The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 3, 2026

SIMULTANEOUS DETERMINATION OF MOXIFLOXACIN AND GLIBENCLAMIDE IN API AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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DOI: https://doi.org/10.5281/zenodo.21713787

Keywords
Moxifloxacin, Glibenclamide, HPLC, method validation

Article History
Received: 19 January 2026
Accepted: 04 March 2026
Published: 18 March 2026

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Abstract

A rapid, cheap, reliable and accurate method has been developed and validated for the simultaneous analysis of Moxifloxacin and Glibenclamide in active and in dosage formulation. Glibenclamide is an antidiabetic drug belonging to the second-generation sulfonylurea. It is indicated for the management of type II diabetes mellitus. Moxifloxacin, an antimicrobial drug belongs to broad spectrum fourth generation flouroquinolone. The simultaneous analysis has been performed using Reverse-Phase High Performance Liquid Chromatography (RP-HPLC). Chromatographic separation was carried out using Purospher star C18 (5 µm, 25 × 0.46 cm) column at room temperature (25± 2 °C) with flow rate of 1 ml /min. The detector was set at 252nm and mobile phase for the experiment was 85:10:5 v/v methanol: water: acetonitrile. The correlation coefficient of both drugs was found to be > 0.990 and 0.999. The lmit of detection (LOD) and limit of quantification (LOQ) was found to be 0.02687 ng/ml, 0.02987 ng/ml and 0.08143 ng/ml, 0.09052ng/ml for moxifloxacin and glibenclamide respectively.

INTRODUCTION

Moxifloxacin (Figure 1) [1 – cyclopropyl -7 {S, S} - 2, 8 –diazabicyclo {4.3.0} non – 8 – y l-6-fluoro – 8 – methoxy – 1, 4 – dihydro – 4 – oxo – 3 - quinolone carboxylic acid hydrochloride] a fluoroquinolne antibiotic of 4th generation equally effective for both gram positive and gram negative microbes [1]. Its mechanism of action includes inhibition of enzyme topoisomerase II and topoisomerase IV. Both of these enzymes are responsible for the synthesis of bacterial DNA [2] .

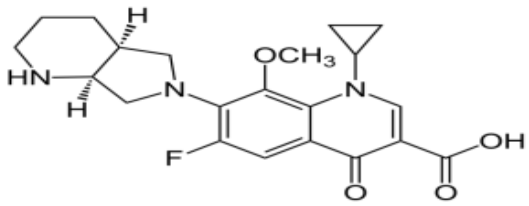

Figure 1 : Moxifloxacin

Glibenclamide also known as glyburide an oral anti diabetic is one of the most commonly used belonging to the second generation sulfonylurea category [3,4]. Structurally (Figure 2), glibenclamide (Euglucon, 1 – {4 – [2 (chloro – 2 - methoxybenzamide) ethyl] – benzene sulphonyl} - 3-cyclo hexyl urea) is a derivative of sulphonyl urea. Glibenclamide is used to treat diabetes which is non-insulin dependent which is of mild to moderate severity. The drug also has an effect on ATP binding cassette where it inhibits many of its activities. In the pancreas it binds to the sulphonyl urea receptors at the beta cells where they lead to the release of insulin by blocking potassium channels which are ATP sensitive [5,3].

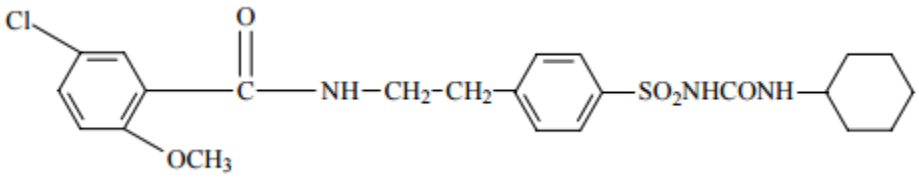

Figure 2: Glibenclamide

Moxifloxacin when given with glibenclamide leads to episode of dysglycemia as moxifloxacin alters the glucose metabolism and can lead to hyperglycemia as well as hypoglycemia. One study even reported that dysglycemia associated with moxifloxacin could be so severe that it can even lead to damage of the brain or even death. Of the other flouroquinolones such as levofloxacin and gatifloxacin, moxifloxacin was found to be causing the most cases of dysglycemia after gatifloxacin and of which hypoglycemia was the most prominent [6]. Numerous determination methods have been employed for the determination of moxifloxacin in pharmaceutical dosage forms and API. These include spectrofluorometric determination and voltammetric determination [7,8]. One method for determining moxifloxacin in drug preparations by RP - High Performance Liquid Chromatography has also been reported by A.P.Dewani et.al [9]. Other assay method used for moxifloxacin determination is UV spectrophotometry [10]. Literature survey reveals that not much work has been done on the determination of glibenclamide alone in dosage forms but a number of analytical methods have been used for the estimation of glibenclamide along with other drugs such as High Performance Liquid Chromatography, reversed phase High Performance Liquid Chromatography, liquid chromatography/tandem mass spectrometry, and liquid chromatographic-electro spray ionization mass spectrometry in dosage forms as well as human plasma [11-15]. Though moxifloxacin has been simultaneously determined with other drugs using a number of analytical techniques. [16-21]. None of the analytical methods have been studied for the simultaneous determination of

moxifloxacin and glibenclamide in drug formulations.

The aim of the present study is to develop a simple, economic, rapid, precise and accurate RP-HPLC method for the simultaneous analysis of Moxifloxacin and Glibenclamide in bulk and in dosage form.

MATERIAL AND METHODS

Instrumentation and analytical conditions:

An HPLC shimadzu system of LC-20AT model, version 1.62used for the analysis equipped with LC-10AT VP pump, Rheodyne manual injector fitted with 20 µL loop and SPD 10AV UV-VIS detector. Column Purospher star C18 having dimensions 5 µm, 25 × 0.46 cm column was utilized for separation. . All analysis was carried out at room temperature (25± 2^0C). The flow rate was set at 1ml/min and detector was set at 254nm.Volulme of injection of solution was 10 µL.

Materials:

Moxifloxacin (Avelox 400 mg) and Glibenclamide (Euglucon 5 mg) both drugs were purchased from local pharmacy of Karachi with expiry of not less than one year. Methanol and ortho phosphoric acid were obtained from RCI Labscan Limited, Darmstadt, Germany. All the reagents used were of analytical grade.

Stock and working solution preparation:

Stock solution of the drugs Moxifloxacin and Glibenclamide were prepared separately by dissolving 100mg of Moxifloxacin and Glibenclamide individually in 100ml volumetric flask using methanol as a diluents. Then further dilutions were prepared from the stock solution in the concentration range of 25-400µg/ml. All solutions were stored in room temperature and 10 µL of the solution was injected into the system for analysis.

Preparation of sample:

To analyze the formulation twenty tablets of each Moxifloxacin and Glibenclamide were weighed and finely grinded to powder. Calculated amount equivalent to 100mg of Moxifloxacin and Glibenclamide were weighed and dissolved in methanol and volume was made to produce 100ml. These solutions were then further diluted to prepare working solutions. Each solution was filtered using filter paper of 0.45um before use.

RESULT AND DISCUSSION

The aim of the work was to design an accurate, precise, robust, simple and convenient method for simultaneous determination of Moxifloxacin and Glibenclamide in bulk and dosage form by RP-HPLC.

Method optimization and chromatographic conditions:

A number of parameters need to be considered to enhance the operating conditions for the detection of analytes using RP-HPLC which includes solvent system composition, pH and flow rate. These parameters were subjected to variation to obtain desirable changes in peak symmetry and other chromatographic parameters. Solvent system for the study was methanol: water, which was run at different ratios (90:10, 80:20, 70:30 v/v). 85:10:5 v/v methanol: water: acetonitrile was found to be most optimal. Sharp peaks were produced when pH of the solvent system was adjusted at pH 3 using ortho phosphoric acid and 1 ml per minute was the set flow rate. The whole operation was carried out at 252 nm. 100 µg per ml of moxifloxacin and glibenclamide were injected individually into the column for their simultaneous quantitation. Retention time was found to be 2.18 min and 4.48 min for MX and GLM respectively.

Wavelength selection:

The common wavelength used for the system was set at 252 nm after recording multiple UV spectra of both the drugs in the wavelength range from 200-400 nm. Thus, 252 nm was found to be most satisfactory allowing sensitive detection of both drugs as shown in figure 3.

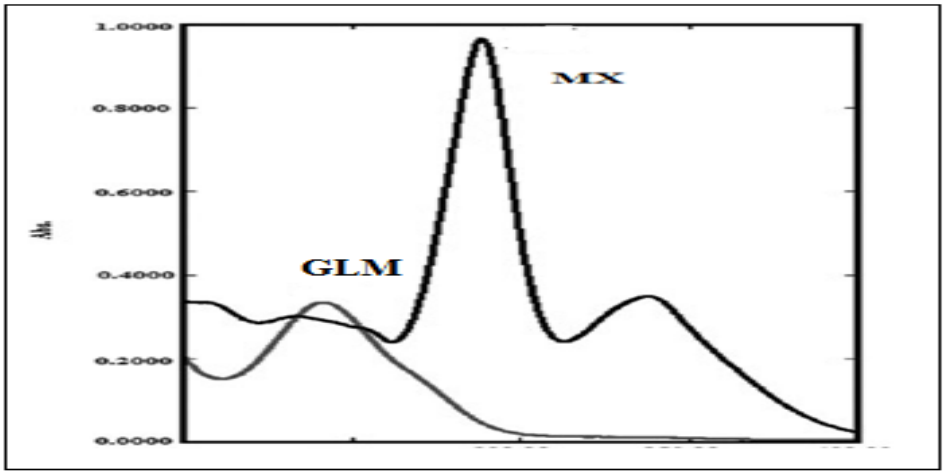

Figure 3: UV spectra for cut point of Moxifloxacin and Glibenclamide

Validation of method:

Developed method has been validated as per guidelines given by ICH (International conference on the Harmonization for the technical requirements of pharmaceuticals for Human use) for assay, accuracy, precision, system suitability, LOD, LOQ, specificity, robustness and ruggedness.

System Suitability:

The aim behind system suitability is to evaluate the degree of agreement between the individual test results. Initially mobile phase composition was used to equilibrate the HPLC system; the same standard was then injected 6 times consecutively to investigate the system suitability every day of method validation process. Capacity factor, theoretical plates of the column, resolution, tailing factor and Retention time are the important parameters to evaluate system suitability as mentioned in Table 1.

Table 1: System suitability of Moxifloxacin and Glibenclamide

Parameters	Moxifloxacin	Glibenclamide
Ret. Time	2.18	4.48
Capacity factor	1.1	1.056
Theoretical Plates	2869.529	2367.227
Tailing factor	1.927	1.289
Resolution	2.66	2.86

Accuracy:

Accuracy of the method developed was obtained by running three different concentrations of the drug solutions (80 %, 100 % and 120 %). Each of the respected concentration solution was injected into the system, a spectrum was obtained and % recovery of each was calculated. Table 2 represents the result of accuracy of moxifloxacin and glibenclamide. Recovery values of both the drugs were found to be in the acceptable range i.e. 98% - 102% when subjected to different concentrations, thus, proving method to be accurate.

Table 2: Accuracy of Moxifloxacin and Glibenclamide

Drugs	Concentration (µg mL^{-1})	RSD (%)	Recovery (%)
Moxifloxacin	80%	0.799	99.99
	100%	0.101	100.15
	120%	1.211	101.00
Glibenclamide	80%	1.881	100.00
	100%	0.561	101.52
	120%	0.112	99.99

Precision:
For our method to be precise that is producing reproducible results, 5 different concentrations of both the drugs were individually injected into system and tested for repeatability and reproducibility. The results obtained were analyzed for inter and intraday variations (Table 3) and it was found that there was no significant difference among the values when tested within a day and between the days.

Table 3: Interday and intraday precision of Moxifloxacin and Glibenclamide

Drugs	Concentration (µg ml^{-1})	Inter-day		Intra-day	
		RSD (%)	Recovery (%)	RSD (%)	Recovery (%)
Moxifloxacin	25	0.00058	99.93	0.00049	100.34
	50	0.00057	100.31	0.01241	100.16
	100	0.00032	100.03	0.18750	99.99
	200	0.00011	99.98	0.00013	100.01
	400	0.00010	100.07	0.00012	99.99
Glibenclamide	25	0.00124	100.13	0.08542	99.99
	50	0.00123	99.98	0.86412	100.00
	100	0.00069	100.19	0.00512	99.99
	200	0.00037	99.99	1.25411	100.01
	400	0.00019	100.08	0.00157	99.99

Specificity and Selectivity:
The above method used is found to be specific as well as specific as the chromatogram obtained (Figure 4) of both the drugs showed no interfering peaks of the excipients and sharp peaks of the drugs were obtained.

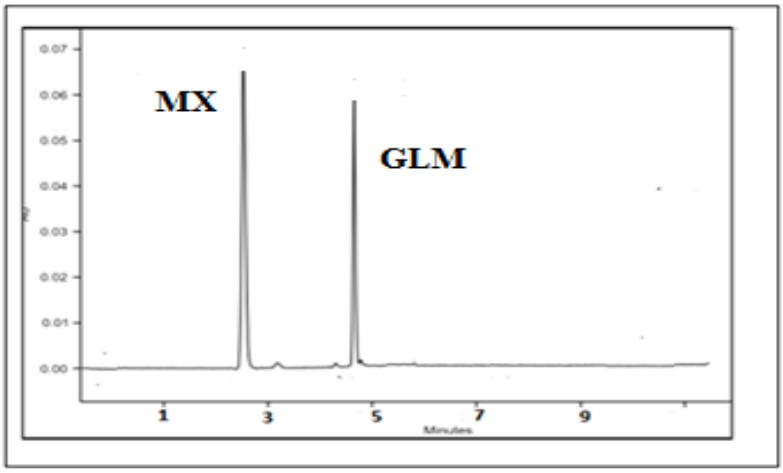

Figure 4: Representative chromatogram of Moxifloxacin and Glibenclamide

Limit of detection (LOD) and Limit of quantization (LOQ):

0.02687 ng per ml, 0.02987 ng per ml are found to be the minimum values that were readily detected, 0.08143 and 0.09052ng/ml are the minimum values quanitified for moxifloxacin and glibenclamide respectively. LOD and LOQ values of moxifloxacin and glibenclamide were calculated and represented in Table 4.

Linearity:

There was a linear relation found to be between different concentrations of moxifloxacin and glibenclamide. Linearity was investigated by analyzing 5 different solutions of moxifloxacin and glibenclamide in the range of 25-400 ng mL^{-1}. Calibration curves were obtained by plotting peak area against different concentrations. Linearity was generated by using linear regression analysis as shown in Table 4. Calibration curve showed excellent linearity with goodness of fit (r^2) > 0.999 as shown in figure 5 and 6.

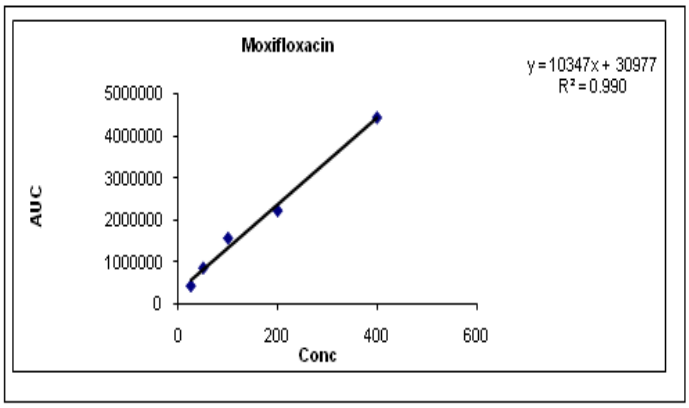

Figure 5: Linearity of Moxifloxacin by HPLC

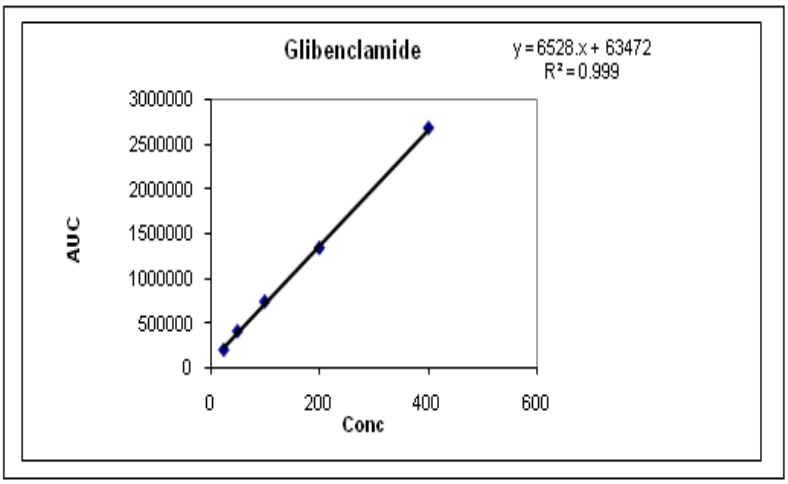

Figure 6: Linearity of Glibenclamide by HPLC

Ruggedness:
To perform the ruggedness of our projected method two analyst in two consecutive days carried out the repeatability in Research lab department of pharmaceutical chemistry, faculty of pharmacy, Jinnah University of women.

Robustness:
To assess the robustness of the analytical method minor but deliberate changes were made in the method parameters i.e. composition of the mobile, flow rate, wavelength and pH of the mobile phase. Results show that %RSD didn't exceed 2% which proves it to be robust.

Table 4: Regression characteristics of Moxifloxacin and Glibenclamide

Drugs	r^2	LOD (ng/ml)	LOQ (ng/ml)	Regression Equation	Concentration (µg mL^{-1})
Moxifloxacin	0.990	0.02687	0.08143	Y = 10347x + 30977	25 – 400
Glibenclamide	0.999	0.02987	0.09052	Y = 6528x + 63472	25 – 400

CONCLUSION
The developed method proves to be of significant importance for routine determination of the moxifloxacin and glibenclamide simultaneously as it is convenient, simple, and rapid. The results proved to be accurate and precise. Sensitivity, good recovery and reliability of the developed method make it a suitable and economical candidate over other methods used for determination.

Funding: None

Conflict of Interest: The authors declare that they have no conflict of interest.

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