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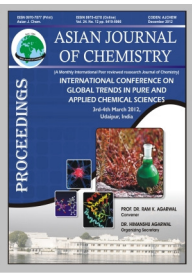

Development and Validation of A Reverse Phase HPLC Method for the Determination of Metformin HCl in Pharmaceutical Dosage Forms†

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Metformin hydrochloride chemically, *N,N*-dimethyl imido dicarbonimidicdiamide hydrochloride is used as antidiabetic drug from the biguanide class used in the management of type 2 diabetes. A rapid specific reverse-phase HPLC method has been developed for assaying metformin HCl in pharmaceutical dosage forms. The method involves an isocratic elution of drug in a Zorbax-SCX, C_{18}, 250 mm × 4.6 mm, 5 mm column using a mobile phase composition of Buffer (pH 3.0) with ammonium dihydrogen phosphate: acetonitrile [50: 50, v/v] with flow rate of 1.0 mL/min at 218 nm. The developed method is found to be linear in the range of 20-60 µg/mL. The method has been validated for specificity, linearity, range, precision, accuracy, limit of detection, limit of quantification, ruggedness and robustness. The % recovery of metformin hydrochloride was found to be in the range of 99.22-100.11 %.

Key Words: Metformin hydrochloride, Antidiabetic, Assay, Isocratic, HPLC, Pharmaceutical formulations.

INTRODUCTION

Metformin chemically, *N,N*-dimethylimido-dicarbonimidicdiamide hydrochloride (Fig. 1) is used as antidiabetic drug from the biguanide class used in the management of type 2 diabetes. Literature survey reveals very few UV spectrophotometric methods[1,2], HPLC[3-5] and ion-pair HPLC[6] method have been reported for the estimation of metformin hydrochloride. The reported techniques were tedious, insufficiently sensitive and required highly dedicated instrumentation.The present study describes a simple, reliable, sensitive and stability-indicating method for the accurate and precise determination of metformin hydrochloride.

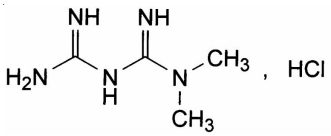

Fig. 1. (Metformin) *N,N*-dimethylimidodicarbonimidicdiamide hydrochloride

EXPERIMENTAL

Metformin hydrochloride was procured from NATCO Pharma Pvt. Ltd., (Hyderabad, India). Commercial pharmaceutical preparations from NATCO Pharma, which were claimed to contain 500 mg of metformin hydrochloride was used in analysis. Different kinds of equipments *viz*. analytical weighing balance (Sartorius), HPLC system (Waters 2690 series) Make: with 2996 PDA detector, empower software equipped with empower software, zorbax column, column consists of zorbax -SCX, C_{18}, 250 mm × 4.6 mm, 5 mm. Data aqusition is done using empower software.

Preparation of Buffer, mobile phase and diluents: Buffer solution is prepared by dissolving 17 g of anhydrous ammonium-dihydrogen phosphate was transferred into 1000 mL volumetric flask and adjust the volume with ortho-phosphoric acid (pH-3.0). Volume was made up to the mark with water. Mobile phase consists of 50:50 Buffer and acetonitrile. Diluent consists of 50:50 Buffer and acetonitrile.

Preparation of standard, sample stock and placebo solution: 40 mg of standard metformin hydrochloride was accurately weighed and transferred into a 100 mL clean dry volumetric flask, about 30 mL of diluent was added, sonicated for 5 min and diluted up to the mark with diluent (stock solution-I). From this stock solution-I (400 µg/mL), 5 mL of solution was transferred into a 50 mL volumetric flask and diluted with the diluent up to the mark and labeled as stock solution-II (40 µg/mL). Twenty tablets of metformin hydrochloride were weighed and powdered in glass mortar. The

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powder equivalent to the amount of active ingredient present in 10 tablets was transferred into a 100 mL volumetric flask, 70 mL of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 min by shaking at intervals of 5 min each and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for further dilution. 1 mL of upper clear solution was transferred to a 100 mL volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 mm filter before injecting into HPLC system. The amount of powdered inactive ingredient suppose to be present in 10 tablets was accurately weighed and transferred in to 100 mL volumetric flask, 70 mL of diluent was added and shaken by mechanical stirrer and sonicated for about 30 min by shaking at intervals of 5 min and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution. 1 mL of upper clear solution was transferred to a 100 mL volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 mm filter before injecting into HPLC system.

System suitability: Stock solution-II of standard was injected six times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections. The results of system suitability studies were given in Table-1.

TABLE-1
SYSTEM SUITABILITY STUDY

	Drug	Theoretical plates	Tailing factor	R.T (Drug)
Mean	11986.41	21705	0.0	0.032
SD	0.14	260.67	0.48	0.28
RSD	8281767	1.201	1.60	11.307

Each mean value is a result of triplicate analysis (n = 6)

Limit of detection and limit of quantification: Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated. The LOD was determined by the formula: LOD = 3.3 s/S, The LOQ was determined by the formula: LOQ = 10 s/S.Where, s = standard deviation of Intercepts of calibration curves, S = Mean of slopes of the calibration curves. The slope S may be estimated from the calibration curve of the analyte.

Linearity (calibration curve): The linearity of the method was demonstrated over the concentration range of 20-60 µg/mL. Aliquots of 20, 30, 40, 50 and 60 µg/mL were prepared from stock solution-II and labeled as solution 1, 2, 3, 4 and 5 respectively. The solutions were injected in to HPLC system as per test procedure. A calibration curve was plotted for concentration *versus* peak area and was given in the Fig. 2. The results of linearity, LOD and LOQ were given in Tables 2-5.

Specificity, repeatability and intermediate precision (analyst to analyst variability): Precision (repeatability) results and the results for analyst-2, which emphasized were and discussed in Table-6. Assay was performed in triplicate for various concentrations of equivalent to 50, 75, 100, 125 and 150 % of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was

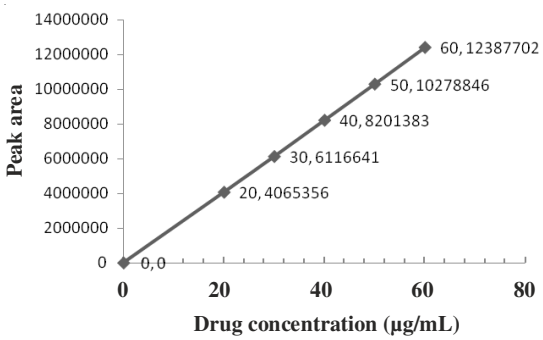

Fig. 2. Calibration curve of metformin hydrochloride

TABLE-2
LIMIT OF DETECTION

Injection no	Drug (area)	Theoretical plates	Tailing factor
Mean	36326	7589	1.60
S.D	572.72	145.92	0.02
RSD	1.58	1.92	1.45

Each mean value is a result of triplicate analysis (n = 5)

TABLE-3
LIMIT OF QUANTIFICATION

Injection no	Drug (area)	TP	TF
Mean	109989	7651	1.63
SD	811.86	83.74	0.02
RSD	0.74	1.09	1.39

Each mean value is a result of triplicate analysis (n = 5)

TABLE-4
LINEARITY RANGE AND AVERAGE AREA VALUES

1	20	4065356
2	30	6116641
3	40	8201383
4	50	10278846
5	60	12387702

TABLE-5
CALIBRATION PARAMETERS FOR METFORMIN HYDROCHLORIDE

Parameter	Results
Slope	20945.13
Intercept	-11227.2000
Correlation coefficient (R^2)	1.000
Percentage of curve fitting	99.9 %

Each mean value is a result of triplicate analysis (n = 3)

calculated and the results were summarized in Table-7. A study to establish the interference of placebo was conducted. A sample of placebo was injected into the HPLC system as per the test procedure. The chromatogram of placebo and standard was represented as Figs. 3 and 4 respectively.

TABLE-6
STANDARD CHROMATOGRAM VALUES FOR REPEATABILITY AND ANALYST TO ANALYST VARIATION

Injection no	Peak area analyst-1	Recovery (%)	Peak area analyst-2	Recovery (%)
Mean	8336050	99.87	8361243	99.24
SD	101248.42	0.501358	103106	0.50
RSD (%)	1.21	0.5	1.23	0.50

Each mean value is a result of triplicate analysis (n=6)

TABLE-7
RECOVERY (%) RESULTS FOR METFORMIN HYDROCHLORIDE

Sample no.	Spiked level (%)	Amount (µg/mL) added	Mean recovery (%)
1	50	20	99.22
2	75	30	99.94
3	100	40	99.83
4	125	50	100.11
5	150	60	99.62

Each mean value is a result of triplicate analysis (n = 3)

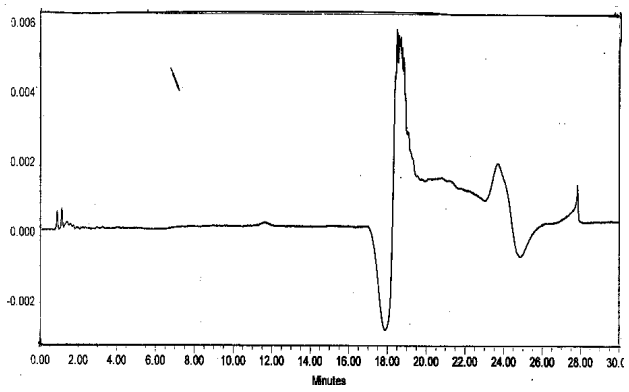

Fig. 3. Chromatogram for placebo interference

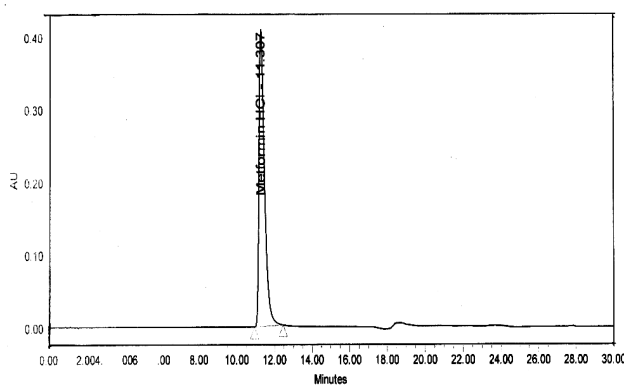

Fig. 4. Standard chromatogram for optimized method

Effect of variation of flow rate and mobile phase composition: A study was conducted to determine the effect of variation in flow rate and mobile phase compositon. The retention time values were measured and are given in Table-8.

TABLE-8
EFFECT OF VARIOUS PARAMETERS IN ASSESSMENT OF METHOD

Parameters	Variation	Retention Time	Tailing
Flow rate	0.9 mL/min	11.129	1.675
Mobile phase	1.0 mL/min	11.122	1.684
	[50:50, v/v, MeoH: Buffer]	11.122	1.675
	[60:40, v/v, MeOH: Buffer]	11.129	1.684

RESULTS AND DISCUSSION

Method validation: From the system suitability studies it was observed that % RSD of retention time was found to be 0.2, % RSD of peak area was found to be 0.2. Theoretical plates were found to be more than 7581. USP tailing factor was found to be 1.6. All the parameters were within the limit. The results of system suitability studies were given in Table- 1.

Determination and quantization limits (sensitivity): The limit of detection and limit of quantification was calculated from the linearity curve method using slope and standard deviation of intercepts of calibration curve. Limit of detection = 1.77 µg/mL. Limit of quantification was found to be 5.39 µg/mL. Tables 2 and 3.

Linearity, accuracy and precision: From the Linearity data it was observed that the method was showing linearity in the concentration range of 20-60 µg/mL. Correlation coefficient was found to be 0.9999. The data of linearity was illustrated in Tables 4 and 5 and the linearity curve was plotted in Fig. 2. The RSD of % recovery for standard chromatograms of repeatability precision was found to be 0.5 % and in intermediate precision it was found to be 0.47 %. The results of precision were summarized in Table-6. The recoveries of pure drug from the analyzed solution of formulation were 99.22 % to 100.11 %. The summary of Accuracy results were expressed in Table-7.

Specificity, robustness and ruggedness study: No interference due to Placebo and Standard at the retention time of analyte, which shows that the method was specific. The chromatograms for specificity studies placebo, standard, were represented as Figs. 3 and 4. Comparison of both the results obtained for two different analysts shows that the assay method was rugged for analyst-analyst variability. The results of ruggedness were found to be within the limits and were discussed in Table-6. The results of robustness for effect of variation in flow rate and mobile phase composition were reported in Table-8.

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