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Analytical Method Development and Validation of Metformin Hydrochloride by using RP-HPLC with ICH Guidelines

Mr. Nilesh Nikam[1], Dr. Avish Maru[2], Dr. Anil Jadhav[3], Dr. Prashant Malpure[4]

[1,4]Assistant Professor, [2,3]Principal
[1,3]Sandip Foundation, Institute of Pharmacy, Mahiravani, Nashik, Maharashtra, India
[2,4]Loknete J. D. Pawar, College of Pharmacy, Manur, Kalwan, Maharashtra, India

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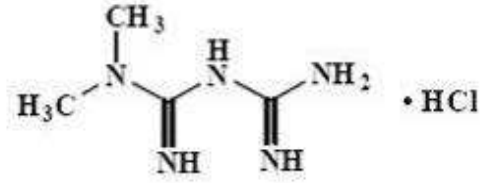
ABSTRACT

A simple and reproducible method was developed for Metformin (MET) by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Metformin was separated on C18 column [4.6x250mm, particle size 5µm], using combination of phosphate buffer with pH of 3.0 and Methanol at the UV detection of 238nm. Isocratic elution of phosphate buffer with pH of 3.0 and Methanol was used as a mobile phase with various ratios and flow rates, eventually 30:70 v/v phosphate buffer with pH of 3.0 and Methanol was being set with the flow rate of 1mL/min. The statistical validation parameters such as linearity, accuracy, precision, inter-day and intra-day variation were checked, assay studies of Metformin were within 98% to 102% indicating that the proposed method can be adoptable for quality control analysis of Metformin.

Keywords: *Metformin, RP-HPLC, ICH Guidelines*

INTRODUCTION:

Metformin is an agent having antihyperglycemic activity and belonging to the biguanide class of antidiabetic drugs. Metformin is analogous with a very low incidence of lactic acidosis. Metformin is the most commonly prescribed drug for the patients having type 2 diabetes mellitus. The literature survey says that, in addition to glucose lowering, several studies have presented evidence propose that some potential role for metformin, such as antitumor effect, cardiovascular protective effect, antiaging effect, neuroprotective effect or an optional treatment for polycystic ovary syndrome. The drug was approved in the United Kingdom in 1958 and in the United States in 1995 and since the doses were ranging from 500 to 2,500 mg/day. According to study of Diabetic guidelines of American Diabetes Association/European Association it is the first-line therapy for patients with T2DM (type 2 Diabetes mellitus). The history of biguanides can be discover from the use of plant namely as *Galega officinalis* (commonly known as galega) for treating diabetes in medieval Europe. Guanidine, the active component of *Galega officinal is*, and it is the parent compound used to synthesize the biguanides. metformin has a superior safety profile and is well tolerated. The other two biguanides, phenformin and buformin, were withdrawn in the early 1970s due to the risk of lactic acidosis and increased cardiac mortality. The incidence of lactic acidosis with metformin at therapeutic doses is rare (less than three cases per 100,000 patient-years) and is not greater than with non- metformin therapies.



Metformin Hydrochloride

$C_4H_{12}ClN_5$ M.W. 165.63

Figure 1: Structure of Metformin Hydrochloride

The Guidelines for analysis method validation include ICH guidelines. By the literature survey a very few methods reported for determination of Metformin HCL in bulk drug as well as pharmaceutical preparation. This research is tries to develop a new sensitive and rapid HPLC method for the

determination of Metformin HCL in Bulk preparation, and this method was also validated according to ICH Q2 (R) guidelines.

MATERIALS AND METHOD:

Instruments:

The chromatographic separation was performed on Analytical Technologies HPLC-3000 series compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used. Model - UV 2012 double beam UV visible spectrophotometer and Wenser High Precision Balance Model: PGB 100 electronic balance were used for Spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals

Pharmaceutical grade pure metformin hydrochloride sample was procured from Swapnaroop Agency. HPLC grade Methanol and HPLC grade Water were used of Merck specialities private limited, Mumbai.

Chromatographic conditions

C18 [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] was used for the chromatographic separation at a detection wave length of 238nm. Methanol, Phosphate buffer pH 3 in a ratio of 70:30 v/v was selected as mobile phase for elution and same mixture was used in the preparation of standard and sample solutions. The elution was monitored by injecting the 20µl and the flow rate was adjusted to 1 ml/min.

Preparation of Mobile phase

Preparation of Phosphate buffer pH 3: Dissolve 1.36g of Potassium dihydrogen orthophosphate & 2ml of triethylamine in 800ml of HPLC water, adjust the pH to 3 with orthophosphoric acid and add sufficient HPLC water to produce 1000ml.The mobile phase was sonicated for 15 min and filtered through a 0.45 µm membrane filter paper.

Preparation of Standard solutions

10mg metformin was accurately weighed and transferred into 10 ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solution of concentration 1000µg/ml of the drug. (Working stock solution).

Preparation of Sample Solution

20 tablets of metformin were initially weighed and powdered and an amount equivalent to 10mg was accurately weighed into a 10ml volumetric flask, mixed with 10ml of mobile phase. The solution was made up to the volume with mobile phase and sonicated for 5 minutes. The solution was then filtered through 0.45µm Millipore membrane filter. The solution contains 1000µg/ml of metformin Hcl. From the above stock solution 0.1ml aliquot was transferred in to a 10 ml volumetric flask, volume was made up to the mark with mobile phase to obtain a final concentration of 10 µg/ml of metformin.

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a estimation of metformin Hcl. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Methanol, Phosphate buffer pH 3 (70:30 v/v) using C18 column [Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)] Table:1.

Parameter	Condition
Column	Cosmosil C18 (250mm x 4.6ID, Particle size: 5 micron)
Mobile Phase	70:30 (Methanol: Phosphate buffer pH-3).
Flow Rate	1 ml/min
Wavelength	238 nm
Injection Volume	20 µl
Detector	UV-3000-M
Run Time	6.5 min
Retention Time	Approx. 4.2 min

Table 1: Optimized parameter

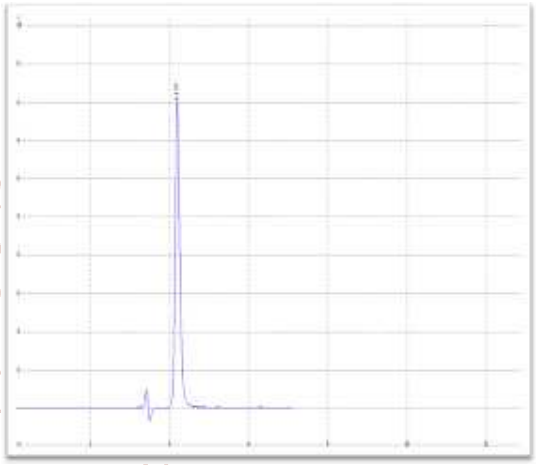


Figure 2: Typical chromatograph of Metformin Hydrochloride

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (R) guidelines.

1. Linearity

For the determination of linearity, appropriate aliquots were pipetted out from 1000µg/ml (working stock solution). 0.1 – 0.5 ml was pipetted out in to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50µg/ml of metformin. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for metformin was shown in figure 2 and their corresponding linearity parameters given in table 2.

2. Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalysed by the proposed method and the percent recovery was reported. The results were given in table 4.

3. Precision

The repeatability of the method was verified by calculating the % RSD of three replicate injections of 100%

concentration (30µg/ml of Metformin) on the same day and for intraday precision % RSD was calculated from repeated studies. The results were given in table 5.

4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 4.2 s/s and LOQ = 9.8 s/s.

5. Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, flow rate, detection wave length, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wave length and ± 0.1 ml/min in the flow rate, were tried individually. Solutions of 100% test concentration with the specified changes in the operational conditions were injected to the instrument in triplicate. % RSD was reported in the table 6.

6. Assay of marketed formulation

20 tablets of teneligliptin were weighed and crushed into fine powder. The average weight of the tablet was calculated and the amount equivalent to 10 mg of pure teneligliptin was dissolved in 10 ml of solvent. From this stock solution 30 ppm dilution was prepared and injected. The % purity was calculated by comparing the result with result obtained from 30 ppm standard drug and are reported in table 7.

7. System suitability

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision**.** System suitability was carried out with three injections of solution of 30 µl/ml of Metformin in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in table 8.

Results and Discussion

Linearity:

Analytical method linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The mean peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study (Figure 1) gave linear relationship over the concentration range of 10 - 50 µg/ml for metformin. From the regression analysis, a linear equation was obtained: y = 31271x + 49523, and the goodness-of-fit (r^2) was found to be 0.9986, indicating a linear relationship between the concentration of analyte and area under the peak.

Table 2: Summary of results of Linearity

Conc. (µg/ml)	Peak Area
10	362718
20	692265
30	956735
40	1308262
50	1618262

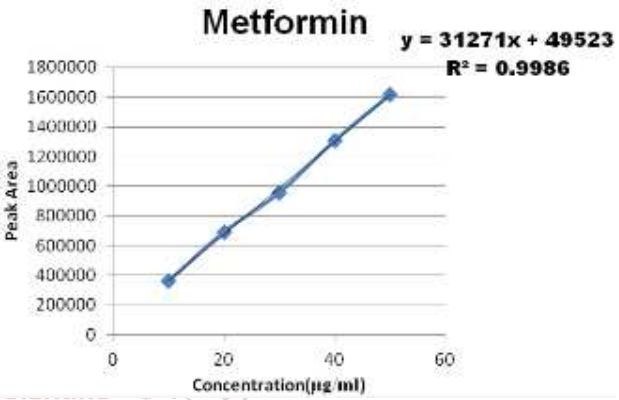


Figure 2: Linearity

Accuracy

The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy testing showed that the method is accurate within the acceptable limits. The RSD is calculated for the metformin and all the results are within limits. Acceptable accuracy was within the range and not more than 2.0% RSD, as demonstrated in Table -3.

			Standard Deviation		Accuracy	Precision
Sr. no.	Conc. (µg/ml)	Area	Mean	SD	%SD	%RSD
	10	363165				
1	10	362718	363846.3333	1583.073698	0.435094	0.435094036
	10	365656				
	30	954648				
2	30	956735	956247.3333	1419.769113	0.148473	0.148473001
	30	957359				
	50	1621245				
3	50	1618262	1617829.333	3650.690939	0.2256536	0.22565365
	50	1613982				

Table 3: summary of Results of Accuracy

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	956735	962945	100.6490826
2	100% Recovery	1308262	1285161	98.2342222
3	150% Recovery	1618262	1635968	101.0941368

Table 4: % recovery

Precision

Precision of an analytical method is defined as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is normally expressed as the relative standard deviation The repeatability, intra-day and inter-day precision results are shown in the table 5. The RSD were calculated for all the results are within limits. Precision was not more than 2.0% RSD, as demonstrated in Table 5.

	Interday	Day 1			Day 2		Mean	%RSD
Injection	1	2	3	1	2	3		
area	956735	963535	956973	955612	958412	956332	956332	0.30%
	Intraday	Morning			Evening			%RSD
Injection	1	2	3	1	2	3		
area	956898	962415	958615	957651	956526	954516	957770.2	0.28%

Table-5: summary of Precision

LOD and LOQ

The LOD and LOQ were calculated by the equations LOD $= \frac{3.3 \times \text{std. Deviation}}{\text{slope}}$ and LOQ $= \frac{10 \times \text{std. Deviation}}{\text{slope}}$ where, std. Deviation taken from accuracy and slope is from linearity. Based on these equations, the calculated LOD and LOQ values for metformin were 0.1502 and 0.4553 µg/ml, respectively.

Robustness

Robustness of the method reflects the reliability of an analysis with respect to deliberate variations in the method parameters. Here, the flow rate and wavelength were slightly changed to lower and higher sides of the actual values to find if the change in the peak area and retention time were within limits. The results obtained with changes in the parameters on a 30µg/mL solution are as shown in Table No. 6.

Sr.no.	Parameter	Condition	Peak Area		Statistical Analysis	Retention Time		Statistical Analysis
1	Flow rate (ml/min)	0.9	973735	Mean=	Mean=	4.296	mean=	mean=
			966735	965818.333	961906.889	4.296	4.29266667	4.22377778
			956985			4.286		
		1	956735		SD=		4.203	SD=
		1.1	964921	Mean=	4671.00377	4.162	mean=	0.06120488
			971366	963167.333	%RSD=	4.186	4.17566667	%RSD=
			953215		0.48559833	4.179		1.44905534
2	Wavelength (nm)	236	950121	Mean=	mean =	4.213	mean=	mean=
			951264	950218	952424.333	4.209	4.20933333	4.206
			949269			4.206		
		238	956735		SD=		4.203	SD=
		240	948963	mean=	3733.49519	4.206	mean=	0.0031798
			949632	950320	%RSD=	4.203	4.20566667	%RSD=
			952365		0.39199914	4.208		0.07560146

Table 6: robustness

Assay of marketed formulation

The % purity obtained from the formulation was given in table 7. And it was found that the Assay results of Metformin are within the limits.

Sr. NO.	% Composition	Area of Standard	Area of Sample	% Assay
1	% Assay	956735	945995	98.8774

Table 7: Assay of tables of Metformin Hydrochloride

System Suitability Parameters:

System suitability was carried out by injecting six replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Metformin hydrochloride at 4.2 min without any interference.

Parameter	Observed Value	limits
No. of Theoretical Plates	5630	> 2000
Tailing Factor	1.2	<1.5
Regression equation	y = 31271x + 49523	
Correlation coefficient (R^2)	0.9986	~1

Table 8: System suitability parameter

CONCLUSION:

The proposed method was found to be simple, precise, accurate, rapid and specific for determination of metformin hydrochloride from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Metformin hydrochloride in pure form and its dosage form.

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