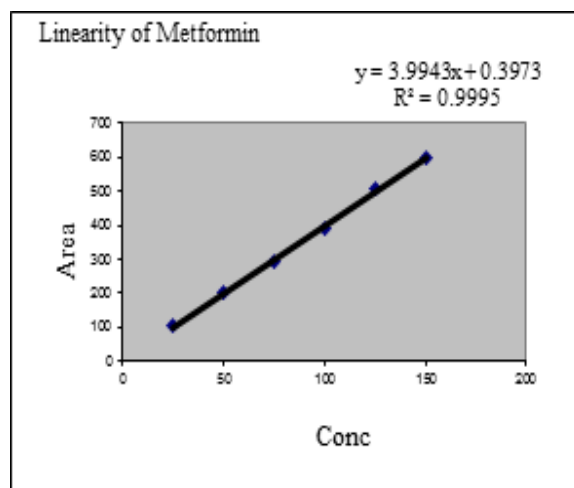


Figure 5: The Linearity chromatogram of both Metformin hydrochloride and Repaglinide



6(a)



6(b)

Figure 6: The chart showing the Linearity of both Metformin hydrochloride and Repaglinide

Table 3: Linearity data of Repaglinide / Metformin Hydrochloride

S. NO	Concentration $\mu\text{g/ml}$	Peak Area	Statistical Analysis
1.	0.1	24.459	Slope (m): 247.29 Intercept (c): 1.0552
2.	0.2	49.334	
3.	0.3	70.556	
4.	0.4	97.251	
5.	0.5	123.888	Correlation coefficient (R^2): 0.999
6.	0.6	147.491	

respectively and made up the volume using 100ml (Standard Stock Solution). The average % Recovery of

Repaglinide and Metformin HCl was found to be 99.80 and 99.70 respectively. The results were tabulated in table 4.

Precision

The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samplings of the same homogeneous sample. Should be investigated using homogeneous, authentic samples

Repeatability

Established the repeatability of the analytical method by estimating the assay for 5 sample proportion of the same batch under normal operating conditions. Calculated the assay for all 5 sample preparation and reported the %RSD for the sample. The results were tabulated in table 4.

Ruggedness

Intermediate precision (ruggedness)

Table 4: Accuracy studies for Repaglinide and Metformin HCl

S. No	Drug	Mixture of pure and formulation	Con. of pure drug, $\mu\text{g/ml}$	Conc. of Formulation, $\mu\text{g/ml}$	% Recovery of pure drug
1.	Repaglinide	0.4+0.05	0.05	0.446	99.16
2.		0.5+0.05	0.05	0.549	99.88
3.		0.6+0.05	0.05	0.652	100.38
1.	Metformin HCl	100+12.5	12.5	111.74	99.33
2.		125+12.5	12.5	137.35	99.89
3.		150+12.5	12.5	162.32	99.89

Table 5: Repeatability of Repaglinide and Metformin HCl

Metformin HCl			Repaglinide		
S. No.	Retention time	Area	S.No.	Retention time	Area
1	2.113	510.122	1	6.007	126.374
2	2.08	511.699	2	5.987	125.187
3	2.077	517.179	3	5.99	127.642
4	2.113	509.128	4	6.007	127.376
5	2.08	519.359	5	5.98	126.796
Average	2.0926	513.4974	Average	5.9942	126.675
Std. Deviation	0.018663	4.517515	Std. Deviation	0.012235	0.967713
%RSD	0.89	0.88	%RSD	0.20	0.76

Table 6: Ruggedness for Repaglinide and Metformin HCl

Analyst	Retention time of Repaglinide (min)	Retention time of Metformin HCl (min)
Analyst 1	5.583	2.193
Analyst 2	5.583	2.193

Table 7: Robustness of Repaglinide and Metformin HCl

Wave length	RT of Repaglinide	RT of Metformin HCl
228 nm	5.437	2.057
232 nm	5.337	2.050
Flow rate	RT of Repaglinide	RT of Metformin HCl
0.9 mL/ min	4.903	5.640
1.1 mL/ min	2.420	1.907

Table 8: Limit of Detection and Limit of Quantization of Repaglinide and Metformin HCl.

S. No	Drugs	Parameters	Values
1	Metformin Hydrochloride	Theoretical Plates (N)	6169.000
2		LOD, $\mu\text{g/ml}$	3.73220
3		LOQ, $\mu\text{g/ml}$	11.64910
1	Repaglinide	Theoretical Plates (N)	7196.000
2		LOD, $\mu\text{g/ml}$	0.01291
3		LOQ, $\mu\text{g/ml}$	0.04030

Intermediate precision study was carried out by repeating the complete experiment with different analysts, on different days in same laboratory as per the following preparation. To 250 mg of Metformin hydrochloride and 1

mg of Repaglinide made up the volume to 100 ml. the sample

Method Precision

Ability to reproduce data within the predefined precision is called method precision. The standard and sample was prepared and readings were taken.

Robustness

Capacity to remain unaffected by small but deliberate variations in method parameters. Variations may include: stability of analytical solution, variation of pH in a mobile phase, different column (lot/supplier), temperature, and flow rate. From the standard stock solution and sample, the robustness can be measured with different flow rate and wavelength. The method remains unaffected by the small deliberate changes that were introduced. the results were tabulated in table 7.

Specificity

Treating with Acids

To 1 ml from the stock solution into a 10 ml volumetric flask. To that 1 ml of 0.1M hydrochloric acid added. Observed for any change took place in the retention of the peak.

Treating with Base

To 1 ml from stocks solution into a 10 ml volumetric flask. To this 1 ml of 0.1 M sodium hydroxide were added and Observed for any degradedness.

Heating

For the specificity study 1 ml from the stock solution should be taken in a 10 ml flask, make up to the volume with the mobile phase. The solution should be heated at 40°C for a period of 30 min. Observed for any degradation occurs or not. From the specificity performed, various degradation products are formed and there is no change in the detection of the analyte in the presence of other components.

Limit of Detection and Limit of Quantization

The Limit of Detection and Limit of Quantification was done as per ICH guidelines for Metformin Hydrochloride and Repaglinide and tabulated in table 8.

LOD = 3 x STDEV / SLOPE

LOQ = 10 x STDEV / SLOPE

CONCLUSION

An HPLC method was developed and validated for various parameters as per ICH guidelines. The system suitability parameters proved that the proposed method is equally suitable for estimation of Metformin HCL and Repaglinide. The chromatogram for Metformin HCL and Repaglinide were found to be satisfactory on RP-18(2), 250 X 4.6mm, 5µm column, using mobile phase combination of ACN: Buffer (58:42 v/v) with flow rate of 1.0 ml/min. The accuracy of the method was determined by recovery with spiked concentration of pure drug at three levels for metformin HCL and Repaglinide. The recovery of drug was well within the acceptance limits of 97-103%. The method was rugged and robust as observed from insignificant variation in the results of analysis on changes in mobile phase composition ratio, pH, flow rate, temperature and analysis being performed by different analysts and on different days respectively. In the all above cases the recovery was found to be within the limit. Hence, this study can be extended by studying the degradation kinetics of Repaglinide and Metformin HCL determination by RP-HPLC method and also its estimation in plasma and biological fluids.

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