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Method Development and Validation of Metformin and Gliclazide in Tablet Dosage Form by Micellar Liquid Chromatography

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ABSTRACT

Micellar Liquid Chromatography (MLC) has emerged as a promising technique for the analysis of pharmaceutical compounds due to its versatility, efficiency, and cost-effectiveness. In this experimental research paper, we explore the application of MLC in the simultaneous analysis of two commonly prescribed antidiabetic drugs, Metformin and Gliclazide. A combination of these medicines is widely used to treat type 2 diabetes mellitus, so accurate quantification of them is essential for efficient therapeutic management. To separate the drug formulation, reverse phase liquid chromatography (RP-HPLC) was employed using surfactant solutions above the Critical Miceller Concentration (CMC). The mobile phase included 0.10M SDS and 10 % Propanol-1, with a pH of 3.50 maintained by o-phosphoric acid, and analysis was done using a Deoxyprobe C18 column (4.6 x 250 mm, 5 µm). The separation was carried out at room temperature with a flow rate of 1.0 ml/min, and detection was performed at 228 nm. Gliclazide has a retention time of 4.46 minutes, compared to 5.57 minutes for Metformin. The percentage composition of the sample under analysis ranged from 90 to 110 percent by Schedule V of the Drug and Cosmetic Act. The suggested practice met ICH requirements. The method was also low-cost, easy to use, secure, and environmentally benign. It can be used to conduct standard quantitative analyses on pills that include Metformin and Gliclazide.

Keywords: Miceller liquid chromatography; Surfactant; RP-HPLC; Critical miceller concentration; Gliclazide; Metformin

1. INTRODUCTION

A popular and easy-to-use subset of high-performance liquid chromatography is Micellar Liquid Chromatography (MLC). MLC is increasingly being used to determine a wide range of chemicals used for pharma research, biological research, the food industry, beverages, and environmental materials[1]. This liquid chromatography has many other advantages over other methods, such as ease of use, low cost, and minimal toxicity[2]. The most significant features of MLC are the ability of isocratic elution to elute chemicals in a range of polarities (neutral and ionic), as well as the possibility of direct infusion of physiological fluids.

MLC is a useful substitute for standard reverse-phase chromatography. It is reverse-phase liquid chromatography, in which micelles are included in the mobile phase along with the surfactant monomers[3, 4]. Surfactants used in MLC can be cationic, anionic, or neutral. The surfactants in MLC behave similarly to those in the stationary phase[5]. It utilises eco-friendly reagents that are safe for the environment and hence meet the principle of green chemistry. In MLC, Sodium Dodecyl Sulfate (SDS), an anionic surfactant, is frequently employed[6]. MLC easily analyses solutes with a wide range of polarities because of its strong elution characteristics[7,8]. Its application is also increasing in the field of bioanalysis of various components, such as serum and urine[9]. Diabetes is a chronic degenerative condition that can have long-term effects on the peripheral and autonomic nervous systems, heart and blood vessels, eyes, and kidneys. In type 2 diabetics, cardiovascular disease leads to the highest mortality, and an increased risk of heart disease and stroke is linked to diabetes. For the treatment of diabetes, two oral medications include Gliclazide and Metformin. To lower blood sugar levels, Gliclazide and Metformin work better together when combined with a healthy diet and exercise. Patients with diabetes type 1 may benefit from it in treating their condition, just as it treats diabetes type 2. Both drugs lower blood sugar levels, although they do it in distinct ways. The creation of insulin in our body is increased by Gliclazide, while the amount of glucose the liver generates is decreased by metformin.

1.1 Metformin Hydrochloride

A biguanide anti-hyperglycemic drug is Metformin. It functions by lowering liver glucose synthesis, raising tissues' sensitivity to insulin, and boosting GDF15 release, which suppresses hunger and calorie intake[10]. Emil Werner and James Bell published the first scientific description

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of Metformin in 1922. In the 1950s, a French doctor named Jean Sterne began conducting human studies. It was first made available as a medicine in France in 1957 and in the US in 1995. The World Health Organisation maintains Metformin, the most popular oral diabetic drug, on its list of essential medicines and makes it accessible as a generic drug. With more than 92 million prescriptions written, it was the third most often prescribed drug in the USA in 2020. Its IUPAC name is *N*, *N*-Dimethylimidodicarbonimidic diamide, and its chemical structure is given in Fig. 1.

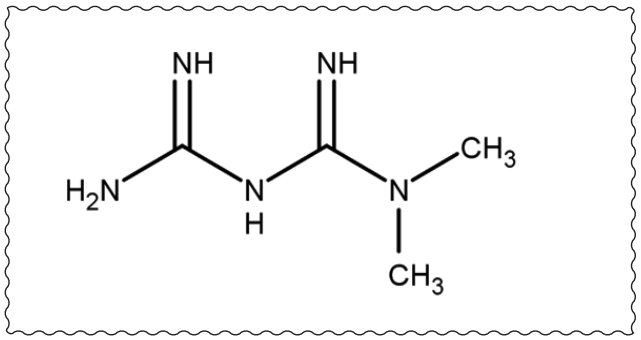

Figure 1. Structure of metformin.

Metformin, a biguanide antidiabetic drug, counters insulin resistance by increasing glucose utilisation and reducing glucose production. Metformin shows therapeutic effects through pathways that are independent of insulin. The exact mechanism through which it benefits glucose metabolism and diabetes-related complications remains unclear[11-12]. Because it is relatively inexpensive, Metformin has drawn attention from scientists due to its potential effects on other illnesses. It has historically operated by both insulin-dependent and non-insulin-dependent mechanisms[13–14].

1.2 GLICLAZIDE

Gliclazide is an oral hypoglycemic medication used to treat Non-Insulin-Dependent Diabetes Mellitus (NIDDM)[15]. Categorisations of Gliclazide vary according to the qualities of the medicine. However, its chemical makeup classifies it as a first-generation sulfonylurea, given its inclusion of an aromatic group and a sulfonamide group capable of releasing a proton. Based on pharmacological efficacy, Gliclazide is considered a second-generation dependent sulfonylurea, displaying increased potency and a shorter half-life. Gliclazide is a member of the sulfonylurea class of insulin secretagogues, which works by inducing the release of insulin from pancreatic beta cells. Sulfonylureas increase the release of insulin in response to meals as well as basal insulin secretion. In this category, drug dosage, absorption rate, duration of effect, elimination route, and binding all vary depending on the receptor of the target pancreatic cell[16-17]. Furthermore, sulfonylureas enhance peripheral glucose uptake, decrease hepatic gluconeogenesis, and potentially increase the quantity and sensitivity of insulin receptors. Sulfonylureas cause weight gain as a side effect, although to a lesser extent than insulin.

Regular food consumption is necessary to reduce the risk of hypoglycemia produced by sulfonylureas due to their mode of action. Gliclazide is demonstrated to lower levels of postprandial blood glucose, fasting plasma glucose, and glycosylated hemoglobin (HbA1c), which is a measure of the past 8–10 weeks of glucose control. The liver extensively metabolizes Gliclazide, which is eliminated as metabolites in urine (60–70 %) and feces (10–20 %). N-(hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4-methylbenzenesulfonamide is the empirical formula for Gliclazide calcium, and its structure is shown in Fig. 2.

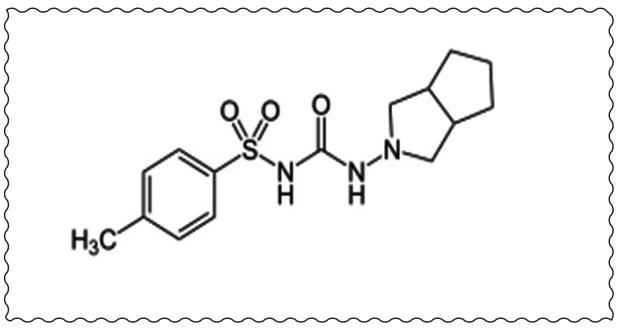

Figure 2. Structure of gliclazide.

Gliclazide may guard against the apoptosis brought on by oxidative stress in pancreatic beta cells. Human cells, both healthy and cancerous, may be protected by Gliclazide from H_2O_2-induced apoptosis. It indicates that the drug's anti-apoptotic action is almost certainly linked to a decrease in oxidative stress.[17] Combination of Gliclazide and Metformin are prominent oral hypoglycemic agents widely used in the management of Type 2 Diabetes Mellitus[18] It was found that Gliclazide with Metformin effectively lowered HbA1C in Indian individuals suffering from type 2 diabetes[19].

Lu *et al.,* reported a 1.4 % reduction in HbA1c after administering Metformin in combination with Gliclazide twice daily or Gliclazide once daily for 20 weeks[20]. When the combined dose of gliclazide + metformin is taken by the patient with HbA1c $\geq$ 7 %, then there is 1.6 % reduction of HbA1c[19-20]. About 80 mg of Gliclazide with 500 mg of Metformin can be taken per day and a maximum dose of Gliclazide 320 mg with Metformin 2000 mg can be taken and after administering the treatment for three months, there is 1.16 % reduction in HbA1c[19-20]. A dose higher than 320 mg of gliclazide + 2000 mg of Metformin per day is toxic and causes hepatic and renal diseases. In clinical practice, Gliclazide and Metformin are frequently used to produce synergistic effects in glycemic management. Research suggests that this combination may increase effectiveness while enabling each medication to be taken at lower levels, thus lowering the frequency of adverse effects. Studies on comparative toxicity indicate that, as compared to monotherapy, the combination does not substantially raise the risk of side effects.

According to a literature review, the pharmaceutical use of a combination of Gliclazide and Metformin is continuously increasing, so a quantitative estimation of these two compounds in their pharmaceutical dosage is

required. RP-HPLC analysis and simultaneous analysis of Gliclazide and Metformin in pharmaceutical combinations have been reported by some researchers[21-23], but MLC analysis has not yet been reported. The objective of this study is to develop and validate an isocratic, quick, sensitive, fast, and simple MLC technique for measuring both Gliclazide and Metformin in tablet formulation.

2. MATERIALS AND METHODS

2.1 Chemical And Reagents

Genclide-80M tablets, whose composition is Gliclazide 80 mg and Metformin 500 mg, manufactured by Infgen Remedies, were procured from the local market. The standard solution of Gliclazide and Metformin used was the Indian Pharmacopeia Reference Standard (IPRS) of Gliclazide and Metformin supplied by the Indian Pharmacopeia Commission. The chemicals and reagents used in the experiment are Sodium Dodecyl Sulfate (SDS), propanol-1, HPLC-grade water, methanol, and o-phosphoric acid.

2.2 Preparation of Stock Solutions of Gliclazide and Metformin

Gliclazide and Metformin reference solutions (1.0 mg/ml) were made separately in methanol. Gliclazide (50 µg ml^{-1}) and Metformin (75 µg ml^{-1}) solutions were combined to create the reference solution by dilution of the stock solution.

2.3 Instruments

The MLC analysis was performed using the Agilent 1260 HPLC with UV detector and C18 column (150 mm x 4.6 mm), 5µm particle size. A flow rate of 1.0 ml/min was used for analysis under isocratic circumstances. All solvents and the mobile phase were sonicated and filtered through 0.45 µm membrane filters to remove any gas before use. All calculations are relevant to the qualitative investigation were completed uniformly by measuring the peak area that is automatically integrated by a computer using DS Chrome Elite software.

2.4 Linearity Study

By examining five standard solutions in the array of 5–25 µg ml^{-1} for Gliclazide and 65–105 µg ml^{-1} for Metformin, the linearity of the proposed approach was confirmed. Injections of solutions were performed three times. Based on calibration curves, the standard concentrations of Gliclazide and Metformin were determined. Additionally, system suitability tests were performed, and they met the requirements for theoretical plate, RSD, tailing factor, and resolution.

2.5 Preparation of Sample Tablets Solution

The average weight was calculated by properly weighing twenty pills, and 20 tablets were then crushed to produce a fine powder. The tablet powder that contained precisely 100 mg of gliclazide and 75 mg of Metformin was dissolved in methanol in a volumetric flask. After that, the mixture was sonicated for 15 minutes with 25 ml of methanol. Then, methanol that had been membrane-filtered was added to dilute the solution to the volumetric flask's mark. Further dilution was done by taking 10.0ml of the above-prepared solution and volume makeup was done to 100 ml by adding methanol, then injected for analysis.

2.6 Accuracy (Recovery Study)

About 1mg of standard Gliclazide was dissolved in 1 ml of methanol, then diluted to 10 ml, and from that solution 8 µL, 10 µL, and 12 µL were injected in HPLC. Similarly, 6mg of Metformin was dissolved in 10ml of methanol, and from that 10 µL, 12.5 µL, and 15 µL. were injected in HPLC. Gliclazide and Metformin standard drug solutions were spiked in a known concentration solution at three distinct concentration levels: 80, 100, and 120 % for each drug to test the method's accuracy.

3. RESULTS AND DISCUSSION

3.1 Method Optimisation

MLC analysis was carried out in isocratic mode in a C18 column with a flow rate of 1 ml/min at 25 °C, and the mobile phase used is orthophosphoric acid (pH = 3.5) with 0.10 M SDS in 10 % Propanol-1. Absorbance was measured at a wavelength of 228 nm. The retention periods for Gliclazide and Metformin during this mobile phase were 4.466 and 5.576 min, respectively. The chromatographs for the test solution of Gliclazide and Metformin tablet powder and the reference Gliclazide and Metformin solution are shown in Figure 3 and 4, respectively.

The test Gliclazide and Metformin powdered tablets were found to have peak areas and retention times that were comparable to those of the standard drug solution taken for analysis. A simple HPLC method using acetonitrile and ammonium formate buffer as the mobile phase was developed and validated for the simultaneous detection of Metformin and Gliclazide[21]. In this method Metformin was detected at r_t 4.1 min and Gliclazide was detected at r_t 6.9 min in isocratic elution using 20 mM ammonium formate buffer (pH 3.5) and acetonitrile (45:55, v/v) as mobile phase. This approach was discovered to be quick, affordable, and reliable and can be applied to routine analyses of the two medications, either together or separately.

Some researchers validated HPLC method for analysis of these medicines in human plasma using acetonitrile, methanol, and sodium dodecyl sulfate as mobile phase in gradient mode, the Lowest Limit of Quantification (LLOQ) for metformin and Gliclazide was found to be 50 µg·mL^{-1} and 49 µg·mL^{-1}, respectively[22]. The peaks of Metformin and Gliclazide appeared at high retention time in this mobile phase. Some researchers developed and validated RP-HPLC technique for Metformin and Gliclazide using methanol and 10mM phosphate buffer (70:30 v/v) as the mobile phase in Kinetics C18 column (250 × 4.6 mm, 5µ)[23]. In this analysis the peaks of

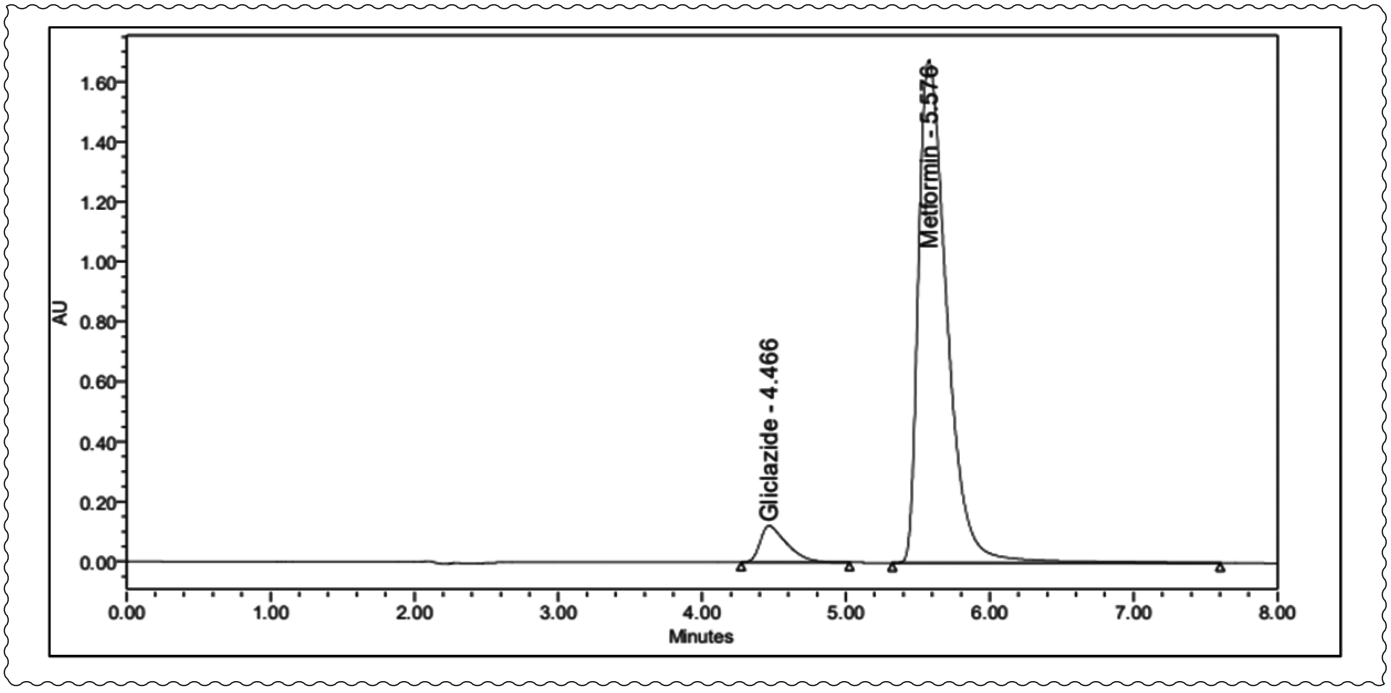

Figure 3. Chromatogram of standard solution of gliclazide and metformin.

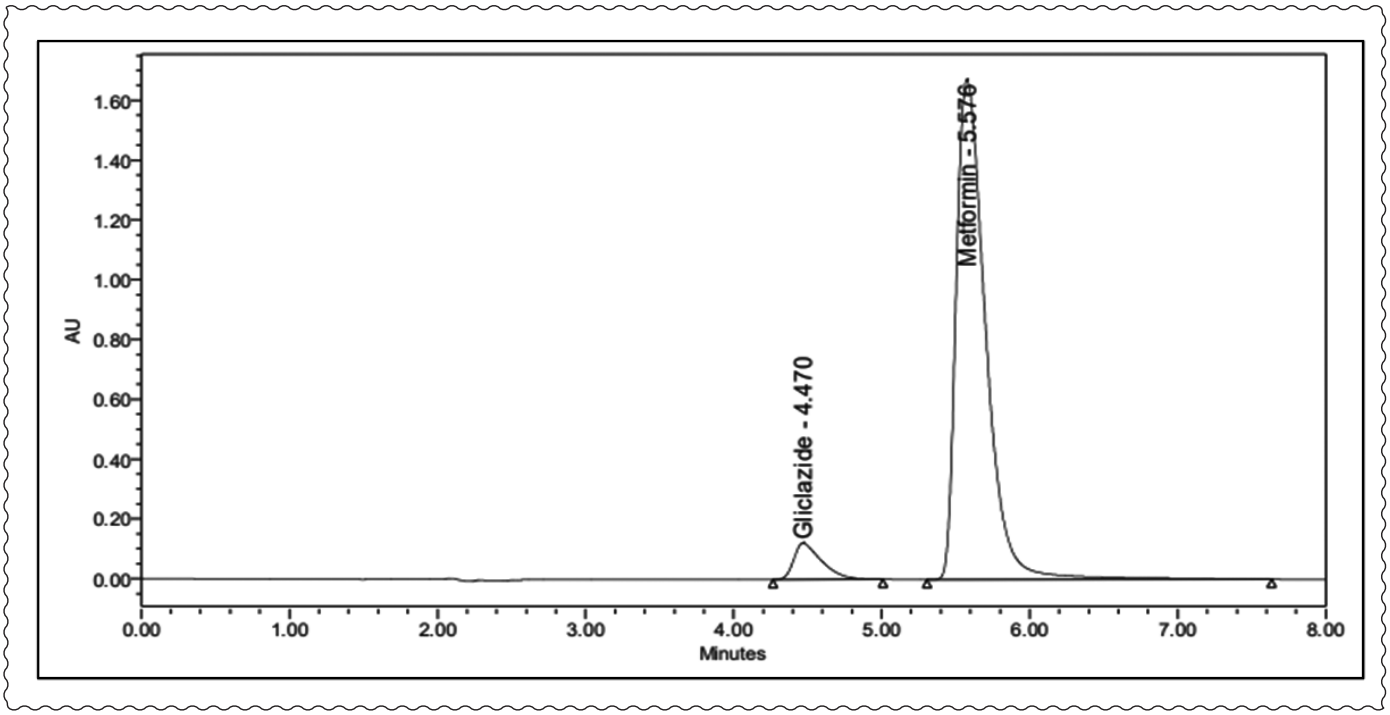

Figure 4. Chromatogram of test solution of gliclazide and metformin.

Metformin and Gliclazide appeared lower retention time 2.2 min and 4.67 min respectively but the limitation of this method is mobile phase is not considered as green solvent.

Shinde *et, al.,* estimated Metformin hydrochloride and Gliclazide quantitatively in their tablet dosage form by RP-HPLC using methanol and water (80:20) as mobile phase and the peaks of metformin and Gliclazide detected at 3.4 min and 5.3 min respectively but the method is not eco-friendly due to use of more volume of methanol[24]. Hossain, *et al.,* developed RP-HPLC method for quantitative estimation of metformin and Gliclazide separately in pharmaceutical formulations using different mobile phase. Metformin was detected at 4.8 min using NaH_2PO_4 (pH-3.0) and CH_3CN (90:10 v/v) and Gliclazide was detected at 6.6 min using same mobile phase at ratio of 20:80 (v/v) [25]. According to ICH Q-R2 guidelines, the above methods were verified, and all of

the validation parameters were confirmed to be within range[26]. Some other researchers estimated Metformin and Gliclazide concentrations using ICH-recommended validation criteria in RP-HPLC using an isocratic mobile phase containing methanol and phosphate buffer[27-28]. This method is sensitive and reliable in nature and, hence, can be successfully applied for the estimation of commercial drugs.

Many researchers used RP-HPLC method for the simultaneous estimation of metformin and Gliclazide as well as a variety of medications as RP-HPLC is a straightforward and quick method for quantitative estimation of pharmaceutical compunds[29-32]. Additionally, numerous studies have demonstrated that RP-HPLC and UHPLC are successful techniques for concurrent quantification of Metformin and Gliclazide in various pharmacological dosage forms[33-34]. HPLC possess several advantages, such as a shorter time requirement and better separation and quantification of various compounds. In the literature, there are several analytical methods such as HPLC, UV-Vis spectrophotometry and gas chromatography, that are used to estimate and quantify Metformin hydrochloride in a single formulation or in a combined formulation with other classes of gliptins in various pharmaceutical drugs[35]. The excellence of the analytical method is ensured throughout the development stage via QbD (Quality by design). The relationship between two parameters, namely pH of the aqueous phase and ratio of acetate buffer and methanol, was studied using Central Composite Design (CCD). Some researchers have analysed metformin in various formulations using the reverse phase HPLC method using isocratic mobile phase and method was satisfactory validated using Central Composite Design (CCD)[36].

Some researchers estimate gliclaside in pharmaceutical dosage forms by RP-UHPLC using an isocratic solvent system, and optimisation was carried out using three factorial designs by utilising the identified Critical Material Attributes (CMA)[37]. The flow rate and medium pH were the key determinants, and their effects on the retention time and peak area were assessed based on the strategy for compound estimation, which is a very sensitive and practical method for gliclaside analysis. Another study used the Box-Behnken design approach to estimate gliclaside by RP-HPLC and it was found that retention time and peak area within the range, which confirmed the robustness of the method validation[38]. Comparing above methods RP-HPLC method with MLC method, the present study suggests that MLC method of separation is preferred over RP-HPLC because it requires less time, a less expensive and non-toxic mobile phase.

3.2 Method of Validation

The optimised method for the simultaneous determination of Gliclaside and Metformin has been validated in compliance with the guidelines set forth by the International Conference of Harmonisation (ICH). This validation includes an assessment of the suitability of the system, specificity, precision, accuracy, linearity, limit of detection, limit of quantitation, and robustness.

3.2.1 Linearity

The analytical procedure is considered linear if test findings are obtained that are exactly proportional to the analyte concentration in the sample, within a specified range. The range of the analytical method is defined as the range between the samples' higher and lower analyte concentrations, indicating an acceptable degree of precision, accuracy, and linearity. To test the method's linearity, five concentrations of 5–25 µg ml^{-1} for Gliclazide and 65–105 µg ml^{-1} for Metformin were utilised. To produce the calibration curve, the response was plotted against the recommended doses of Gliclazide and metformin. The regression obtained for both was 0.9998, which is shown in Fig. 5

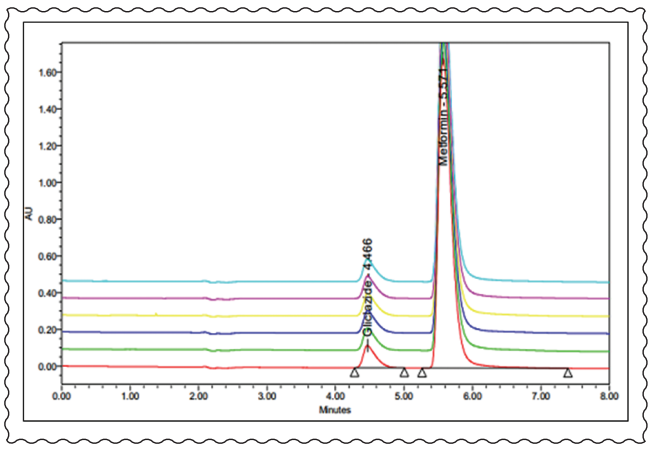

Figure 5. Linearity curve of standard doses of gliclazide and metformin in MLC analysis.

3.2.2 System Suitability Study

The study satisfied the requirements for theoretical plate, RSD, tailing factor, and resolution, according to the results of the system suitability test shown in Table 1. It was observed that the parameters were within the allowed range based on the results of six replicate injections. With an RSD % of the stated retention times of 0.2, Gliclaside and Metformin were consistently retained and distinct at 4.466 and 5.576 min, respectively, indicating strong resolution among both peaks. This suggests that replicate injections during MLC analysis have good reproducibility. The tailing factors of the Gliclazide and Metformin peaks are both less than 2, indicating strong peak symmetry for all of the peaks. It is found that there is adequate column efficacy throughout the intended separation process; the number of theoretical plates in every chromatographic run was consistently more than 2000. Theoretical plate count (N) is calculated from the equation given below.

$N=⟦16(t\_R⁄W)⟧2$

where N= Theoretical Plate count, tR= retention time and W= peak width.

The acceptance limit for RSD % in system suitability and precision results is < 2, which is reflected in Table 1.

Table 1. Systesm appropriateness and precision findings of MLC analysis (RSD % <2)

S. No.	Time of Retention		Tailing Factor		No. of Theoretical Plates		Resolution
	Gliclazide	Metformin	Gliclazide	Metformin	Gliclazide	Metformin	
1	4.470	5.576	1.72	1.64	2570.4	4108.5	3.1
2	4.434	5.496	1.71	1.63	2587.1	4127.2	3.1
3	4.428	5.581	1.71	1.64	2564.9	4119.3	3.2
4	4.491	5.527	1.73	1.65	2578.2	4087.9	3.1
5	4.508	5.601	1.73	1.64	2589.4	4197.1	3.1
6	4.398	5.543	1.72	1.64	2565.0	4210.7	3.1
Mean	4.455	5.554	1.72	1.64	2575.8	4141.78	3.11
SD	0.0418	0.03907	0.008	0.0063	10.7982	50.078	0.041
RSD%	0.939	0.704	0.52	0.386	0.42	1.21	1.31

3.2.3 Accuracy

The degree to which a method's test findings are close to its true value is referred to as the method's accuracy. The proportion of the assay known quantity recovered is used to measure accuracy. Gliclazide and Metformin standard drug solutions were spiked in a known concentration solution at three distinct amounts, such as 80, 100, and 120 % for each drug, to test the method's accuracy. Recovery ranged from 98.0 to 102.0

3.2.4 Precision

Precision is the degree to which measurements taken from different samples of the same homogeneous sample taken under identical conditions agree with one another. Six separate injections of the combination of Gliclazide and Metformin samples yielded peak areas that were reproducible and accurate for 3 days. Results of intraday and interday analysis confirm that the developed approach possesses remarkable repeatability and precision.

Table 2. Recovery results for gliclazide and metformin (Recovery % limit = 98 - 102%)

		Gliclazide		
Sample No.	**Theoretical conc. in µg/ml**	**Observed conc. in µg/ml**	**% of Recovery**	**Statistical Parameters**
S1 80%	64	79.61	99.51	Mean= 99.77 Standard Deviation=0.812 RSD= 0.81%
S2 80%		79.34	99.12	
S3 80%		80.54	100.68	
S1 100%	80	99.22	99.22	Mean= 100.07 Standard Deviation=1.25 RSD=1.25%
S2 100%		101.51	101.51	
S3 100%		99.49	99.49	Mean= 99.38 Standard Deviation=1.00 RSD= 1.01%
S1 120%	96	120.62	100.52	
S2 120%		118.79	98.99	
S3 120%		118.35	98.6	
		Metformin		
S1 80%	400	592.27	98.71	Mean=99.63 Standard Deviation=1.34 RSD=1.35%
S2 80%		594.43	99.00	
S3 80%		607.78	101.17	
S1 100%	500	758.21	101.09	Mean=99.93 Standard Deviation= 1.09 RSD=1.35%
S2 100%		742.07	98.94	
S3 100%		748.11	99.75	
S1 120%	600	884.54	98.28	Mean=99.06 Standard Deviation=1.10 RSD=1.1%
S2 120%		902.88	100.32	
S3 120%		887.12	98.57	

It is observed that all data articulated in the percentage of relative standard deviation is within the recognised limit and not exceeding 0.25 % (recognised limit RSD % <2), as shown in Table 3. The maximum threshold value was set at 2.0 %, with the RSD of the standard and test solutions being 0.6 and 0.8 %, respectively. Different analysts conducted intermediate precision tests on different days. The RSD was measured to be 0.9 %.

3.2.5 LOD and LOQ

The limit of detection was determined by using a low concentration of standard solution. In this case, it was measured to be 1.0 µg. By employing a modest concentration of standard solution, the limit of quantification was established. In this instance, it was measured to be 15 µg ml^{-1} for Metformin and 5 µg $ml^{-1}$for Gliclazide.

Table 3. Precision findings as the peak area of several analytes on three separate days (Gliclazide 80 µg ml^{-1}, Metaformin 500 µg ml^{-1}) (n=6), acceptance limit RSD % <2)

	1st Day		2nd Day		3rd Day	
	Gliclazide	Metformin	Gliclazide	Metformin	Gliclazide	Metformin
1	1567243	23130132	1609876	22190872	1526090	21281194
2	1570989	23098441	1600835	22109865	1529038	21250089
3	1565265	23289868	1599807	22017609	1528859	21399722
4	1556802	23180984	1610028	22098736	1549391	21279873
5	1580891	23109099	1601980	22098782	1528539	21188308
6	1569798	23198096	1617893	22189012	1530399	21297127
Mean	156849.8	2316777.0	160673.6	22117479.3	1532052.7	21282718.8
Standar Deviation	7877.8837	71648.6053	7079.9419	65171.11	8608.59	69117.10
RSD%	0.502	0.309	0.441	0.295	0.56	0.325

Table 4. Analysis of metformin and gliclazide's robustness (acceptance limit RSD % <2)

Parameters	Retention Time		Tailing Factor		Theoretical Plates		Resolution
Flow rate (ml/ min)	Gliclazide	Metformin	Gliclazide	Metformin	Gliclazide	Metformin	
0.9	4.412	5.611	1.71	1.64	2586.9	4138.2	3.1
1.0	4.470	5.576	1.72	1.64	2570.4	4108.5	3.1
1.1	4.481	5.486	1.72	1.65	2568.1	4128.1	3.1
Mean	4.454	5.557	172	1.64	2575.1	4124.9	3.1
Standard Deviation	0.037	0.064	0.0058	0.0058	10.2549	15.1011	0.0
RSD%	0.87	1.160	0.34	0.35	0.398	0.37	0.0
SDS 0.12M	4.397	5.424	1.72	1.64	2566.5	4152.9	3.2
SDS 0.10M	4.470	5.576	1.72	1.64	2570.4	4108.5	3.1
SDS 0.14M	4.495	5.501	1.72	1.65	2591.8	4087.8	3.1
Mean	4.454	5.500	1.72	1.64	2576.2	4116.4	3.1
Standard Deviation	0.051	0.076	0.0058	0.0058	13.62	33.2612	0.058
RSD%	1.143	1.382	0.34	0.35	0.53	0.81	1.84
pH 3.45	4.411	5.601	1.71	1.65	2591.9	4139.9	3.1
pH 3.50	4.470	5.576	1.72	1.64	2570.4	4108.5	3.1
pH 3.55	4.501	5.511	1.72	1.64	2581.7	4120.4	3.2
Mean	4.460	5.562	1.54	1.64	2581.3	4122.9	3.1
Standard Deviation	0.457	0.046	0.0058	0.0058	10.7546	15.8525	0.058
RSD%	1.025	0.835	0.34	0.35	0.42	0.38	1.84

3.2.6 Range and Specificity

The range of an analytical technique is the variation between the higher and lower amounts of analytes in the standard solution for which the investigative technique possesses a sufficient level of precision, accuracy, and linearity. It has been thoroughly observed that this MLC analysis has a sufficient level of exactness, accuracy, and linearity within the range of 80–120 %. At different ranges, the RSD was estimated to be 0.4, 0.8, and 1.1 %. Specificity is the ability to reliably access the analyte in the presence of potential co-existing components. In this approach, where Metformin and Gliclazide were found to be between 80 and 120 %, none of the excipients interfered with the Gliclazide and Metformin peak or the outcome.

3.2.7 Robustness

The robustness of the procedure was confirmed by injecting the standard and sample solution on multiple days in Table 4. The robustness of the procedure is indicated by the high degree of reproducibility of detection response and retention time.

The method is resilient to minor intentional adjustments made to the flow rate, the buffer's pH, or the variable concentration of the mobile phase because no substantial changes were found after making minor adjustments to the chromatographic conditions. The peaks of Gliclazide and Metformin retention times were reported to be consistently symmetric (tailing factor 2), well-separated (resolution > 2), and with standard deviations of 0.1, which indicates the analytical method's tolerance to slight variations.

4. CONCLUSIONS

Gliclazide and Metformin can be determined using micellar liquid chromatography in the pharmaceutical business. The analysis was completed quickly and accurately according to the MLC approach. MLC can be used to analyse a variety of medications, including antipyretic and anti-cardiac ones. Gliclazide and Metformin in dose formulation can be assayed (quantitatively analysed) using the established method. This technique was determined to be legitimate. It can be used by ICH guidelines regularly for quality control of Gliclazide and Metformin.

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