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HPLC METHOD FOR THE QUANTIFICATION OF METFORMIN HYDROCHLORIDE IN BULK AND DOSAGE FORMS

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ABSTRACT: A simple, selective, rapid and economical reversed phase high performance liquid chromatographic method for the determination of metformin hydrochloride in bulk and dosage forms has been developed and validated. The separation was carried out on a Discovery reversed phase C-18 column (250 mm x 4.6 mm, 5.0 μm) with UV detection at 233 nm. The mobile phase contained 34% acetonitrile and 66% aqueous phase. Aqueous phase contained 10 mM KH_2PO_4 and 10 mM sodium lauryl sulfate. Mobile phase pH was adjusted to 5.2. The mobile phase was run isocratically. The flow rate of the mobile phase was maintained at 1.3 ml/min. The linearity of the calibration curve was obtained in the concentration range of 2.5 to 20 $\mu\text{g ml}^{-1}$ and coefficient of determination (R^2) was found to be 0.9985. The % RSD value for intraday and interday precision was below 1 which indicated that the method was precise. Limit of detection and limit of quantification were 0.1 and 0.3 $\mu\text{g/ml}$ respectively.

INTRODUCTION: Metformin hydrochloride (1, 1-dimethyl biguanide hydrochloride) is an oral antidiabetic drug¹.

The structural formula is as shown:

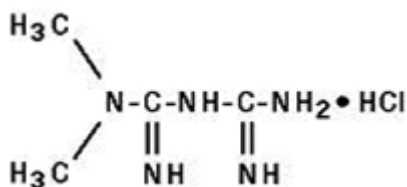


FIGURE 1: CHEMICAL STRUCTURE OF METFORMIN HYDROCHLORIDE

This is the first line of choice in the management of diabetes in obese persons having good renal function. It suppresses excessive hepatic glucose production and improves glucose clearance, predominantly by decreasing fasting plasma glucose¹.

Unlike sulfonylureas, metformin does not produce hypoglycemia in either patients with type 2 diabetes or normal subjects and does not cause hyperinsulinemia.

With metformin therapy, insulin secretion remains unchanged while fasting insulin levels and day-long plasma insulin response may actually decrease. Metformin hydrochloride is formulated as tablet and liquid dosage forms. The European and Indian Pharmacopoeia describe a potentiometric titration method for metformin hydrochloride bulk assay^{2, 3} and a UV-visible spectrophotometric method for the assay of metformin hydrochloride tablets.

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In this context, a method for the analysis of metformin hydrochloride in bulk and pharmaceutical dosage forms by HPLC is needed.

Several methods have been reported for the determination of metformin by capillary electrophoresis⁴, NMR spectrometry⁵, spectrofluorometry⁶, potentiometry⁷, UV-visible spectrophotometry⁸, conductometry⁹, voltammetry¹⁰ and by HPLC in human serum^{11, 12}. These methods are either complex, expensive, meant for plasma or do not use an internal standard. In this context, a simple, sensitive and economic reversed phase HPLC method has been developed and validated for the quantification of metformin hydrochloride in bulk and dosage forms.

As this method is quite sensitive it can be used to validate the cleaning of equipments like: double cone blender, mass mixer, tablet compression machine etc. after the manufacturing of metformin tablets. This method uses a commonly available reversed phase C-18 column with UV detector and phenytoin sodium has been used as an internal standard (IS) to minimize the errors of sample preparation.

MATERIALS AND METHODS

Reagents and chemicals: Metformin hydrochloride was obtained from ABC Chemicals and phenytoin sodium from FAGRON Belgium. Potassium dihydrogen phosphate, sodium lauryl sulfate, acetonitrile were purchased from Merck-Germany, ACROS Organics and VWR Prolabo Belgium respectively. Metformin tablets (500mg) were purchased from the Belgian market.

Instrumentation: The LC system consisted of a pump (Merck Hitachi L-6200 intelligent pump) with auto sampler (Spectra SERIES AS 100) having 20 μ l injector. The detector used was a UV-vis (ELITE Lachrom Hitachi L-2400 model). The wavelength was set at 233 nm. The software used was Chromeleon. The column used was Discovery Reversed Phase C-18 column (250 mm x 4.6 mm, 5.0 μ m).

Preparation of standard and sample solutions: Stock solution of metformin hydrochloride bulk was prepared by dissolving 20 mg of metformin hydrochloride in methanol and the final volume was made to 100 ml with the same solvent.

Similarly phenytoin sodium, the internal standard, was also prepared by dissolving 20 mg of it in 100 ml methanol. To each of 0.125, 0.25, 0.5, 0.75, and 1.0 ml of metformin hydrochloride stock solution, 1 ml of phenytoin sodium stock solution was added and final volume was made to 10 ml with methanol. Thus the final concentration of metformin hydrochloride was made 2.5, 5, 10, 15, and 20 μ g/ml respectively. The final concentration of internal standard was 20 μ g/ml.

For the analysis of the tablet, twenty tablets were weighed separately and average weight was calculated. All the tablets were crushed by using mortar and pestle. Powder equivalent to 20 mg of metformin hydrochloride was weighed, dissolved in methanol for 15 minutes and volume was made to 100 ml with methanol. 8, 10 and 12 μ g/ml of metformin was prepared from the tablet powder similar to the bulk described above and internal standard was also added to get a final concentration of 20 μ g/ml.

RESULTS AND DISCUSSION: A reversed phase HPLC method was developed for the analysis of metformin hydrochloride in bulk and tablet dosage forms. For this, a method for analysis of the drug in human plasma by Zarghi et al. has been modified and validated for the analysis of metformin hydrochloride in bulk and dosage form¹³.

Optimization of HPLC method: The HPLC method was optimized for the analysis of metformin hydrochloride in bulk and tablet dosage forms. Different mobile phases were tested to find the best condition to quantify metformin hydrochloride in bulk and dosage forms. Different ratios of methanol, acetonitrile, sodium lauryl sulfate and potassium dihydrogen phosphate were tried at different molarities and the optimum mobile phase was finalized.

The mobile phase composition finally selected contained 34% acetonitrile and 66% aqueous phase. The aqueous phase contained 10 mM KH_2PO_4 and 10 mM sodium lauryl sulfate. Mobile phase apparent pH was adjusted to 5.2 by using dilute orthophosphoric acid or sodium hydroxide. The mobile phase was run isocratically. The flow rate of the mobile phase was maintained 1.3 ml/min. Injection volumes were 20 μ l.

The mobile phase was degassed by using helium gas prior to its use. The HPLC column was kept at ambient temperature. Initially, the samples were prepared in water but the recovery of the drug from dosage form was found higher with methanol. So, all the samples were prepared in methanol.

Validation of the method: The method was validated by following the ICH¹⁴ and USP guidelines¹⁵ for system suitability, linearity, accuracy, precision, specificity and limit of detection (LOD). The system suitability was checked by analyzing the repeatability of retention time, tailing factor and theoretical plates of the column. The robustness of the method was checked by deliberately changing the experimental conditions like: flow rate, mobile phase composition and temperature of the column.

System suitability: System suitability parameters were recorded from the typical chromatogram of the metformin hydrochloride. The typical chromatograms of metformin hydrochloride show an average retention time of 9.26 ± 0.02 mins for five replicate injections. The retention time of the IS was found to be 7.44 ± 0.01 mins with a resolution factor 5.44. This shows that there is a good resolution between the internal standard and metformin hydrochloride peaks. The typical chromatogram of metformin hydrochloride and internal standard is shown in **figure 2**. The tailing factor was found to be 1.13, which indicated the symmetric nature of the peaks. The number of theoretical plates was found to be 14469 for metformin hydrochloride and 6728 for the internal standard. This high number of theoretical plates suggests the efficient performance of the column.

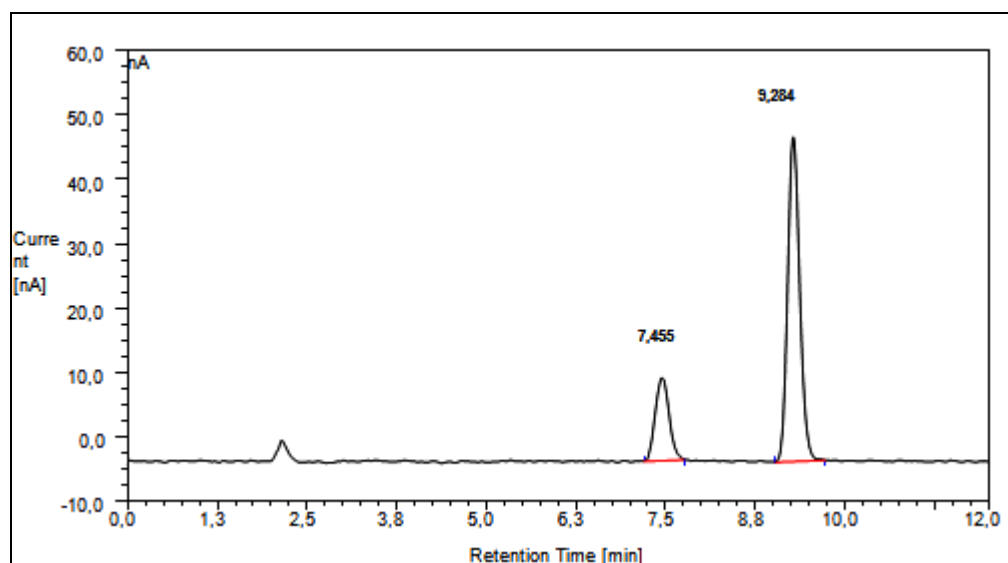


FIGURE 2: TYPICAL CHROMATOGRAM OBTAINED UNDER THE CONDITIONS DESCRIBED IN MATERIALS AND METHOD SECTION

Accuracy: As per the ICH guideline, accuracy is inferred once linearity, specificity and precision of the method are established¹⁴. This approach was followed to establish the accuracy of the method and the method was found to be accurate.

Linearity: To evaluate the linearity of this method, five standard solutions covering the range of 2.5 to 20 $\mu\text{g/ml}$ were analyzed using the method. The plot of peak area ratio of drug and IS against respective concentration of the drug was found to be linear in the range as shown in figure 3. The regression equation was found to be $Y=0.3305X+0.1646$ and the coefficient of determination R^2 of the standard curve was found to be 0.9985.

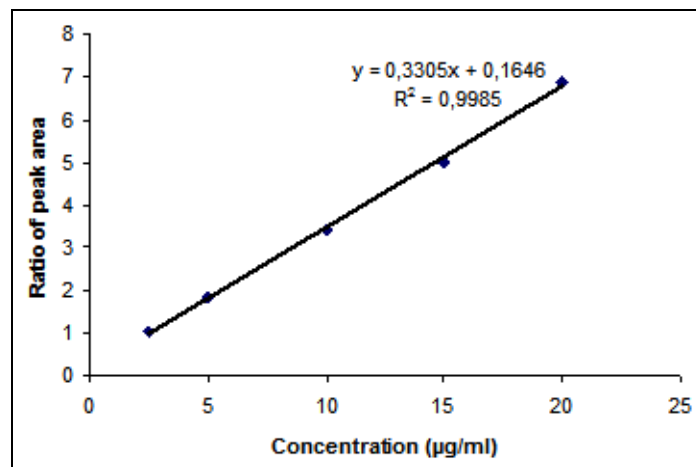


FIGURE 3: CALIBRATION CURVE

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present¹⁴. A mixture that contained metformin hydrochloride (10 µg/ml), phenytoin sodium (20 µg/ml), starch (0.6 µg/ml) and hydroxypropylmethylcellulose (0.6 µg/ml) was prepared and filtered (0.25 µm membrane filter) and 20 µl of it was injected. Separate excipient solutions were also injected. There were no interfering peaks of the excipients. Also, during the analysis of the drug in marketed tablet no interfering peaks were observed. This shows that the developed method is specific to the metformin hydrochloride and internal standard.

Precision: Precision of the method was considered at two levels: repeatability and intermediate precision. Repeatability was determined by repeating six determinations at 10 µg/ml whereas intermediate precision was determined by intra-day and inter-day variations.

Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time¹⁴. The relative standard deviation (RSD) of the peak areas of six replicate injections of a 10 µg/ml solution of metformin hydrochloride was calculated and it was found to be 0.31%. This small RSD value indicates the good precision under the same operating conditions.

Intermediate precision: The intermediate precision of the method was determined by the intra-day and inter-day variation of the results. Three concentrations- 8, 10 and 12 µg/ml of metformin hydrochloride respectively were analyzed in triplicate on three consecutive days. The % RSD of the concentration obtained was calculated within and in between days as shown in **table 1**. As the value is less than 1% in both the cases, the method was considered precise.

TABLE 1: INTRA-DAY AND INTER-DAY PRECISION

Concentration (µg/ml)	Intra-day precision (n=3)	Inter-day precision (n=3)
	RSD (%)	RSD (%)
8	0.66	0.90
10	0.45	0.80
12	0.35	0.72

Robustness of the method: There was no marked change in the retention time of metformin hydrochloride by small variations like flow rate, pH,

mobile phase ratio and temperature. This demonstrates that the proposed method was robust.

Stability: The stability of metformin hydrochloride in methanol was checked by determining the concentration of the solution after 48 hours at room temperature in comparison with the concentration at zero time. The result showed no marked variation and the RSD was less than 2% of the initial amount. This shows a good stability of metformin hydrochloride in methanol over a period of 48 hours at room temperature.

Limit of detection (LOD) and Limit of Quantification (LOQ): The lowest concentration of the drug that can be detected is expressed as a concentration that gives signal to noise ratio of 3:1¹⁴. The lowest amount of analyte that can be determined with acceptable precision and accuracy under the experimental condition or LOQ was measured in terms of signal to noise ratio of 10:1¹⁴. The LOD and LOQ were found to be 0.1 µg/ml and 0.3 µg/ml respectively.

Application of the method to assay metformin hydrochloride tablet: The method was used for the determination of metformin hydrochloride in marketed tablet formulations. The result showed that the percentage recoveries were high and RSD values were low (**Table 2**). This confirms that the method is suitable for the routine determination of metformin hydrochloride in its tablet dosage forms.

TABLE 2: PERCENTAGE LABEL CLAIM IN COMMERCIAL DOSAGE FORM (n=3)

Expected (µg/ml)	Obtained (µg/ml)	RSD (%)	% Label claim
10.2	10.4	1.79	101.9%

CONCLUSION: The HPLC method developed is accurate, precise, repeatable and specific. The method is linear over a wide range and utilizes a mobile phase which can be easily prepared. The column used is a widely available reversed phase C-18. This method is suitable for the routine quantification of metformin hydrochloride in bulk drug and in the tablet dosage forms.

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