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QUANTITATIVE ANALYSIS OF METFORMIN HYDROCHLORIDE AND DAPAGLIFLOZIN USING HPLC: METHOD VALIDATION AND APPLICATION IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT
This study presents a validated method for the quantitative analysis of dapagliflozin and metformin hydrochloride (MET), two pivotal medications in the management of type 2 diabetes mellitus. High-Performance Liquid Chromatography (HPLC) equipped with a C18 column and a mobile phase comprising acetonitrile and potassium dihydrogen phosphate buffer solution was employed. Method validation encompassed the establishment of linear calibration curves, determination of accuracy, and calculation of limits of detection (LOD) and quantification (LOQ). Excellent linearity, as indicated by regression coefficients of 0.9998 for dapagliflozin and 0.9997 for metformin hydrochloride, underscores the reliability of the analytical approach. Accuracy assessments yielded results within the acceptable range, affirming the precision of the method. Practical application in pharmaceutical quality control laboratories was demonstrated through the analysis of both pure doses and pharmaceutical preparations, highlighting the method's utility in real-world settings. While the study offers a robust methodology, further exploration of potential limitations and method robustness factors would enrich its contribution to the field.

Keywords: Method Validation, Quality Control, Pharmaceutical Preparation

INTRODUCTION
Dapagliflozin and metformin hydrochloride are key medications used to manage type 2 diabetes. Dapagliflozin, an SGLT2 inhibitor, helps lower blood sugar by promoting glucose excretion through urine, and it also aids in weight loss and blood pressure reduction. Metformin hydrochloride, a biguanide, decreases glucose production in the liver and enhances insulin sensitivity, making it a staple in diabetes treatment without causing weight gain. These medications are crucial in controlling blood sugar levels and minimizing complications, offering significant benefits for those living with type 2 diabetes.

Diabetes is a metabolic disorder caused by the body's inability to produce or respond to insulin, resulting in disrupted blood glucose levels. It is classified into two main types: Insulin-dependent diabetes mellitus (IDDM) and Non-insulin-dependent diabetes mellitus (NIDDM). Treatment strategies vary depending on the type of diabetes, ranging from insulin administration to lifestyle modifications, oral anti-diabetic medications, and dietary control. In 2015, an estimated 415 million people were living with diabetes mellitus, with urban areas having the highest concentration. South Asia, including Mauritius, faces a significant burden of undetected diabetes cases and associated deaths. The Middle East's diabetes prevalence is expected to rise significantly by 2040, further exacerbating the public health challenge. Oral hypoglycemic drugs play a crucial role in regulating blood glucose levels, offering a less invasive alternative to insulin injections. The escalating prevalence underscores the urgent need for effective diabetes management strategies and therapeutic interventions.

Pharmaceutical medicines, containing synthetic chemical structures, are essential for managing, preventing, and alleviating symptoms of various diseases. With advancements in technology, researchers have developed synthetic drug manufacturing methods, emphasizing the importance of drug quality. The World Health Organization (WHO) urges governments to develop national drug policies addressing affordability, quality, and accessibility. Pharmaceutical analysis is crucial for ensuring drug quality, involving quantitative and qualitative assessments of raw materials, active pharmaceutical ingredients, and excipients. Regulatory bodies like the Drug Regulatory Authority of Pakistan and provincial government agencies enforce stringent quality control measures to combat substandard drug products. Newly developed medications often lack nationally and internationally accepted standard analytical procedures, necessitating the development of validated methodologies to identify and quantify these medications effectively. Dapagliflozin is a sodium-glucose transporter inhibitor used to manage glycemic blood levels in adults with type 2 diabetes. It prevents glucose reabsorption in the nephron's superficial tubule, causing glycosuria and reduced blood glucose levels. FDA approval was granted in 2014, making it a crucial anti-diabetic medication.

Dapagliflozin is often combined with insulin, thiazolidinediones, and sulfonylureas for type 2 diabetic patients unresponsive to individual treatments. However, women using dapagliflozin may experience higher rates of sexual mycotic infections compared to the placebo. The drug's mechanism of action involves glucose excretion in urine, leading to reduced blood glucose levels and osmotic diuresis, contributing to blood pressure and body weight reduction. Metformin Hydrochloride, an oral anti-hyperglycemic medicine, is often used in combination therapies. However, caution is advised in patients with cardiovascular disease due to potential increased cardiovascular risk associated with certain medications. The administration of metformin Hydrochloride, a molecular class III drug, typically occurs two to three times daily, with a half-life ranging from 1.5 to 4.5 hours. While metformin Hydrochloride effectively reduces hepatic glucose production and improves insulin sensitivity, it may induce adverse effects such as lactic acidosis, gastrointestinal symptoms, and skin allergic reactions. Notably, women using metformin Hydrochloride may experience sexual organ mycotic infections at higher rates compared to placebo.

The primary objective of anti-diabetes drugs is to regulate or lower blood glucose levels in hyperglycemic conditions, aiming to restore patients to a normal physiological state. Both traditional and allopathic treatments stimulate pancreatic islet cells to produce insulin naturally, thereby reducing blood sugar levels. These medications also inhibit hormones that elevate blood glucose levels and enhance the sensitivity of insulin receptors, thereby decreasing glycogen release and increasing glucose utilization in various tissues and organs. Sulfonylureas, a common class of oral anti-diabetics, exert their action by stimulating pancreatic Langerhans islets to produce insulin. By binding to sulfonylurea receptors on islet cells, they induce depolarization of the cell membrane, facilitating the release of stored insulin. Conversely, biguanides, another class of anti-diabetic drugs, restore insulin sensitivity in peripheral tissues and reduce hepatic gluconeogenesis, leading to enhanced insulin-stimulated glucose uptake and utilization. However, biguanides are ineffective in the absence of insulin. Furthermore, SGLT-2 inhibitors like dapagliflozin, while offering promising results in

diabetes mellitus treatment, have been associated with various side effects beyond blood glucose management. These medications, including canagliflozin, empagliflozin, and dapagliflozin, have been linked to increased risks of cancer, genital and urinary tract infections, and diabetic ketoacidosis, as evidenced by post-marketing studies. Despite their efficacy in controlling blood glucose levels, the potential risks associated with SGLT-2 inhibitors warrant careful consideration in clinical practice.

The growing prevalence of diabetes mellitus presents a significant challenge to global healthcare systems, necessitating the continual improvement of treatment strategies and analytical methods to ensure optimal patient care. In this context, the development and validation of precise and cost-effective analytical techniques for the quantitative analysis of anti-diabetic medications have garnered considerable attention. This literature review delves into various studies aimed at refining analytical methods for assessing medications like Dapagliflozin and Metformin Hydrochloride. By examining these studies, this review seeks to identify advancements, challenges, and opportunities in pharmaceutical analysis, ultimately contributing to the enhancement of diabetic therapy and patient outcomes. Debata et al. [1] established a highly sensitive HPLC method for dapagliflozin analysis, adhering to ICH standards. Vankalapati et al. [2] expanded this analysis to include metformin Hydrochloride (MET), dapagliflozin (DAPA), employing a Kromasil C18 column. Meanwhile, Mohsin Kazi et al. [3] introduced a rapid HPLC method for sitagliptin and dapagliflozin. Donepudi et al. [4] devised a rapid RP-HPLC approach for dapagliflozin and empagliflozin. Vinutha Kommineni et al. [5] developed a UV spectrophotometric method for setagliptin and dapagliflozin. Maruthi et al. [6] isolated dapagliflozin using RP-UFLC. Rafaela Zielinski Cavalheiro de Meira et al. [7] proposed a spectrophotometric method for dapagliflozin. Kumar et al. [8] suggested a precise RP-HPLC method for metformin and empagliflozin. Zhang et al. [9] underscored dapagliflozin's effectiveness and safety. Rizk et al. [12] explored electrochemical detection of dapagliflozin. Patel et al. [13] emphasized the utilization of herbal remedies for diabetes management. Gahlan et al. [14] and Sunkara et al. [17] focused on cost-effective electrode modification and chromatographic methods. Xie et al. [18] utilized UPLC-MS/MS for sitagliptin and ertugliflozin detection. Finally, Sohrabi et al. [19] proposed a colorimetric method for sitagliptin and metformin Hydrochloride detection using gold nanoparticles. These studies collectively contribute to advancing analytical methodologies for anti-diabetic medications, facilitating precise drug quantification and ensuring better patient care. The study by Padmaja et al. [15] introduced a distinctive UV spectrophotometric method for the simultaneous quantification of Empagliflozin and Dapagliflozin, emphasizing its cost-effectiveness and accuracy. Ayoub et al. [16] employed LC-MS/MS for the detection of Empagliflozin and Metformin, ensuring precise validation and quality control of Synjardy tablets. Additionally, the innovative approach by Gahlan et al. [14] demonstrated the modification of pencil graphite electrodes with copper microparticles, enhancing sensitivity for detecting Dapagliflozin. Meanwhile, Sunkara et al. [17] presented a comprehensive RP-HPLC method for Ertugliflozin and Metformin, emphasizing its practicality and reliability for routine quality control tests. Xie et al. [18] introduced UPLC-MS/MS spectrometry for the simultaneous measurement of Sitagliptin and Ertugliflozin in rat plasma, showcasing its utility in pharmacokinetic studies. Furthermore, the colorimetric method developed by Sohrabi et al. [19] utilizing gold nanoparticles offered a rapid and simple approach for the simultaneous detection of Metformin Hydrochloride and Sitagliptin. Additionally, the studies by Fioretto et al. [11], Rizk et al. [12], and Patel et al. [13] provided valuable insights into the efficacy, safety, and alternative remedies for diabetes management, thus broadening the scope of diabetic treatment strategies. These diverse methodologies collectively underscore the continuous efforts and advancements in pharmaceutical analysis, ultimately aiming to enhance patient care and treatment outcomes in diabetic therapy.

This study aimed to develop and validate a cost-effective and efficient HPLC method for the analysis of Dapagliflozin and Metformin Hydrochloride. The objective was to address the need for a reliable analytical technique that could accurately quantify these pharmaceutical substances in dosage forms. By studying the potential overlapping effects of other raw materials with the active ingredients, the research sought to ensure the specificity and accuracy of the developed method. Moreover, the

application of the validated protocols aimed to facilitate the analysis of Dapagliflozin and Metformin Hydrochloride in dosage forms, thereby reducing the daily pill burden for patients undergoing diabetic therapy. By streamlining the analytical process and potentially minimizing the number of pills required, the study aimed to enhance patient adherence to treatment regimens, ultimately maximizing the therapeutic benefits of these medications. This research was driven by the pressing need to optimize pharmaceutical analysis techniques, with a focus on improving patient outcomes and treatment adherence in diabetic therapy.

MATERIALS AND METHODS

Instrumentation utilized in the analysis included Shimadzu's LC-2030C series of Liquid Chromatography, comprising a column oven, auto-sampler, PDA detector, and quaternary low-pressure gradient pump. A column with specifications of 4.6 mm x 250 mm, L1, 5-micron ODS C18 was consistently used for separations, with chromatographic data recorded using laboratory solution software. The degassing process was facilitated by Elma Sonics' Model E60H sonicator, while chemical substances were accurately measured using Shimadzu's analytical balance ATX 224.

Required Chemicals and Reagents

Throughout the entire analysis, analytical-grade chemicals were meticulously employed. These included working standards of Dapagliflozin and Metformin Hydrochloride, potassium dihydrogen phosphate, acetonitrile, orthophosphoric acid, and distilled water. These chemicals were sourced from reputable suppliers such as Merck (Darmstadt, Germany) and Ferozsons Laboratories (Nowshera, Pakistan), ensuring the highest quality and reliability in the experimental procedures.

Required Chromatographic Conditions

Separations were carried out using a conventional column (4.6 mm x 250 mm; 5 µm packing ODS C18), with temperature control maintained at 30°C using a column oven. The mobile phase comprised a mixture of acetonitrile and a potassium dihydrogen phosphate buffer solution with a pH of 4.5, flowing at a rate of 1.0 mL/min. Detection of dapagliflozin and metformin Hydrochloride was achieved using a PDA detector at wavelengths of 223 nm and 233 nm, respectively. Injection volumes of 20 µL were utilized to optimize chromatographic conditions.

Preparation of Buffer Potassium Dihydrogen Phosphate Ph 4.5

Potassium dihydrogen phosphate was accurately dissolved in deionized water, with the pH adjusted to 4.5 using diluted phosphoric acid, ensuring the precise composition of the buffer solution.

Mobile Phase Preparation

Acetonitrile and buffer solution of pH 4.5 were meticulously mixed in a 50:50 ratio to prepare the required mobile phase. The mixture was then filtered and degassed to eliminate any impurities or gas bubbles, maintaining the stability and consistency of the mobile phase.

Preparation of Diluents

Diluents for the standards and sample solutions were prepared by mixing acetonitrile and buffer solution of pH 4.5 in a 50:50 ratio, ensuring uniformity and compatibility with the chromatographic system.

Standard and Sample Preparation

Standard stock solutions of Dapagliflozin and Metformin Hydrochloride were meticulously prepared by weighing and transferring the respective working standards into volumetric flasks, followed by sonication and dilution with mobile phase to achieve desired concentrations. Standard working solutions were then prepared by pipetting appropriate volumes of the stock solutions and diluting to volume with mobile phase. Similarly, stock samples were prepared by weighing and averaging the

weight of 20 tablets, followed by dissolution in mobile phase and sonication. Sample solutions were then prepared by pipetting appropriate volumes of the stock sample solutions and diluting to volume with mobile phase.

System Suitability

System suitability tests were conducted to ensure compliance with the requirements outlined in the SOP for "Good Laboratory Practices", with parameters including RSD and tailing factor not exceeding 2.0.

RESULTS AND DISCUSSIONS

HPLC methods were used for the analysis, requiring careful fine-tuning to ensure optimal separation and detection of dapagliflozin and metformin hydrochloride. Adjustments were made to the mobile phase composition, flow rate, temperature, and wavelengths for detection. The chosen wavelengths, 233 nm for metformin hydrochloride and 223 nm for dapagliflozin, were selected to maximize sensitivity and minimize interference. Through systematic experimentation, a mobile phase flow rate of 1.0 mL/min was found to be ideal after testing rates from 0.9 to 1.1 mL/min. The mobile phase, composed of a 650:350 ratio of buffer to acetonitrile, consistently produced well-resolved chromatograms for both compounds. Importantly, variations in flow rate within the tested range did not affect peak resolution, highlighting the robustness of the chromatographic method. Chromatographic analysis revealed complete resolution of the metformin hydrochloride and dapagliflozin mixture within a runtime of 12 minutes, with retention times of 3.4 and 4.9 minutes, respectively. This efficient separation of the target compounds is depicted in *Figure 1*, illustrating the chromatogram obtained using the optimized mobile phase composition and flow rate. Acetonitrile was chosen as the solvent for the mobile phase due to its high elution strength, low viscosity, and excellent compatibility with the C18 column, which together ensure efficient separation of dapagliflozin and metformin hydrochloride. Its UV transparency allows for accurate detection at the specified wavelengths, minimizing background interference. Additionally, the good solubility of both drugs in acetonitrile ensures consistent and reliable results throughout the HPLC process.

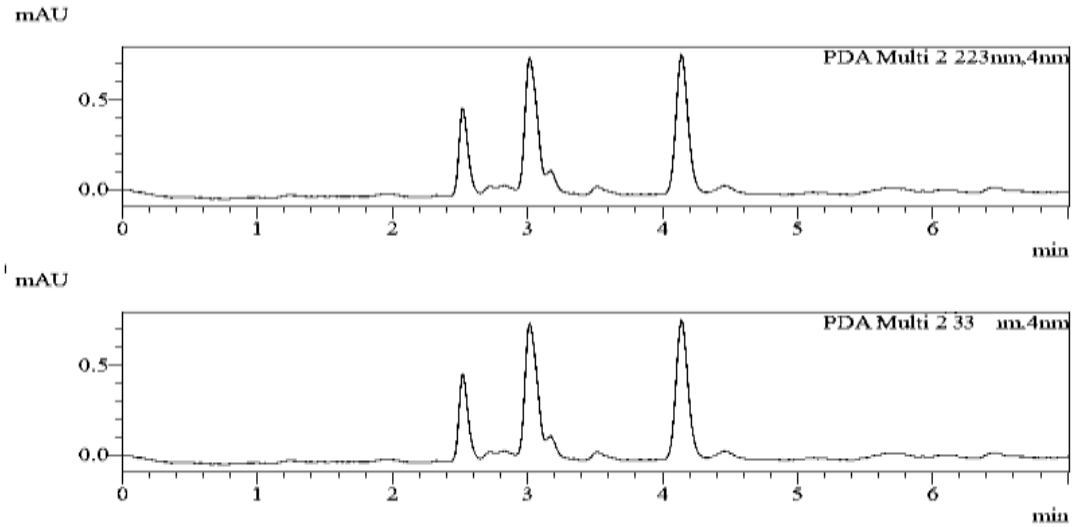

Figure 1 – Chromatogram of the mobile phase used in analysis of MET and dapagliflozin

The successful optimization of HPLC parameters underscores the efficacy of the methodology in accurately quantifying dapagliflozin and metformin hydrochloride in pharmaceutical formulations. The precise control of experimental variables ensures reproducibility and reliability, facilitating the generation of meaningful analytical data essential for pharmaceutical quality control and pharmacokinetic studies. These findings contribute to the advancement of analytical techniques in

pharmaceutical research and underscore the importance of method validation in ensuring the integrity and accuracy of analytical results. The chromatogram obtained from the analysis of bulk powder containing metformin hydrochloride and dapagliflozin provided valuable insights into the composition and purity of the bulk powder sample, further enhancing our understanding of the pharmaceutical formulation. *Figure 2* and *Figure 3* illustrates the absorbance signals of metformin hydrochloride and dapagliflozin at their respective wavelengths of 233 nm and 223 nm. These signals serve as a visual representation of the compounds' concentration levels, validating the effectiveness of our selected detection wavelengths in accurately quantifying the target analytes. These figures complement our discussion by providing visual evidence of the chromatographic separation and detection of metformin hydrochloride and dapagliflozin, thereby bolstering the robustness and reliability of our analytical methodology.

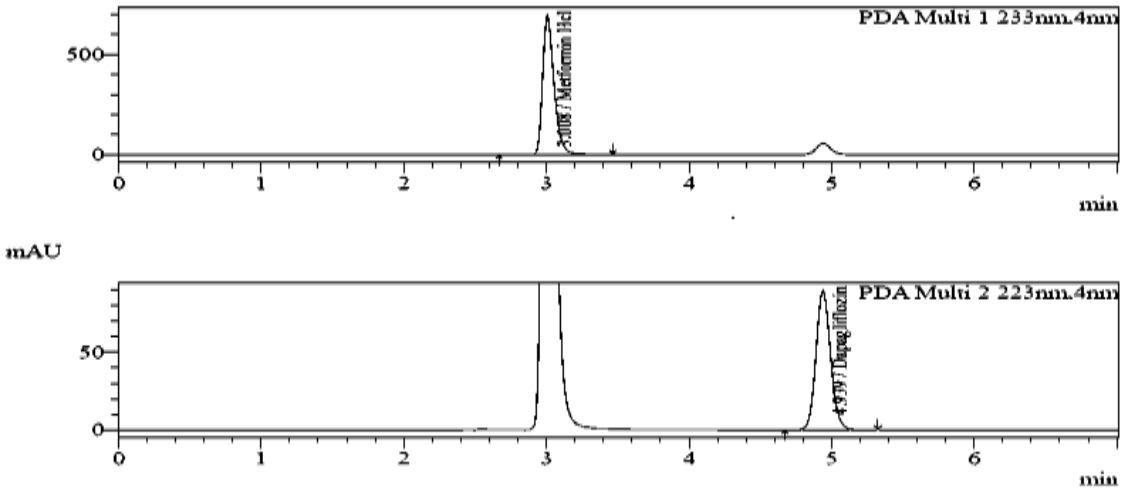

Figure 2 – Absorbance signals of metformin hydrochloride at of 233nm and 223nm respectively

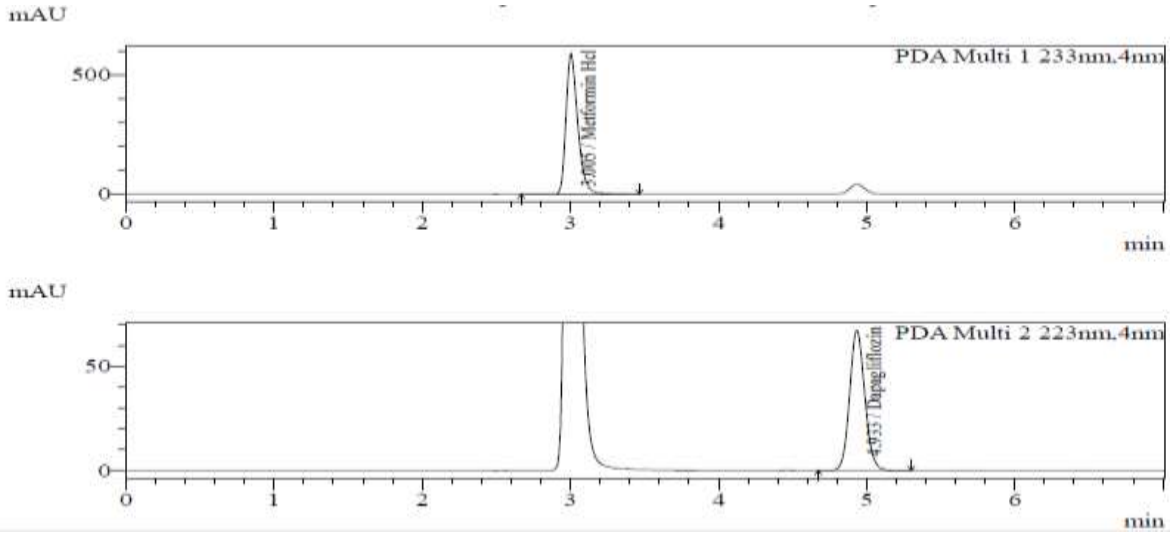

Figure 3 – Absorbance signals of metformin dapagliflozin at of 233nm and 223nm respectively

Validation

We assessed the linearity of our analytical method according to the International Conference on Harmonization (ICH) guidelines. Six distinct concentrations spanning from 50% to 150% of the predicted working range for both metformin hydrochloride and dapagliflozin were examined to establish linearity. The results demonstrated a consistent linear increase in peak height with increasing concentration, as depicted in *Table 1* and illustrated in *Figure 4*, affirming the method's linearity for metformin hydrochloride. Similarly, for dapagliflozin, linearity was investigated across the same

concentration range, with *Table 2* showcasing the corresponding increase in absorbance with concentration.

Table 1 – Linearity data of MET

Dilution Factor *(ppm)*	**Conc of Solution** *(mg/mL)*	**Area / Abs**
0.80	40.080	1992851
1.24	62.124	3293589
1.68	84.17	4747643
2.12	106.212	6043170
2.56	128.256	7394790
	R²	0.9997
	Y-intercept	-480548.473

Table 2: Linearity data of dapagliflozin

Dilution Factor (ppm)	**Conc of Solution (mg/mL)**	**Area / Abs**
0.50	12.60	322704
0.75	18.90	475737
1.00	25.20	646719
1.25	31.50	801793
1.50	37.80	963011
	R²	0.9997
	Y-intercept	-675.200

Analysis confirmed the linearity of dapagliflozin, as depicted in *Figure 5*. The linear increase in absorbance observed for metformin hydrochloride and dapagliflozin as their concentrations range from 50% to 150%. These findings underscore the method's ability to reliably quantify both analytes over a wide concentration range, essential for accurate pharmaceutical analysis.

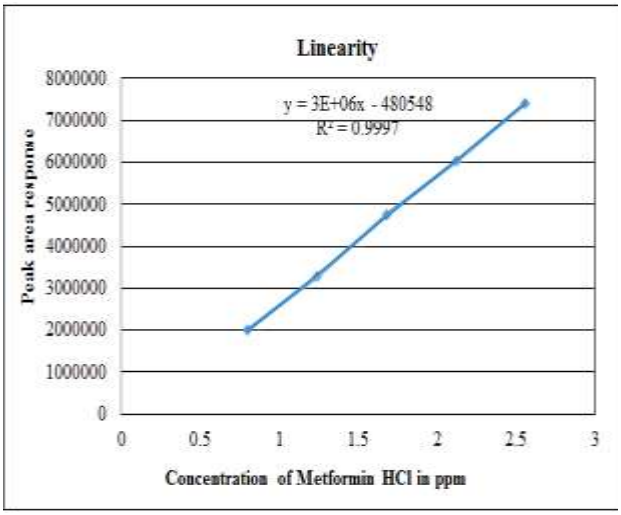

Figure 4 – Linearity Confirmation of MET

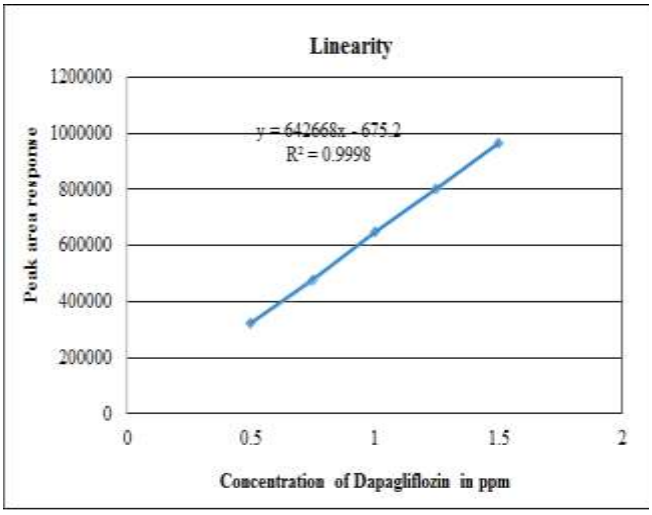

Figure 5 – Linearity Confirmation of dapagliflozin

Accuracy

We scrutinized the accuracy and precision of our method to ensure reliable results. Accuracy was evaluated by spiking known quantities of dapagliflozin and metformin hydrochloride into powdered placebo, followed by sample and standard solution preparation. The percent recoveries were calculated using the formula provided, with *Tables 3* and *Tables 4* illustrating the accuracy of the data and *Figures 6* and *Figures 7* presenting histograms of accuracy. Acceptance criteria stipulated that percentage recovery should fall within ±2%.

Table 3 – Accuracy data for metformin hydrochloride

Theoretical value *(%)*	**Quantity Added** *(mg)*	**Area / Abs**	**Quantity Observed** (*mg)*	**Recovery** *(%)*	**Mean Recovery**
50%	484.5	2142069	491.5394632	101.5	101.3
	482.2	2130437	488.8702742	101.4	
	483.0	2127516	488.1999939	101.1	
100%	950.6	4202896	964.4363668	101.5	101.4
	950.0	4195616	962.7658290	101.3	
	951.2	4208624	965.7507680	101.5	
150%	1425.3	6162561	1414.1196790	99.2	99.4
	1425.6	6178411	1417.7567700	99.4	
	1425.1	6172364	1416.3691680	99.4	

Table 4 – Accuracy data for dapagliflozin

Theoretical value *(%)*	**Quantity Added** *(mg)*	**Area / Abs**	**Quantity Observed** (*mg)*	**Recovery** *(%)*	**Mean Recovery**
50%	484.4	309827	476.2097841	98.3	98.6
	482.2	309873	476.280487	98.8	
	483.0	309916	476.3465788	98.6	
100%	950.6	625439	961.3112194	101.1	101.2
	950.0	628988	966.7660975	101.8	
	951.2	623649	958.5599566	100.8	
150%	1425.3	937954	1441.652509	101.1	100.7
	1425.6	931785	1432.170643	100.5	
	1425.1	930570	1430.303165	100.4	

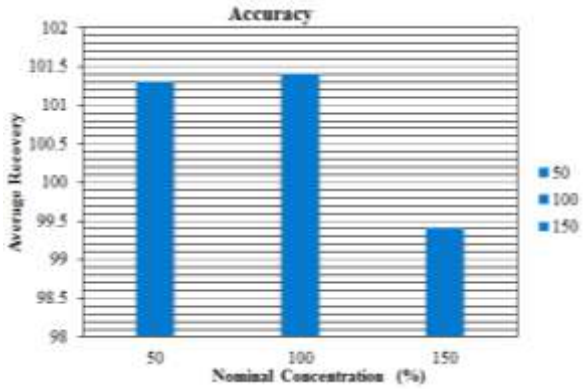

Figure 5 – Histogram accuracy for metformin hydrochloride

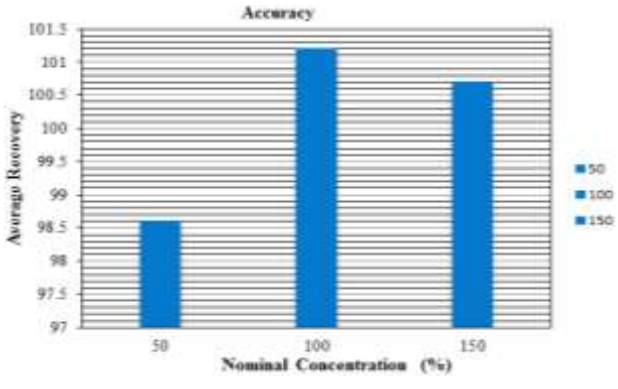

Figure 6 – Histogram accuracy for dapagliflozin

Precision

The study then evaluated precision by assessing both repeatability (consistency across repeated measurements under identical conditions) and intermediate precision (variations observed under different conditions such as different days, operators, and equipment). Repeatability was assessed by

preparing sample solutions (in mixture of water and acetonitrile) with the required analyte concentration, followed by six replicates using the finalized method. Additionally, six assays of dapagliflozin tablets against standards were conducted to confirm testing technique accuracy, with the relative standard deviation (RSD) calculated for each assay, as detailed in *Table 5* and *Table 6*, along with the respective histograms in *Figure 7* [1-4].

Table 5 : Repeatability data for metformin HCl [1-4]

No.	**Weight of sample** *(mg)*	**Area / Abs**	**Amount Found** *(mg)*	**Recovery** *(%)*
1	960.5	827062	5.10	102.1
2	961.2	825306	5.09	101.8
3	952.5	821346	5.11	102.2
4	952.7	812577	5.05	101.1
5	955.6	823898	5.11	102.2
6	950.9	819095	5.11	102.1
			Average	101.9
			Std. Deviation	0.43
			CI 95% ±	0.37
			%RSD	0.42

Table 6 – Repeatability data for dapagliflozin [1-4]

No.	**Weight of sample** *(mg)*	**Area / Abs**	**Amount Found** *(mg)*	**Recovery** *(%)*
1	960.5	4273485	862.07	101.4
2	961.2	4255663	857.85	100.9
3	952.5	4263530	867.29	102
4	952.7	4258198	866.02	101.9
5	955.6	4249008	861.53	101.4
6	950.9	4255582	867.13	102.0
			Average	101.6
			Std. Deviation	0.45
			CI 95% ±	0.39
			%RSD	0.44

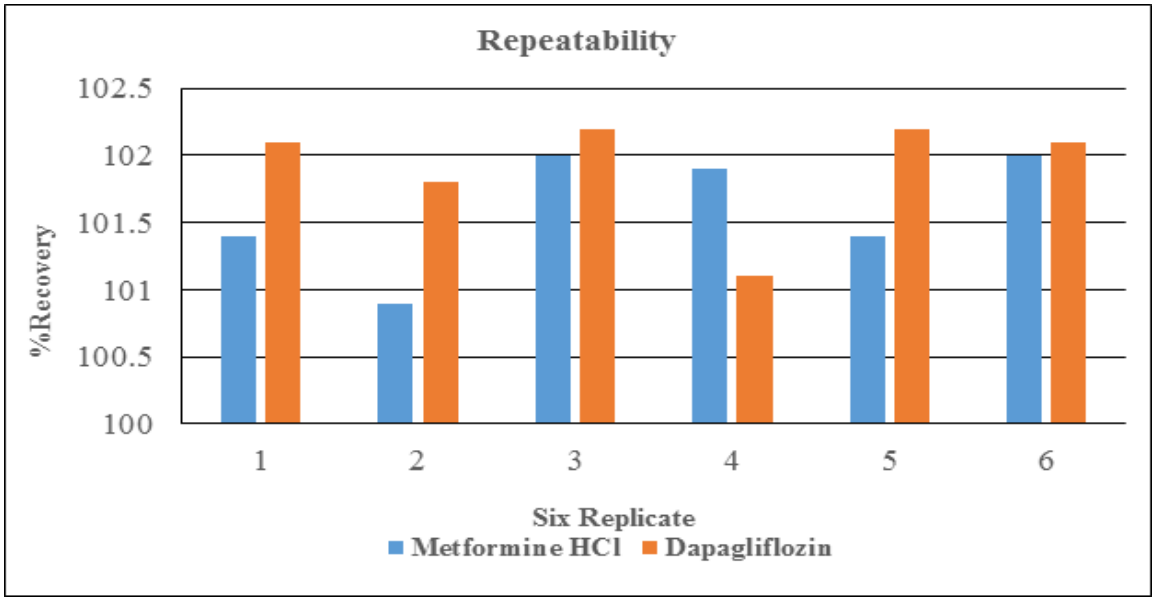

Figure 7 – Histogram of repeatability for metformin HCl and dapagliflozin [1-4]

Intermediate Precision

Intermediate precision was evaluated by the same analyst on different days using two distinct HPLC systems to estimate within-laboratory variance [1-4]. Means and standard deviations of the drug components were determined using the same sample preparation method. *Table 7* and *Table 8* present the intermediate precision data, while *Figure 8* and *Figure 9* depict the corresponding histograms for metformin hydrochloride and dapagliflozin.

Table 7 – Intermediate precision data for metformin hydrochloride [1-4]

No.	**Specimen Quantity** *(mg)*	**Area / Abs**	**Quantity Observed** *(mg)*	**Day 2 Analyst 2** *Recovery (%)*	**Day 1 Analyst 1** *Recovery (%)*
1	950.9	4287579	861.63	101.4	101.4
2	951.5	4330198	869.65	102.3	100.9
3	955.4	4316798	863.42	101.6	102.0
4	955.9	4344258	868.46	102.2	101.9
5	961.2	4483444	891.34	104.9	101.4
6	955.6	4267454	853.37	100.4	102.0
			Average	102.1	101.6
			Std. Deviation	1.51	0.44
			CI 95% ±	1.21	0.35
			%RSD	1.48	0.44

Grand Average	101.9
Std. Deviation	1.09
CI 95% ±	0.62
%RSD	1.07

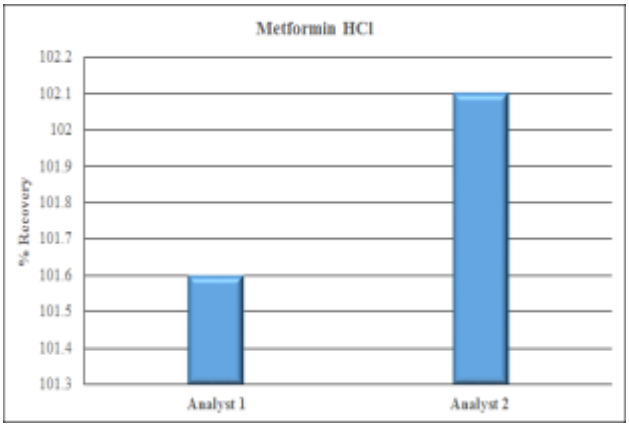

Figure 8 – Histogram of intermediate precision for metformin hydrochloride

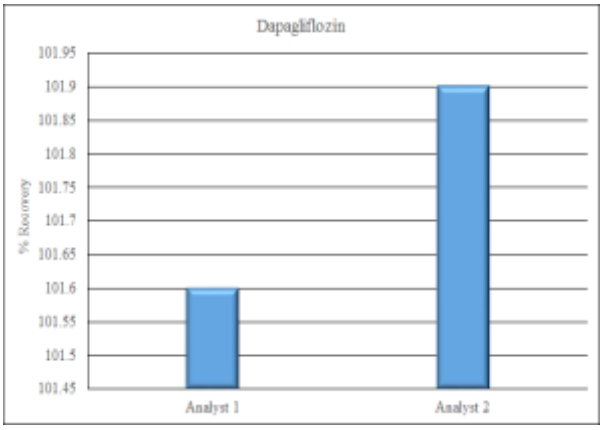

Figure 9 – Histogram of intermediate precision for dapagliflozin

Table 8 – Intermediate precision data for dapagliflozin [1-4]

No.	Specimen Quantity *(mg)*	Area / Abs	Quantity Observed *(mg)*	Day 2 Analyst 2 *Recovery (%)*	Day 1 Analyst 1 *Recovery (%)*
1	950.9	828641	5.07	101.3	101.4
2	951.5	824657	5.04	100.8	100.9
3	955.4	846990	5.15	103.1	102.0
4	955.9	842470	5.12	102.5	101.9
5	961.2	848081	5.13	102.6	101.4
6	955.6	832134	5.06	101.3	102.0
			Average	101.9	101.6
			Std. Deviation	0.92	0.44
			CI 95% ±	0.74	0.35
			%RSD	0.90	0.44

Grand Average	101.8
Std. Deviation	0.71
CI 95% ±	0.40
%RSD	0.70

Method Robustness

The robustness of our analytical technique was assessed by intentionally modifying experimental parameters to assess its adaptability and reliability. Each parameter was individually altered while others remained constant, and the retention time of the peak was measured. *Table 9* to *Table 12* demonstrate the plus and minus changes in adaptability and dependability for metformin hydrochloride and dapagliflozin analysis.

Table 9 – Plus-changes in adaptability and dependability for metformin hydrochloride analysis

No.	Area / Abs	Wave length	Flow Rate	Column Oven
1	3887528	3531394	4581016	3546573
2	3900791	3537842	4580538	3552734
3	3898512	3539378	4613204	3553964
Avg.	3895610	3536205	4591586	3551090
SD	7091.65	2789.40	21324.71	7115.17
%RSD	0.18	0.12	0.41	0.11

Table 10 – Minus-changes in adaptability and dependability for metformin hydrochloride analysis

No.	Area / Abs	Wave length	Flow Rate	Column Oven
1	3887528	3449105	5124773	3558362
2	3900791	3450272	5155842	3557888
3	3898512	3454413	5165611	3570442
Avg.	3895610	3451263	5148742	3562230
SD	7091.65	2789.40	2132471	7115.17
%RSD	0.18	0.08	0.41	0.20

The Solution Stability

The solution stability was finally assessed, which pertains to the consistency of standard and sample solutions ready for injection after extraction from the sample or matrix [1-6]. *Table 13* and *Table 14* presents the stability of metformin hydrochloride and dapagliflozin solutions respectively, it is emphasizing the importance of proper storage conditions at room temperature for both metformin hydrochloride and dapagliflozin solutions.

Table 11 – Plus-changes in adaptability and dependability for dapagliflozin analysis

No.	Area / Abs	Wave length	Flow Rate	Column Oven
1	630496	559918	576620	575757
2	630395	560680	576399	577133
3	630181	560700	569429	576496
Avg.	630357	560432	577483	576462
SD	160.84	445.83	1689.19	688.63
%RSD	0.03	0.08	0.29	0.12

Table 12 – Minus-changes in adaptability and dependability for dapagliflozin analysis

No.	Area / Abs	Wave length	Flow Rate	Column Oven
1	630496	577039	705912	574596
2	630395	577151	706655	575745
3	630181	576688	708216	577214
Avg.	630357	576959	706928	575851
SD	160.84	241.56	1175.95	1312.73
%RSD	0.03	0.04	0.17	0.23

Table 13 – Stability of metformin hydrochloride solution

No.	Area / Abs Freshly prepared	Area / Abs After Defined Time
1	3887528	3987418
2	3900791	3968100
3	3898512	3996025
Avg.	1534324	1496364
% Diff.	-2.5	

Table 14 – Stability of dapagliflozin solution

No.	Area / Abs Freshly prepared	Area / Abs After Defined Time
1	630496	642701
2	630496	643374
3	630181	643369
Avg.	1534324	1496364
% Diff.	-2.0	

CONCLUSION

This study successfully developed and validated an HPLC method for the analysis of the metformin hydrochloride and dapagliflozin, through systematic validation procedures, including assessments of accuracy, precision, linearity, and robustness, the reliability and suitability of our approach was demonstrated. The developed method exhibited accuracy, precision, and linearity within the range of 50% to 150% of the nominal concentration, indicating its effectiveness in quantifying the target compounds. We identified the flow rate as a critical parameter influencing the method's robustness, further emphasizing the importance of meticulous method optimization. Both standard and sample solutions were found to be stable for at least 24 hours under ambient conditions, enhancing the practical applicability of our method. Beyond the technical aspects of our study, we gained valuable insights into the analytical characterization of pharmaceutical compounds and the importance of method validation in ensuring the accuracy and reliability of analytical results. This study not only contributes to the body of knowledge in pharmaceutical analysis but also provides practical implications for quality control and assurance in the pharmaceutical industry. This method can serve as a valuable tool for the quantitative analysis of metformin hydrochloride and dapagliflozin in tablets, facilitating research and development efforts aimed at improving healthcare outcomes for patients with diabetes.

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