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Determination of metformin hydrochloride by reverse phase high performance liquid chromatography (RP-HPLC)

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ABSTRACT

A Rapid, precise, accurate, specific and simple HPLC method was developed for estimation of metformin hydrochloride in its commercial formulations. A High performance liquid chromatograph 10AT SHIMADZU-SPD10A, using Phenomenex - Luna RP-18(2), 250X4.6mm, 5 μm column, with mobile phase composition of methanol: water and flow rate was 1 mL min^{-1} with UV detection at 233 nm. The retention time of metformin hydrochloride was 2.0 min. The total elution time was less than 5 minutes. Linearity was observed over concentration range of 4-20 μg mL^{-1} for metformin hydrochloride. The Limit of detection and Limit of quantitation for metformin hydrochloride was found to be 20.895 μg mL^{-1} and 63.319 μg mL^{-1} respectively. The accuracy of the proposed method was determined by recovery studies and found to be 101.48 % and 102.24 % for metformin hydrochloride respectively. Commercial formulations and laboratory prepared mixtures were successfully analyzed using the developed method. The proposed method was validated for various ICH parameters like linearity, limit of detection, accuracy, precision, ruggedness, robustness, and system suitability.

KEYWORDS

Metformin hydrochloride;
HPLC;
Method validation;
Standard addition.

INTRODUCTION

The quality control of active pharmaceutical ingredients (APIs) in the formulation is always a thrust area for the pharmaceutical industries. So the development of reproducible, sensitive, simple and extremely inexpensive methods for the determination of APIs in the formulation is always challenging.

Metformin (N, N dimethylimidodicarbonimidic diamide) is an oral antidiabetic drug. It is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function[1,3].

Evidence is also mounting for its efficacy in gestational diabetes, although safety concerns still preclude its widespread use in this setting. It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor. When prescribed appropriately, metformin causes few adverse effects. The most common is gastrointestinal upset and unlike many other anti-diabetic drugs, does not cause hypoglycemia if used alone. Lactic acidosis (a buildup of lactate in the blood) can be a serious concern in overdose and when it is prescribed to people with contraindications, but otherwise, there is no significant risk. Metformin helps reduce

LDL cholesterol and triglyceride levels and is not associated with weight gain, and is the onlyanti-diabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. As of 2009, metformin is one of only two oral anti-diabetics in the World Health Organization Model List of Essential Medicines (the other being glibenclamide)[4]. First synthesized and found to reduce blood sugar in the 1920s,metforminwas forgotten for the next two decades as research shifted to insulin and other anti-diabetic drugs. Interest in metformin was rekindled in the late 1940s after several reports that it could reduce blood sugar levels in people, and in 1957, French physician Jean Sterne published the first clinical trial of metformin as a treatment for diabetes. It was introduced to the United Kingdomin 1958, Canada in 1972, and the United States in 1995.Metformin is now believed to be the most widely prescribed anti-diabetic drug in the world; in the United States alone, more than 40 million prescriptions were filled in 2008 for its generic formulations[5,6]. Number of analytical methods have been reported for measuring metforminin biological fluids and tissue extracts. These methods involved colorimetry techniques[7,9].

Aim of this work was to develop a sensitive and simple spectrophotometric method for the quantification of metformin is finding new dimensions of clinical importance. To support its investigation an appropriate analytical method (sensitive, selective, reproducible and simple) for quantification of metformin is essential. For the drugs that obey the beer Lambert's law, spectrophometric methods of analysis of single component in solution are usually rapid, sensitive and economical.

EXPERIMENTAL

Apparatus and software

Filtration unit, Glass apparatus & a model Shimadzu UV-1601 double beam spectrophotometer with a fixed slit width of 2nm using a pair of 1cm matched quartz cells was used for spectrophotometric analysis. The Shimadzu HPLC system consisting Phenomenex - Luna RP-18(2), 250X4.6mm, 5 μm column

Reagents and pharmaceutical preparations

Metformin hydrochloride was kindly gifted by Dr. Reddy's Laboratories India and Biocon India Ltd, certified to contain 99.6% and 99.8% purity respectively. The drugs are used without further purification. All the solvents used in analysis were of HPLC grade.

HPLC method

Preparation of stock solution

An accurately weighed 100.0 mg of pure drug Metformin hydrochloride was taken in clean, dry 100 ml volumetric flask and dissolved in small volume mobile phase-methanol: water (90:10). The solution is diluted to 100.0 ml with mobile phase-methanol: water (90:10), resulting solution in 1000.0 mcg/ml of drug concentration.

Preparation of mobile phase

The mobile phase methanol: water (90:10) was selected.

Pharmaceutical sample solution (from Formulation)

Weigh accurately 20 tablets of metformin hydrochloride. An amount of the powder equivalent to content of one unit of tablet was dissolved separately in 60 ml of mobile phase. The solutions were sonicated for 10 min and filtered into a 100 ml volumetric flask through 0.45μ nylon membrane filter. The residue was washed 2 times with 10 ml of mobile phase, and then the volume was completed to 100 ml with the same solvent. The proposed RP-HPLC method was applied and the concentration of component in the formulations was determined.

Chromatography

The mobile phase methanol: water (90:10) was selected, because it was found that it ideally resolves the peaks with retention time (RT) 1.17 min and Wavelength was selected by scanning all standard drugs over

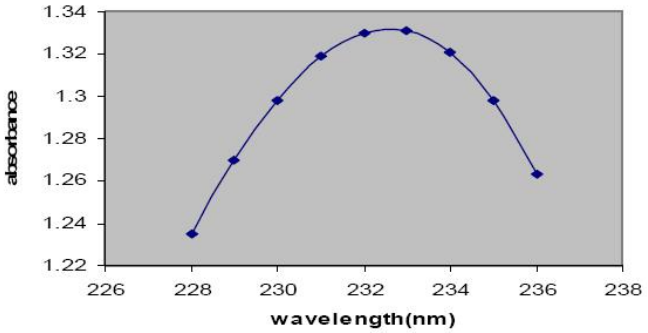

Figure 1 : Metformin HCL: 8 mcg/ml in mobile phase-methanol: water (90:10).

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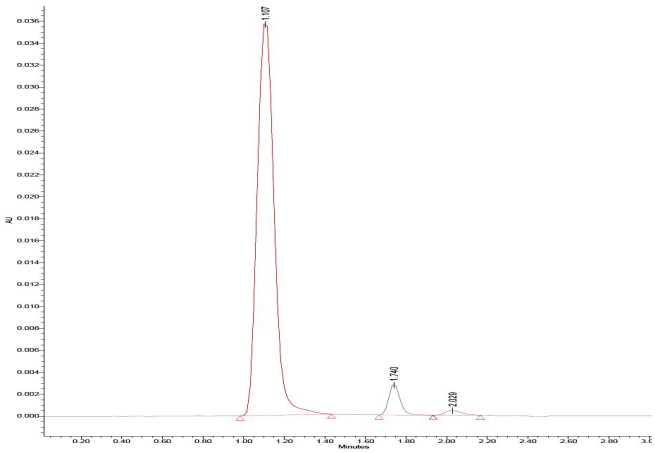

Figure 2 : Chromatogram showing retention time (R_t) 4 µg ml^{-1} of metformin (1.40 min) in laboratory-prepared mixture.

a wide range of wavelength 200nm to 400nm. The component shows reasonably good response at 233 nm. The absorbance values are represented graphically in Figure 1 and Figure 2.

Selection of sampling wavelength

The equivalent of 10 mg of Metformin was accurately weighed in 100 ml of volumetric flask separately after the immediate dissolution. The volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 µg/ml of Metformin. From the above stock solution, working standard solution having concentration 20 µg/ml was prepared by appropriate dilution. Working standard solution of 20 µg/ml of drug was scanned in the range of 233 nm in the spectrum mode at the low scan speed to obtain the overlain UV spectra for drug.

Selection of mobile phase

Different column chemistry, solvent type, solvent strength, detection wavelength and flow rate were varied to determine the chromatographic condition giving the best result. The mobile phase conditions were optimized so that the drugs were not interfering with solvent and excepients.

For the Metformin the best result was obtained at Methanol: water (90:10). Further the model was optimized by changing the concentration of mobile phase. From the study it was found that the best result was obtained in quality separation in terms of peak symmetry, reasonable, run time and other parameters by use of Methanol: Water (90:10) ratio mixture as mobile phase. The flow rate was determined the testing the effect of different flow rate on the peak area and resolution, flow rate of 1 ml /min found optimum.

CALIBRATION

Preparation of stock solution

An accurately weighed 100.0 mg of pure drug Metformin hydrochloride was taken in clean, dry 100 ml volumetric flask and dissolved in small volume mobile phase-methanol: water (90:10). The solution is diluted to 100.0 ml with mobile phase-methanol: water (90:10), resulting solution in 1000.0 mcg/ml of drug concentration. Now pipette out 5 ml of this solution and diluted to 100 ml with mobile phase-methanol: water (90:10), resulting in 50 µg/ml of the drug concentration.

Procedure

Aliquots of 0.4, 0.8, 1.2, 1.6, 2.0 ml of 50 µg/ml solution of Metformin Hydrochloride was pippeted into each of five 10 ml of volumetric flask. The volume was made up to 10.0 ml with mobile phase-methanol: water (90:10). The absorbance of the solution was measured at 233.0 nm. All stock and working solutions were sonicated for 5 min then filtered through the nylon mem-

TABLE 1 : Calibration table of metformin

SI.NO.	Conc. [mcg/ml]	*Peak area
1.	4	0.2067
2.	8	0.3774
3.	12	0.5229
4.	16	0.6843
5.	20	0.8242

***Average of three experiments**

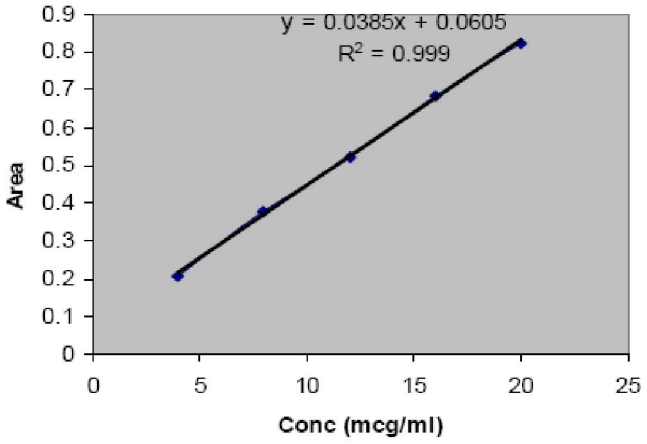

Figure 3 : Calibration curve of metformin

brane filter (0.45µ) prior to use. The absorbance values are recorded in TABLE 1 and represented graphically in Figure 3

VALIDATION OF ANALYTICAL METHOD

Method validation

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application.

To check the validity (predictive ability) of the calibration models, the simultaneous analysis of the prediction set containing five samples of various concentrations of Metformin was carried out and the results are given in TABLE 2. The mean recoveries and the relative standard deviations of our proposed method was computed and indicated in table. Their numerical values were completely acceptable because of good recovery values and hence found satisfactory for the validation.

Pharmaceutical sample solution (from Formulation)

Aliquots of 0.4, 0.8, 1.2, 1.6, 2.0 ml of 50 µg/ml solution of Metformin Hydrochloride was pippeted into each of five 10 ml of volumetric flask. The volume was made up to 10.0 ml with mobile phase-methanol: water (90:10). The absorbance of the solution was measured at 233.0 nm. All stock and working solutions were sonicated for 5 min then filtered through the nylon membrane filter (0.45µ) prior to use. The absorbance values are recorded in TABLE 2 and represented graphically in Figure 4

TABLE 2 : Validation table of metformin

SI.No.	Conc [mcg/ml]	*Peak area
1.	4	0.2405
2.	8	0.4305
3.	12	0.5996
4.	16	0.7964
5.	20	0.9915

***Average of three experiments**

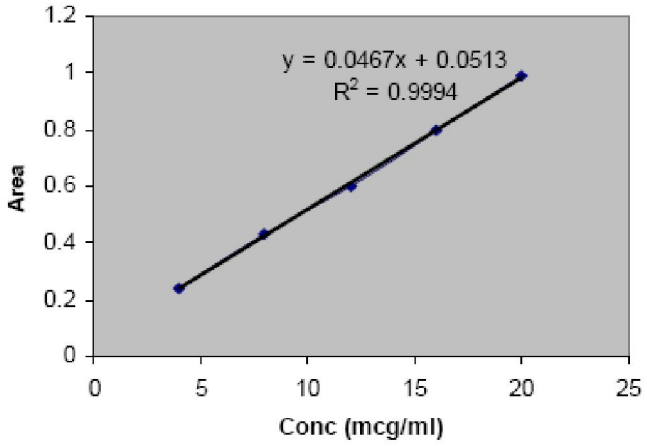

Figure 4 : Validation curve of metformin

Linearity

The linearity of the proposed HPLC method for determination of Metformin was evaluated by analysing a series of different concentrations of standard drug. In this study five concentrations were chosen, ranging between 4-20 µg mL^{-1} of Metformin. Each concentration was repeated three times and obtained information on the variation in the peak area response ratio of the internal standard to pure analytes is presented in TABLE 3. Area is plotted graphically as a function of analyte concentration. The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value. A linear relationship was obtained for Metformin in the range of 4-20 µg mL^{-1}. The absorbance values are recorded in TABLE 3 and represented graphically in Figure 5

TABLE 3 : Linearity table of metformin

SI.NO.	Conc [mcg/ml]	*Peak area
1.	4	0.2085
2.	8	0.3875
3.	12	0.5724
4.	16	0.7675
5.	20	0.9654

***Average of three experiments**

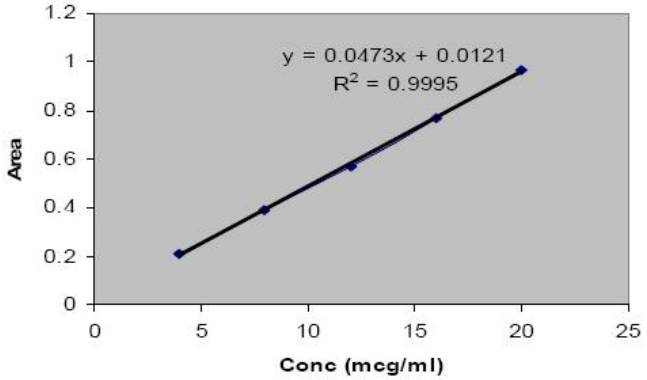

Figure 5 : Linearity curve of metformin

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

The detection limit of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value and the limit of quantitation is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. The values of LOD and LOQ of Metformin are given in TABLE 4.

TABLE 4 : Characteristic parameters of calibration equation for the proposed HPLC method for determination of metformin.

Parameters	HPLC
Calibration range (µg mL-1)	4-20 µg/ml
Detection limit (µg mL-1)	20.895
Quantitation limit (µg mL-1)	63.319
Regression equation (Y)a	0.9995
Slope (b)	0.0473
Intercept (a)	0.0121
Correlation coefficient	14.365

Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay of Metformin tablets was performed in different condition, different analyst, and different dates. As the results in TABLE 5 are within the acceptance limit, the proposed method is found to be rugged.

TABLE 5 : Ruggedness

Parameter	Result observed
Percentage Area	102.24%
SD between set of analysis on same date	0.85
SD between set of analysis on different date	1.65
RSD between set of analysis on same day	0.78%
RSD between set of analysis on different days	1.75%

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions. The robustness of the proposed HPLC method was assessed for peak resolution and symmetric factor. The parameters investigated are:

- Mobile phase organic content (± 3%)
- Mobile phase flow rate (± 0.6 ml min^{-1})

The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust. The absorbance values are recorded in TABLE 6 and represented graphically in Figure 6.

TABLE 6 : Robustness

Sl. No.	Conc.(mcgml)	*Peak area
1	4	0.2080
2	8	0.3870
3	12	0.5722
4	16	0.7175
5	20	0.9454

***Average of three experiments.**

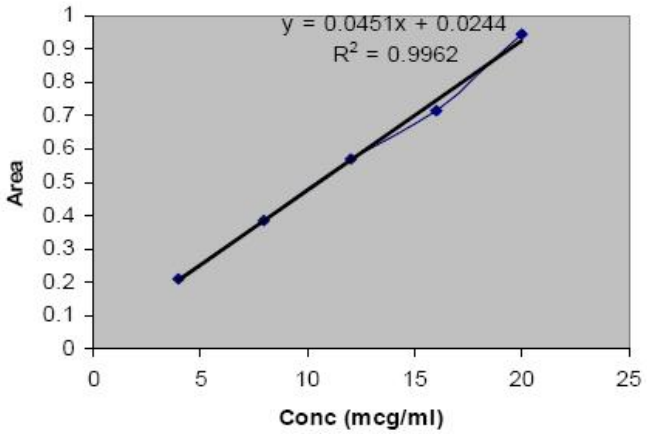

Figure 6 : Robustness curve of metformin.

CONCLUSION

For routine analytical purpose it is desirable to establish method capable of analyzing large numbers of samples in a short time period with good accuracy and precision without any prior separation step. The run time of the HPLC procedure is only two minutes. Good agreement was seen in the assay results of pharmaceutical formulations as well as in laboratory prepared mixtures by developed method. It can be concluded that the proposed method was good approach for obtaining reliable results and were found to be suitable for the routine estimation of metformin in pharmaceutical formulations.

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