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Development and Validation of RP- HPLC Method for Estimation of Metformin in Formulation

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ABSTRACT

A new sensitive and rapid RP-HPLC method was developed for the determination of Metformin in pharmaceutical dosage forms and it was validated according to ICH guidelines. The HPLC analysis was performed on the ACCLAIMED mix mode HILIC-1 (5µ, 150 X 4.6mm. ID). Ammonium acetate-Acetonitrile (35:65 v/v) as mobile phase, at the flow rate of 1.0 mL/min. The detection was performed at the wavelength (λ) of 210nm, and the retention time of Metformin was around 8.4 min. The total run time was 15 min. The calibration plot gave a linear relationship over the concentration range of 3.9–62.5µg/ml. The LOD and LOQ were 1.3 and 4.5µg/ml, respectively. UV detection was monitored at 210nm for Metformin as the compound exhibited optimum absorption at this selected wavelength. The accuracy of the proposed method was determined by recovery studies and was found to be in the range of 99.43 to 100.35%. The repeatability testing for both standard and sample solutions showed that the method is precise, and accurate within acceptable limits.

INTRODUCTION

Metformin is used with a proper diet and exercise program and possibly with other medications to control high blood sugar. It is used in patients with type 2 diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems. Proper control of diabetes may also lessen your risk of a heart attack or stroke. Metformin works by helping to restore your body's proper response to the insulin you naturally produce. It also decreases the amount of sugar that your liver makes and that your stomach absorbs. In this research, a new sensitive and rapid RP-HPLC method was developed for the estimation of Metformin in tablet dosage forms, and this method was validated according to ICH Q2 (R1) guidelines.[1]

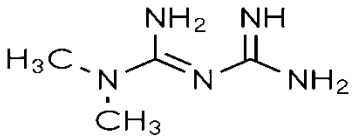

Figure No.1 Metformin

MATERIALS AND METHODS

Research work was carried out in an Acclaimed mix-mode HILIC-1 column (5µ, 150 X 4.6 mm. ID) with an isocratic elution technique, consisting of Ammonium acetate-Acetonitrile (35:65 v/v).

Column:	ACCLAIMED mix mode HILIC-1; 5µ, 150 X 4.6mm. ID.
Mobile Phase:	Ammonium acetate-acetonitrile (35:65 v/v)
Flow rate:	1ml/min
Elution mode:	Isocratic elution mode
Wavelength selected:	210nm
Temperature:	Room temperature
Run time:	15 minutes

Method Validation

The method was validated as per ICH Q2 (R1) guidelines, and the validation parameters included Specificity, Linearity, Range, Accuracy, Precision, System suitability, LOQ, LOD, and Robustness.[2]

System suitability

The purpose of the system suitability test is to ensure that the complete testing system, including instruments, reagents, columns, analysts, etc., is adequate for the intended analysis. The following parameters are usually determined: theoretical plate count, tailing factors, resolution, and reproducibility.[3]

Peak	Ret. Time	Area	Area%	T.Plate	Resolution	Tailing F.	Separation
Metformin	8.7	12613074	45.80	3281	7.2	1.1	2.2

Specificity

Specificity is the ability of the analytical method to discriminate between the analyses and the other components in the mixture.[4]

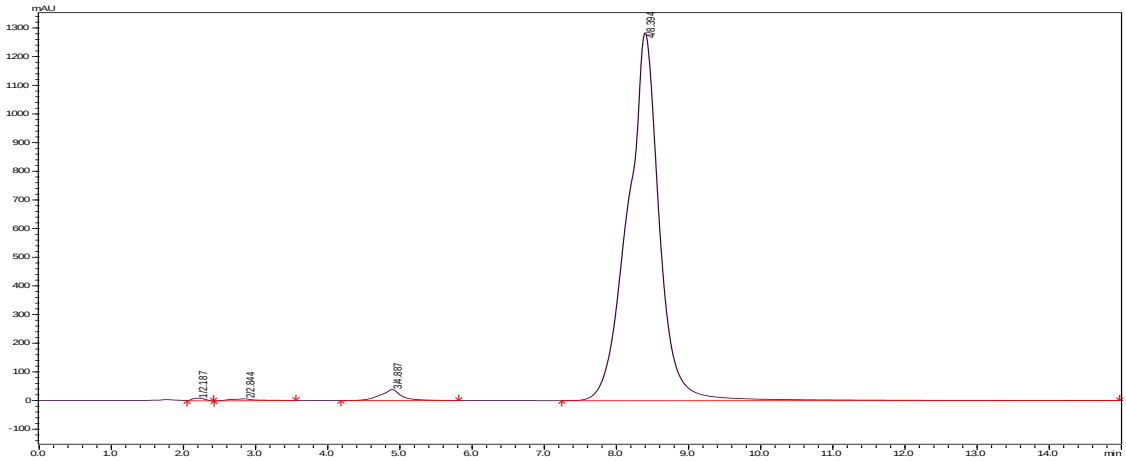

Figure no.2 Chromatogram of Metformin standard solution

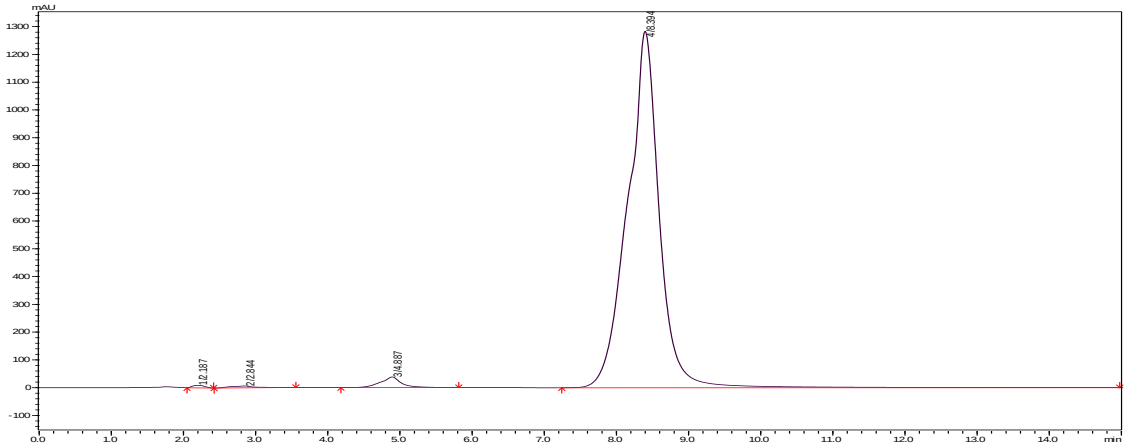

Figure 3: Chromatogram of Metformin sample solution

Repeatability

Implementing the procedure, the homologous mixture Metformin (100 ug/ml) was tested for 6 injections within the same day. The% RSD was calculated and found it is less than 2%.[5]

Table 1 Repeatability data of Metformin

Sr. No.	**Metformin**
1	10563458
2	10891336
3	10656305
4	10639080
5	10611007
6	10423112
Mean	**10330716**
STD. DEV.	**168747.53**
RSD (%)	**1.64**

Precision studies

The precision of the RP-HPLC method reflects its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions.[6]

Table 2 Precision data of Metformin

S. No.	Concentration (ppm)	Area
1	100 PPM	10563458
2	100 PPM	10891336
3	100 PPM	10656305
1	100 PPM	10639080
2	100 PPM	10611007
3	100 PPM	10423112
1	100 PPM	10841098
2	100 PPM	10639475
3	100 PPM	10674741
	Mean % RSD	**1.42**

Linearity and range

The linearity of any RP-HPLC Method represents its ability to explicit the results that should proportional to the concentration of studied analytes within a selected range. Therefore, over the tested range of 6.25-100µg/ml for Metformin. [7]

Table 3 Linearity data of Metformin

S. No.	Concentration ($\mu g.mL^{-1}$)	Area	Average (Mean)
1	100	9275218	9275218
2	50	4644200	4644200
3	25	2400945	2400945
4	12.5	1192777	1192777
5	6.25	596388	596388

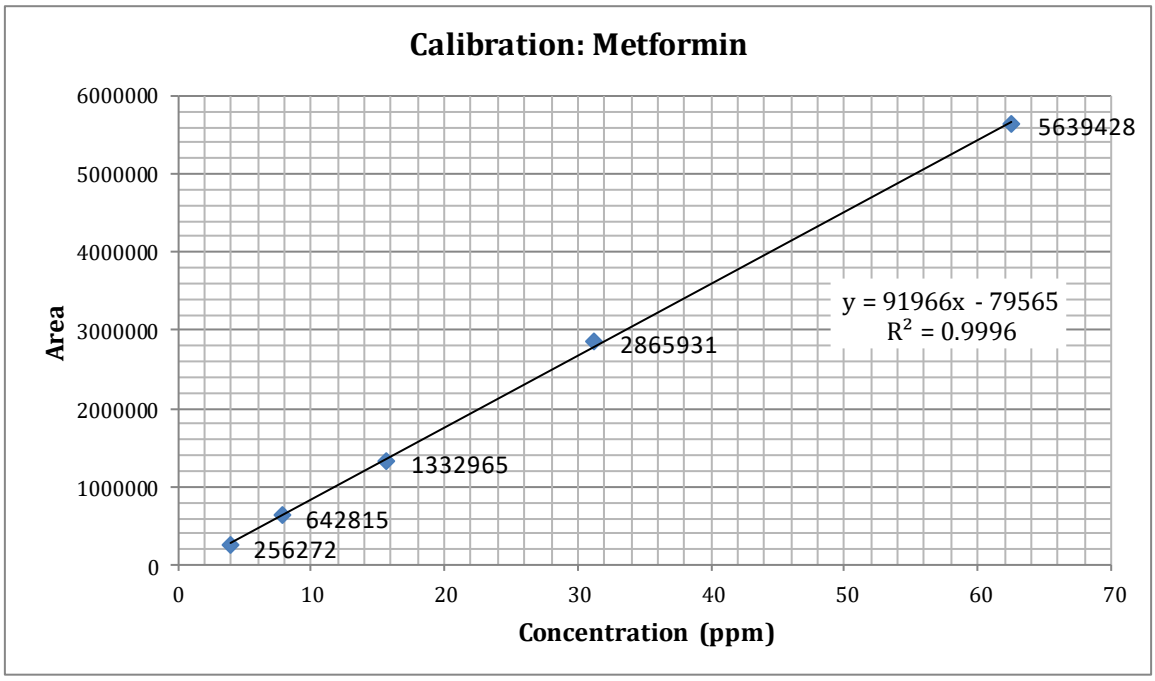

Figure no. 4 Calibration curve of Metformin

Robustness for the chromatographic method

The robustness of the RP-HPLC Method represents its ability to remain unaffected by small but deliberate variations in separation parameters to ascertain its reliability during routine analysis. In this method, robustness was established by making deliberate changes in flow rate (1.0 ± 0.2 ml/minutes), and temperature (28°C ± 2°C). capacity factor (k'), resolution (Rs), and peak tailing (*Tf*) of selected Evogliptin were almost unchanged which signified that the proposed RP-HPLC Method obliged all minimum requirements led by the ICH guidelines.[8]

Table No.4 Robustness data of Metformin

Variables	tR(min)	k'	Tf	Rs	N
Flowrate(+0.2mL.min^{-1})	4.13	1.99	1.08	985	4.13
Flowrate(-0.2mL.min^{-1})	5.97	2	1.14	1135	5.97
Temperature(+2˚C)	4.77	1.95	1.11	2047	4.77
Temperature(-2˚C)	4.77	1.95	1.08	2116	4.77
Mean±S.D.	5.28 ± 1.10	1.96 ± 0.04	1.13 ± 0.09	5.28 ± 1.10	

Limit of quantification (LOQ) and Limit of detection (LOD)

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the regression equation. As observed, the LOD and LOQ of Metformin were 1.3 and 4.5µg/ml.[9]

Accuracy

Percentage recoveries of three different concentrations; 80%, 100%, and 120% (injected thrice) to determine the Metformin were calculated to determine the drug recovery (%) and variation in RSD% and results obtained were reported.[10]

Table No.5 Accuracy data of Metformin

Conc. (%)	S. N.	Drug. added	Amt. rec.	% recovery	Peak Area (50 ppm)	Mean Rec %	% RSD
80%	1	4	4.11	102.75	629149	104	1.05
	2	4	4.18	104.50	639864		
	3	4	4.19	104.75	641395		
100%	1	5	5.09	101.80	779165	102	0.82
	2	5	5.11	102.20	782227		
	3	5	5.03	100.60	769980		
120%	1	6	6.13	102.17	938366	102	0.52
	2	6	6.18	103.00	946020		
	3	6	6.12	102.00	936835		

CONCLUSION

The developed HPLC method is fast & simple and found specific, linear, accurate, precise, and robust. Hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Metformin In addition, the main features of the developed method are short run time and retention time of around 4.8 min. The method was validated following ICH guidelines.

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