International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 14, Issue 5, 2022

Original Article

A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF A POTENTIAL ANTI-DIABETIC DRUG METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM

JAYASHREE A. HIREMATH[1*], HARISH KUMAR[1]

[*1]Department of Pharmaceutical Chemistry M. S. Ramaiah University of Applied Sciences, Bengaluru 560054, Karnataka, India
Email: jaya98862@gmail.com

Received: 16 Jun 2022, Revised and Accepted: 22 Jul 2022

ABSTRACT

Objective: This study was conducted to develop a simple, economical, linear, rapid method for the assay studies of Metformin HCl by RP-HPLC method and to carry out the method validation.

Methods: A simple, robust and accurate method to carryout assay of Metformin hydrochloride tablet(500 mg) by RP-HPLC method in which the stationary phase used is Shimadzu shim-pack GIST C_{18} column with specification (5µm ×4.6×250 mm). This method involves isocratic elution of mobile phase containing 70% buffer and 30% acetonitrile. The buffer used for analysis is Tetra-Butyl Ammonium Hydroxide (0.002%), the flow rate was maintained at 0.5 ml/min. detection was done at 232 nm. Principal peak for Metformin was observed at 3.5 min and the runtime for each injection was set to 10 min. The standard solutions of Metformin were prepared using purified water (milli-Q water) and scanned from 190 nm to 400 nm. Sharp peaks were observed in the range of 232 nm and thus, wavelength of 232 nm was selected and used throughout the validation process.

Results: Linearity graph generated was found acceptable and accurate and the graph was generated in the range of 50% to 150% concentration. The regression coefficient was found to be 0.999(acceptable range). Validation was carried out according to ICH guidelines and found to be acceptable.

Conclusion: This developed method was found to be simple, robust, economical, accurate, linear and can be used in the assay of Metformin tablet using RP-HPLC.

Keywords: Reverse phase-HPLC (RP-HPLC), Limit of detection, Limit of quantification, Method validation, System suitability, Relative standard deviation, Specificity, Linearity, Range, Precision, Intermediate precision, Accuracy, Solution stability and system suitability

DOI: https://dx.doi.org/10.22159/ijcpr.2022v14i5.2017 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Analytical chemistry helps for the determination of quality, purity, safety of the chemicals and drugs by separating, quantifying and identifying the sample using certain methods and instruments. It helps in both quantitative as well as qualitative analysis of the sample, in qualitative analysis, it determines the purity and quality of the sample whereas, in quantitative analysis the concentration of the sample i.e., the amount of the expected content present within the sample can be found [1].

HPLC is High-Performance Liquid Chromatography is mainly used for the separation, identification and quantification of the components present in a mixture. Till 1960 liquid chromatography in which only glass columns were used and it was used to work in low pressure was developed to HPLC later with metal columns and high pressure. The basic working principle of HPLC is that it separates the constituent of the mixture based on relative affinities of the constituents for the stationary phase and mobile phase, which are used for the separation. Mainly there are two types of HPLC they are; reverse phase uses polar mobile phase and the non-polar stationary phase and the normal phase uses the non-polar mobile phase and polar stationary phase. Major applications of HPLC are analysing drugs, pollutants and synthetic polymers, isolation of components and used in industries in quality control departments to check the purity of the drug samples and chemicals [2].

Analytical method validation

Method validation provides evidence in documented form and gives high degree of assurance whether the developed method is suitable to produce a consistent result or not. ICH (International Conference on Harmonization) guidelines are issued by regulatory authorities to validate the newly developed method by using parameters such as accuracy, precision, intermediate precision(ruggedness), specificity, linearity and range, system suitability, robustness to test whether the developed method posess all the requirements [3].

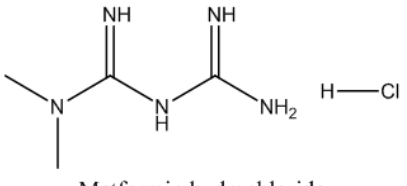
Metformin HCl

Metformin HCl is an antidiabetic drug with a chemical name, 1-carbamimidamido-N,N-dimethylmethanimidamide hydrochloride falls under biguanide class of antihyperglycemic agent. Works by reducing production of hepatic glucose and also increases glucose uptake, it also helps to prevent complications related to cardiovascular system. Its also used for PCOS problems and also decreases levels of triglycerides and low-density lipoprotein cholesterol [4].

As per pharmacopeia only potentiometry and UV methods are mentioned for Metformin, as per European pharmacopeia and Indian pharmacopeia and as per literature HPTLC [5], HPLC [6, 8], UV [6, 9, 10], LC-MS/MS [11] were reported. Even in the mentioned HPLC method, there was a need for an improved and stable method.

Drug profile

Molecular structure



Metformin hydrochloride

Fig. 1: Structure of metformin HCl

Chemical name: 1-carbamimidamido-N, N-dimethyl-methanimidamide hydrochloride

Molecular Formula: $C_4H_{12}ClN_5$

Molecular Weight: 165.62

Appearance: White or almost white crystalline powder

State: solid

Melting point: 223-226 °C

pKa: 12.4

Category: Biguanide class of anti-hyperglycemic agent

MATERIALS AND METHODS

Instrumentation

This work describes a method development and validation of Metformin HCl by the RP-HPLC method. Used shimadzu: LC10AD HPLC and by using Lab solutions software, chromatography was performed by Shimadzu shim-pack GIST C_{18} column (5µm×4.6×250 mm) with the mobile phase composed of buffer solution (700 ml) and acetonitrile (300 ml). The flow rate was 0.5 ml/min, the Injection volume was 20 µl, and the detection is at 232 nm where column temperature was 15 ˚C. The retention time of Metformin HCl is 3.5 min and the run time for each injection was 10 min.

Preparation of buffer

Tetra butyl ammonium hydroxide was taken in a minute quantity i.e.,2 ml in 1000 ml of HPLC grade water, sonicated for better mixing of solutions using sonicator and pH was adjusted to 3 using ortho-phosphoric acid and filtered using a 0.45 µm filter paper.

Preparation of mobile phase

Mobile phase was prepared by mixing the buffer and acetonitrile in the ratio of 70:30

Standard and sample preparation

Preparation of standard solution: 50 mg of Metformin HCl (working standard sample) was weighed approximately and transferred to a 100 ml volumetric flask dissolved using mobile phase solution and made up to the volume using the same solution.

Preparation of reference solution (a): 10 ml of the prepared standard solution was transferred to 50 ml volumetric flask and diluted up to the mark using mobile phase.

Preparation of sample solution: Approximately about 5 equivalent tablets were crushed and 61.5 mg of the sample was weighed and transferred to a 100 ml volumetric flask and diluted upto the mark using the mobile phase sonicated to dissolve the drug contents and finally filtered and diluted further by taking 10 ml of this solution in a 50 ml volumetric flask and diluted upto the volume using mobile phase.

Validation of the developed method

Validation was done according to ICH guidelines for the determination of the developed RP-HPLC method for Metformin HCl in the tablet dosage form. Validation was carried out for the parameters like precision, intermediate precision (ruggedness), specificity, linearity and range, system suitability, and robustness.

RESULTS AND DISCUSSION

New method was developed for the assay studies of Metformin HCl tablet (500 mg); the following are conditions stabilized.

Table 1: Chromatographic conditions

Chromatographic mode	Isocratic (70:30) Buffer: Acetonitrile
Detector wavelength	232 nm
Flow rate	0.5 ml/min
Injection volume	20µl
Column	Shimadzu shim-pack GIST C_{18} column (5µm×4.6×250 mm).
Column oven	25 °C
Run time	10 min

Specificity

Interference study

The blank, standard solution and sample solutions were prepared as described previously and injected in HPLC system. The retention time of all the corresponding peaks observed in the chromatogram were recorded. The obtained results are, as given in table 2. Based on the obtained result it is concluded that there is no interference due to the blank solution and placebo at a same retention time of the main peak of standard solution and sample solution chromatograms. The peak purity of Metformin HCl is 1.0

Table 2: Specificity studies

Type of solution	Retention time (min)	Peak purity
Blank	No interference	NA
Placebo	No interference	NA
Standard	3.5	1.0
Sample	3.5	1.0

Linearity

To evaluate the linearity of the method, 5 levels of concentrations 50%, 80%, 100%, 120% and 150% of Metformin HCl were taken. The correlation coefficient for Metformin HCl was found to be within the acceptable limit of not less than 0.999, which passes the linearity parameter.

Table 3: Linearity studies

Concentration (%)	Area
50	9677559
76	15483262
101	21162424
119	25130352
150	32649189

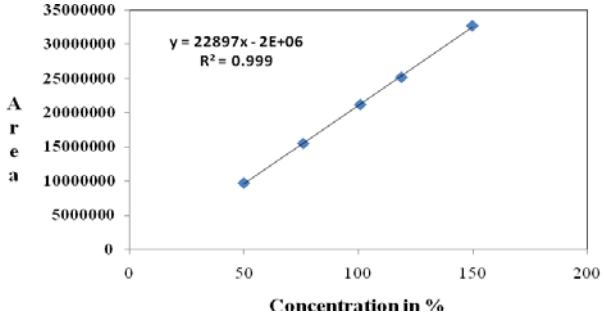


Fig. 2: Linearity graph

Range

For range, injected six replicates of lower and higher concentration levels of the linearity level and calculated the Mean and relative standard deviation. The relative standard deviation for Metformin HCl range levels was found to be within acceptable limits of not more 2.0%.

Table 4: Range of metformin HCl

No. of injections	Retention time	Range (50)
1	3.517	9656375
2	3.517	9650591
3	3.517	9653005
4	3.517	9653793
5	3.517	9654915
6	3.517	9657619
Mean	3.517	9654383
SD	0.000	2504
%RSD	0.000	0.026

Table 5: Range of metformin HCl

No. of injections	Retention time	Range (150)
1	3.508	32520257
2	3.500	32508290
3	3.500	32467103
4	3.500	32527585
5	3.508	32510855
6	3.508	32518020
Mean	3.504	32508625
SD	0.005	25565
%RSD	0.130	0.091

Precision

Repeatability

Six sample preparations were prepared and repeatability was checked. The relative standard deviation for Metformin HCl was found to be within acceptable limits of not more 2.0%.

The % Assay of Metformin HCl was within the acceptable limit. i.e. 98%-102% of label claim.

Table 6: Precision studies

S. No.	Sample weight (mg)	Metformin HCl area	Assay (%)
1	497.79	19003498	99.56
2	493.80	18851319	98.76
3	492.99	18823564	98.60
4	491.13	18749274	98.23
5	495.57	18820469	99.11
6	501.66	18918691	100.33
Mean		18869580	99.098
SD		88605	4061
%RSD		0.470	0.02

Intermediate precision

The intermediate precision was checked using a different instrument on a different day and compared with repeatability results.

Table 7: Intermediate precision/ruggedness studies

S. No.	Sample weight (mg)	Metformin HCl area	Assay (%)
1	509.48	18775093	101.90
2	509.17	18763514	101.83
3	507.34	18696083	101.47
4	506.31	18658191	101.26
5	504.34	18585724	100.87
6	501.62	18847912	100.32
Mean		18716818	100.272
SD		93494	6016
%RSD		0.499	0.03

Table 8: Comparative studies of repeatability and intermediate precision

Parameters	Assay (%)
Mean assay in repeatability	99.098
Mean assay in intermediate precision	100.272
Absolute difference	1.174

The % Assay of Metformin HCl was within the acceptable limit. i.e. 98%-102% of label claim.

The relative standard deviation of assay obtained from 12 sample preparations (Repeatability and Intermediate precision) is within the acceptance criteria of not more than 2.0%.

The absolute difference between the mean assay results obtained in repeatability and intermediate precision is within the acceptance criteria of not more than 2.

Based on the above results, it is concluded that the proposed method for assay by RP-HPLC is rugged.

Accuracy

The accuracy was carried at 3 levels, at 50%, 100% and 150%. The % recovery at 50%, 100% and 150% levels is within the acceptance criteria 98.0% to 102%

Based on the below-obtained recovery results as in table 8, it is concluded that the method for assay by HPLC is accurate.

Robustness

Robustness was carried out on standard solution by making the following alterations:

- By changing wavelength
- By changing the oven temperature
- By changing the flow rate

Table 9: Accuracy/recovery studies

Levels	Test area	Std area	Potency	Mg/tablet	% recovery	Mean recovery (%)
1	9873462	188444044	100.1	250.04	100.02	
	9535003	188444044	100.1	253.25	101.30	100.52
	9436227	188444044	100.1	250.63	100.25	
2	18897573	188444044	100.1	501.92	100.38	
	18925005	188444044	100.1	502.65	100.53	100.43
	18896952	188444044	100.1	501.91	100.38	
3	28074970	188444044	100.1	745.67	99.42	
	28521507	188444044	100.1	757.53	101.00	100.39
	30271908	188444044	100.1	755.78	100.77	

Table 10: Robustness studies

Condition	Retention time (min)	Tailing factor	Theoretical plates	%RSD
Normal	3.500	1.249	3081	0.024
Flow rate (0.4 ml)	4.4	1.266	3186	0.031
Flow rate (0.6 ml)	2.9	1.300	2434	0.046
Column oven(20℃)	3.55	1.255	2603	0.0
Column oven(30℃)	3.53	1.280	2910	0.085
Wavelength (230 nm)	3.53	1.308	2786	0.009
Wavelength (234 nm)	3.53	1.272	2767	0.070

Table 11: Stability studies

Time (h)	Standard solution	Time	Sample solution		
	25 ℃		25 ℃		
	Retention time (min)		RT	%Assay	Absolute difference with respect to initial
Initial	3.558	Initial	3.558	99.47	NA
6.0	3.567	6.0	3.567	99.49	0.02
12.0	3.567	12.0	3.567	100.17	0.70
18.0	3.567	18.0	3.567	100.99	1.52
24.0	3.558	24.0	3.558	101.90	2.43
30.0	3.567	30.0	3.567	102.85	3.38
36.0	3.558	36.0	3.558	103.85	4.38
42.0	3.558	42.0	3.558	104.49	5.02
48.0	3.558	48.0	3.558	105.39	5.92

Table 12: System suitability studies

Condition	Retention time	Tailing factor	Theoretical plate	%RSD
Specificity	3.5	1.161	2285	0.129
Linearity	3.5	1.317	2741	0.026
Range	3.5	1.317	2741	0.026
Repeatability	3.5	1.249	3081	
Intermediate precision	3.5	1.202	2583	0.499
Accuracy	3.5	1.205	3113	0.470

Solution stability

The solution stability is the parameter used to verify the stability of the prepared solutions such as mobile phase, standard solution and sample solution and to check the duration of time till the prepared solutions are stable. From this analysis, we come to the conclusion that the prepared solution is stable upto 24 h

System suitability

Standard solution was prepared as per the methodology and injected into the HPLC system before starting every validation parameter. The percentage RSD for 5 replicate injections, tailing factor and theoretical plates of standard solution are considered.

The tailing factor of Metformin HCl fulfills the acceptance criteria of not more than 2.0.

The theoretical plates of Metformin HCl fulfills the acceptance criteria of more than 2000.

CONCLUSION

The proposed method describes the analytical method development and validation of the potent antidiabetic drug Metformin HCl developed method is validated according to ICH guidelines found to be accurate, precise, simple and economical, the developed method can be used for the quantification of Metformin HCl in regular analysis.

ACKNOWLEDGEMENT

It is also my privilege to thank dr. S. Bharath, dean Ramaiah university of applied sciences, department of pharmaceutical chemistry for allowing me to carry out this work and their guidance and technical advice in Analytical study and for providing all information and documents for my project.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Stuart J. Practical HPLC method development by Lloyd R. Snyder (L. C. Resources, Inc.), Joseph J. Kirkland (Rockland Technologies, Inc.), and Joseph L. Glajch (DuPont Merck Pharmaceutical Co.). $84.95. J Am Chem Soc. 1998;120(14):3540.
2. Metzger S, Kolbesen BO. Application of HPLC for the analysis of organic additives in cleaning chemicals and cleaning mixtures. Solid State Phenom. 2005;103-104:221-6. doi: 10.4028/www.scientific.net/SSP.103-104.221.
3. Rathinavel G, Uma Nath U, Valarmathy J, Samueljoshua L, Thanuja C, Ganesh M. RP-HPLC method for the simultaneous estimation of rosiglitazone and gliclazide in tablets. E-Journal of Chemistry. 2009;6(4):1188-92.
4. Neelima K, Prasad YR. Analytical method development and validation of metformin, voglibose, glimepiride in bulk and combined tablet dosage form by gradient RP-HPLC. Pharm. 2014;5(1):27-33. doi: 10.5530/phm.2014.1.5.
5. Bhole RP, Shinde SS, Chitlange SS, Wankhede SB. A high-performance thin layer chromatography (HPTLC) method for simultaneous determination of diphenhydramine hydrochloride and naproxen sodium in tablets. Anal Chem Insights. 2015;10:47-51. doi: 10.4137/ACI.S31506, PMID 26692760.
6. Musmade BD, Baraskar ML, Ghodke VN, Bhope SG, Padmanabhan S, Lohar KS. Impurity profiling method development and validation of metformin hydrochloride and teneligliptin hydrobromide hydrate in their combination tablet dosage form by using RP-HPLC with UV/PDA detector. Futur J Pharm Sci. 2021;7(1). doi: 10.1186/s43094-021-00362-9.

7. Chhetri HP, Thapa P, Van Schepdael A. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharm J. 2014;22(5):483-7. doi: 10.1016/j.jsps.2013.12.011, PMID 25473337.
8. Chhetri HP, Thapa P, Van Schepdael A. Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharm J. 2014;22(5):483-7. doi: 10.1016/j.jsps.2013.12.011, PMID 25473337.
9. Mubeen G, Noor K. Spectrophotometric method for analysis of metformin hydrochloride. Indian J Pharm Sci. 2009;71(1):100-2. doi: 10.4103/0250-474X.51947, PMID 20177473.
10. Arayne MS, Sultana N, Zuberi MH, Siddiqui FA. Spectrophotometric quantitation of metformin in bulk drug and pharmaceutical formulations using the multivariate technique. Indian J Pharm Sci. 2009;71(3):331-5. doi: 10.4103/0250-474X.56022, PMID 20490308.
11. Chunduri RH, Dannana GS. Development and validation of a reliable and rapid LC-MS/MS method for simultaneous quantification of sacubitril and valsartan in rat plasma and its application to a pharmacokinetic study. Biomed Chromatogr. 2016;30(9):1467-75. doi: 10.1002/bmc.3707, PMID 26876742.
12. PS, Vidyadhara S, Sasidhar RLC, Chakravarthi KK. Method development and validation for the estimation of entecavir in bulk and pharmaceutical dosage forms by RP-HPLC. Int J Curr Pharm Sci. 2017:107-11. doi: 10.22159/ijcpr.2017v9i5.22151.