Development and Validation of RP-HPLC Method for Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate, Glimepiride and Metformin Hydrochloride in Tablet Dosage Form

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Abstract: The given RP-HPLC technique was found to be clear-cut, specific, exact, and economical for estimation of Dapagliflozin Propanediol Monohydrate, Glimepiride and Metformin Hydrochloride in Tablet Dosage Form. The method was validated according to the international conference on harmonization guidelines. A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed utilizing an Agilent Zorbax SB-CB column (250 x 4.6 mm, 5 µm) as the stationary phase. The mobile phase consisted of a buffer, acetonitrile (ACN), and methanol in a 35:5:60 v/v ratio. The sample injection was performed at a flow rate of 1.0 mL/min, with detection carried out at a wavelength of 228 nm. The developed method demonstrated linearity in the concentration ranges of 5–15 µg/mL for Dapagliflozin Propanediol Monohydrate, 1–3 µg/mL for Glimepiride, and 500–1500 µg/mL for Metformin Hydrochloride. The correlation coefficients (r^2) were found to be 0.9993, 0.9991, and 0.9989, respectively, indicating excellent linearity. Precision studies showed that the percentage relative standard deviation (% RSD) was below 2%, confirming method repeatability. Robustness evaluation also yielded % RSD values below 2%, demonstrating method reliability under small variations in conditions. Accuracy was assessed through a recovery study, ensuring the method's suitability for quantitative analysis.

Keywords : Dapagliflozin Propanediol Monohydrate, Glimepiride, Metformin Hydrochloride, RP-HPLC, Method Development and Validation.

Introduction

Diabetes mellitus is a syndrome of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. This disorder is often associated with long term complications, involving organs like eyes, kidneys, nerves, heart and blood vessels. Glucose penetrates most tissues slowly unless insulin is present to facilitate its uptake: however, central nervous system (CNS) cells, pancreatic cells, and renal medullary cells are freely permeable to glucose.[1] Permission given to FDC of Dapagliflozin, Glimepiride and Metformin Hydrochloride to conduct phase III trials by CDSCO on 13 Dec 2021.[2]

Dapagliflozin Propanediol Monohydrate (DAPA) is a highly selective, orally active and reversible inhibitor of the human Sodium-Glucose Co-Transporter 2 (SGLT2), the major transporter responsible for the renal glucose reabsorption. It improves glyceamic control in patients with Type 2 Diabetes Mellitus by inhibiting the Sodium-Glucose Co-Transporter 2, intern by reducing glucose reabsorption. Dapagliflozin's mechanism of action is complementary to and different from the mechanisms of currently available antidiabetic drugs as it involves the direct and insulin independent elimination of glucose by the kidney.[3] Dapagliflozin selectively block for SGLT2 over SGLT1. It is chemically known as (2S)-propane-1,2-diol (2S,3R,4R,5S,6R)-2-{4-chloro-3[(4-ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol hydrate. It has a

molecular formula $C_{24}H_{35}ClO_9$ with molecular weight 502.99 g/mol. Dapagliflozin is a white to off-white crystalline powder which is soluble in ethanol, methanol, dimethylsulfoxide and dimethyl formamide.[3]

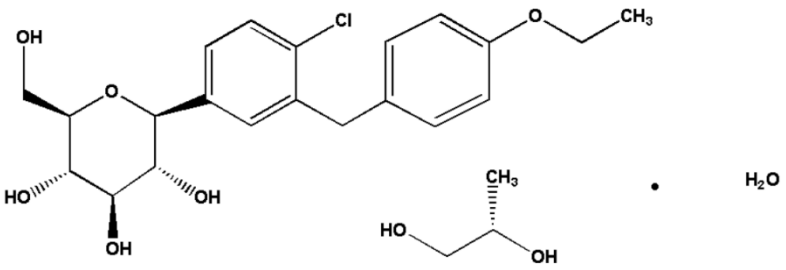


Figure No.1- Structure of Dapagliflozin Propanediol Monohydrate

Glimepiride is a third-generation sulfonylurea that is used as an oral antidiabetic agent for the treatment of Type 2 Diabetes Mellitus (T2DM). It acts by stimulating insulin secretion from the pancreatic β-cells and increasing the sensitivity of peripheral tissues to insulin. Glimepiride binds to the sulfonylurea receptor (SUR1), a subunit of the ATP-sensitive potassium (KATP) channels, leading to their closure, membrane depolarization, and subsequent calcium influx, which triggers insulin release. Glimepiride is chemically known as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulfonyl]-3-(trans-4-methylcyclohexyl)urea. Its molecular formula is C24H34N4O5S and molecular weight is 490.62 g/mol with Appearance as White to off-white crystalline powder and slightly soluble in ethanol, methanol, and acetone, but practically insoluble in water. [4-5]

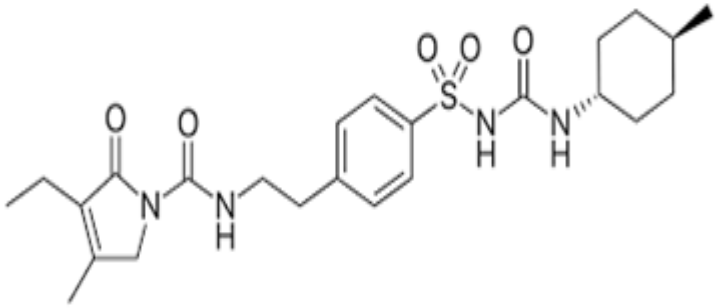


Figure No.2- Structure of Glimepiride

Metformin Hydrochloride is a biguanide-class oral hypoglycemic agent used to improve glycemic control in patients with Type 2 Diabetes Mellitus (T2DM). Unlike sulfonylureas, metformin does not increase insulin secretion but works by reducing hepatic glucose production (gluconeogenesis), increasing insulin sensitivity, and enhancing peripheral glucose uptake. Its mechanism of action primarily involves activation of AMP-activated protein kinase (AMPK), leading to improved insulin signaling and reduced glucose output by the liver. Metformin selectively lowers blood glucose levels without causing hypoglycemia, making it a first-line treatment for T2DM. Metformin Hydrochloride is chemically known as 1,1-dimethylbiguanide hydrochloride. Its Molecular formula is C4H11N5·HCl and Molecular weight: 165.62 g/mol with Appearance of White crystalline powder and Freely soluble in water, slightly soluble in ethanol (96%), and practically insoluble in acetone and chloroform. [6]

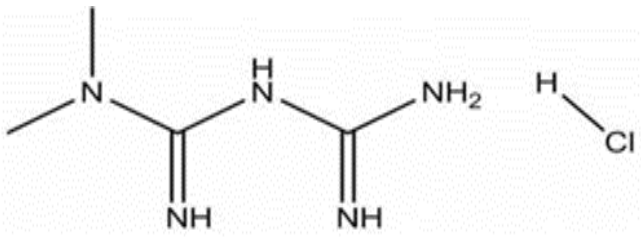


Figure No.3- Structure of Metformin Hydrochloride

From the Literature review it was indicated that the drugs have been estimated from bulk by RP-HPLC and UV- spectroscopy for individual and combination with other drugs. But no method was reported with this combination. The developed method was validated as per ICH guidelines. Stress testing under various conditions such as pH (acid/base), temperature, light, oxidation, etc. was also carried out. The present work objective is to develop novel, accurate and precise method for simultaneous estimation.

Material and Methods

Chemicals and Reagents

Dapagliflozin Propanediol Monohydrate (DAPA) API was received as a gift sample from Precise Chemipharma Pvt. LTD., Navi Mumbai, India. Glimepiride (GLIM) API was received as a gift sample from care Lifescience Pvt. LTD., Bagdol, Gujarat. Metformin Hydrochloride (METF) was received as a gift sample from Chemdyes Corporation, Rajkot, Gujarat.
Milli-Q Water, Methanol HPLC Grade, Acetonitrile HPLC Grade, Buffer AR Grade.

Instruments:

HPLC Shimadzu LC 2010 CHT instrument used for method development and validation and LC Solution software used. Shimadzu UV-1700 double beam UV-VIS spectrophotometer used for UV absorbance.

Preparation of mobile phase:

2.72 g of Potassium Dihydrogen Orthophosphate was dissolved in 1 liter of Milli-Q water. The pH of the solution was adjusted to 4.0 using diluted orthophosphoric acid. The mixture was thoroughly stirred and sonicated for 5 minutes, followed by filtration through a 0.45-micron filter under vacuum.

Diluent

Mobile phase was used as a diluent.

Standard solution Preparation:

A. **DAPA Stock Solution (50 µg/ml):** Approximately 5 mg of Dapagliflozin working standard was precisely weighed and placed into a 100 mL volumetric flask. Methanol (50 mL) was added, and the mixture was sonicated to ensure complete dissolution. The solution was then diluted to volume using the diluent to prepare the stock solution, which was subsequently further diluted with methanol to the desired concentration.
B. **GLIM Stock Solution (20 µg/ml):** Approximately 5 mg of Glimepiride working standard was carefully weighed and transferred into a 250 mL volumetric flask. To dissolve the substance, 2 mL of 0.1N NaOH was added. Subsequently, 100 mL of methanol was added, and the mixture was sonicated for complete dissolution. The solution was then diluted to volume with methanol to prepare the stock solution.
C. **METF Stock Solution (5000 µg/ml):** Approximately 250 mg of Metformin Hydrochloride working standard was accurately weighed and placed into a 50 mL volumetric flask. Methanol (50 mL) was added, and the mixture was sonicated to ensure complete dissolution. The solution was then diluted to the mark with methanol to prepare the stock solution.

Working Standard Solution Preparation

A. **DAPA (5 µg/ml):** Pipette out 1 ml from stock solution into 10 ml flask was diluted with diluent up to the mark in the flask to prepare the working standard solution.
B. **GLIM (2 µg/ml):** Pipette out 1 ml from stock solution into 10 ml flask was diluted with diluent up to the mark in the flask to prepare the working standard solution.
C. **METF (500 µg/ml):** Pipette out 1 ml from stock solution into 10 ml flask was diluted with diluent up to the mark in the flask to prepare the working standard solution.
D. **Mixed Ternary Mixture (5 µg/ml DAPA + 2 µg/ml GLIM + 500 µg/ml METF):** Pipette out 1 ml from each stock solution DAPA, GLIM and METF respectively, into 10 ml volumetric flask. Volume was made upto the mark with diluent (as mobile phase).

Sample Preparation:

Five tablet of Oxramet-G were weighed and average weight of a single tablet was calculated. Weight equivalent to one tablet was taken into 100 ml flask containing 50 ml of methanol. The mixture was sonicated for 15 minute to dissolve the content. Then volume was made upto the mark

with methanol with intermittent shaking. The resultant solution was filtered through Whatman 0.45 µm syringe filter. Further aliquot of 1 ml of the clear filtrate was taken into 10 ml volumetric flask and diluted upto the mark with diluent to get a final concentration of 500 ppm metformin + 5 ppm dapagliflozin + 2 ppm glimepiride.

Method Development and Optimization of Chromatographic Conditions

The primary objective of this study was to develop an RP-HPLC method for the simultaneous estimation of Dapagliflozin (DAPA), Glimepiride (GLIM), and Metformin (METF) in tablet formulations. The solubility of the active pharmaceutical ingredients was evaluated in various solvents, including methanol, water, acetonitrile (ACN), and buffer. Based on solubility testing, the active ingredients were found to dissolve effectively in buffer, methanol, and ACN, making these suitable as diluents.

During method development, chromatographic parameters such as mobile phase composition, flow rate, detection wavelength, analytical column, and column temperature were optimized. The RP-HPLC analytical column Agilent Zorbax SB-CB (250 × 4.6 mm, 5 µm) was selected for method development based on its performance

Chromatographic Mode	Reversed Phase
Column	Agilent Zorbax SB-CB (250 x 4.6 x 5 µ)
Detection Wavelength	228 nm
Column Temperature	25 °C
Flow Rate	1.0 ml/min
Injection Volume	10 µl
Run Time	10 min
Mode of Elution	Isocratic
Diluent	As per mobile phase
Mobile Phase	**Buffer:ACN:Methanol (35:5:605v/v)**

System suitability parameters, including retention time, theoretical plates, tailing factor, and resolution, were assessed. Based on these criteria, the Agilent Zorbax SB-CB column was finalized for the study. Various mobile phase combinations, such as water, methanol, acetonitrile, buffer, and ion-pairing agents in different proportions, were tested. The optimized mobile phase consisting of Buffer (Potassium Dihydrogen Orthophosphate): ACN: Methanol in a ratio of 35:5:60 (v/v/v) provided the ideal polarity, ensuring proper migration, separation, and resolution of DAPA, GLIM, and METF. Under these conditions, well-defined and resolved peaks were achieved for all three components.

Validation of RP-HPLC Method:

"Validation is the process of providing documented proof that a certain method will regularly result in a product that fulfills the desired standard and quality criteria." [7]

A method's performance parameter and limitation, as well as the variable that may have an impact on these qualities and to what extent are all determined through the process of method validation. Method compatibility for the use that it is intended for is also verified. The validation procedure makes sure that analytical techniques are carefully examined and approved for usage or rejected if they don't adhere to the required criteria.

The proposed RP-HPLC method was validated in accordance with the ICH Q2 (R2) guidelines. The validation parameters evaluated included system suitability, precision, accuracy, specificity, linearity, range, limit of detection (LOD), limit of quantification (LOQ), robustness, and stability of the analyte in the analytical solution. Each parameter was assessed to ensure the method's reliability, reproducibility, and suitability for the simultaneous estimation of Dapagliflozin (DAPA), Glimepiride (GLIM), and Metformin (METF) in tablet formulations.

Result & Discussion:

Selection of Wavelength

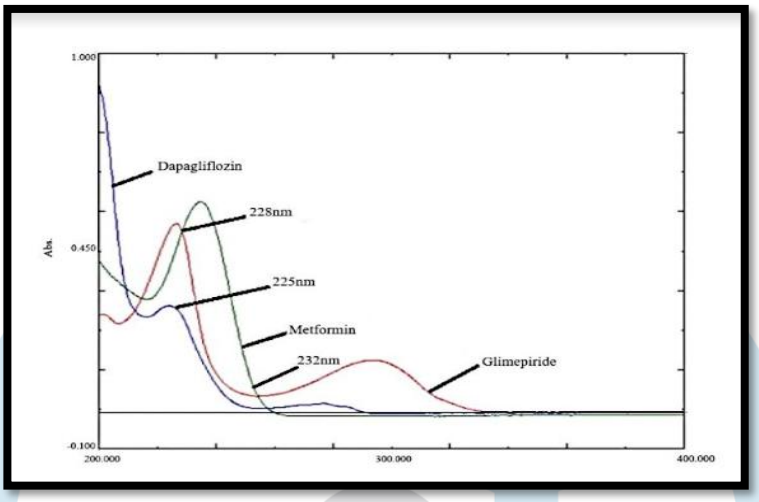


Figure No.4- Overlain UV spectra of DAPA, GLIM and METF

System Suitability Study

Before proceeding with the validation, system suitability tests were conducted to ensure the method's performance met the required criteria.

The retention times for DAPA, GLIM, and METF were 4.677 minutes, 6.595 minutes, and 3.431 minutes, respectively. The theoretical plate count exceeded 2000, the tailing factor was less than 2, and the resolution was greater than 2. Additionally, the %RSD of the peak areas from six injections did not exceed 2%, indicating the system's suitability for accurate analysis.

Table No.1- System Suitability Study Data

Sr. No.	System Suitability Parameters	DAPA		GLIM		METF	
		Mean ± SD (n = 6)	% RSD	Mean ± SD (n = 6)	% RSD	Mean ± SD (n = 6)	% RSD
1	Theoretical Plate	6405 ± 1.03	0.01	6639 ± 4.082	0.06	5560 ± 4.08	0.07
2	Retention Time	4.677 ± 0.006	0.13	6.595 ± 0.121	1.85	3.431 ± 0.004	0.11
3	Tailing Factor	1.10 ± 0.016	1.47	1.12± 0.012	1.08	1.12± 0.005	0.45
4	Resolution	6.2 ± 0.0516	0.82	7.1 ± 0.081	1.14	0.00	0.00

Specificity and Selectivity

The specificity of the developed method was checked to ensure that no other compounds or excipients interfered in the analysis were shown in Figure 5.

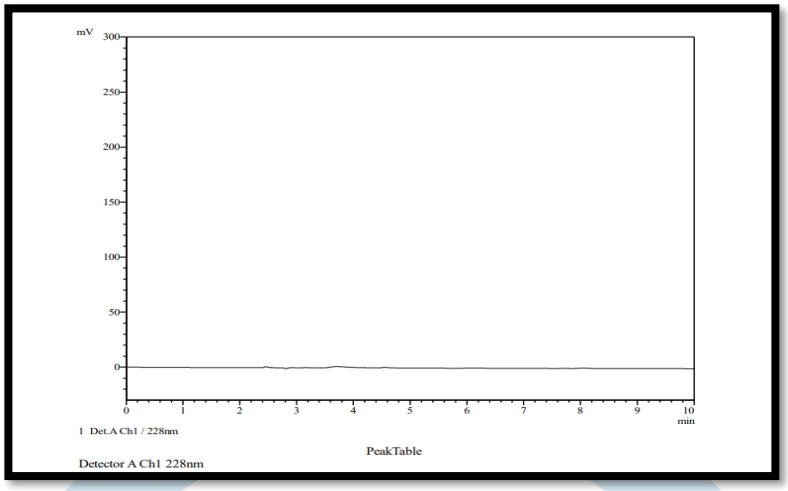
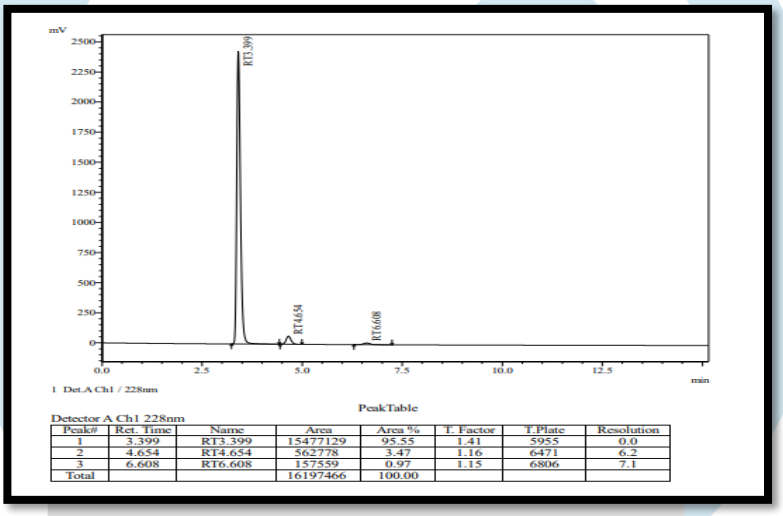


Figure No.5- Chromatogram of Diluent



Peak#	Ret. Time	Name	Area	Area %	T. Factor	T.Plate	Resolution
1	3.399	RT3.399	15477129	95.55	1.41	5955	0.0
2	4.654	RT4.654	562778	3.47	1.16	6471	6.2
3	6.608	RT6.608	157559	0.97	1.15	6806	7.1
Total			16197466	100.00			

Figure No.6- Chromatogram of Standard

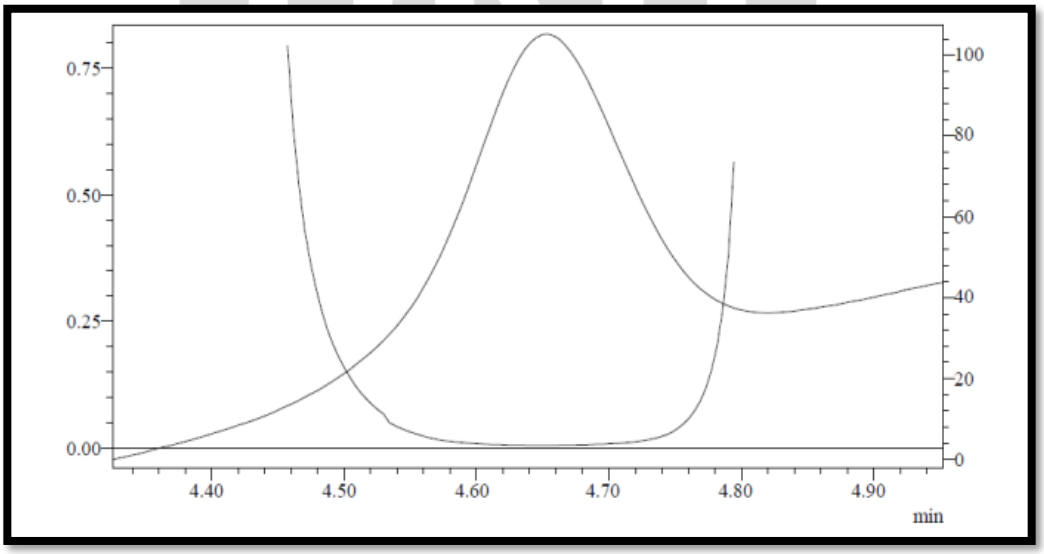


Figure No.7- Peak purity of DAPA (Standard)

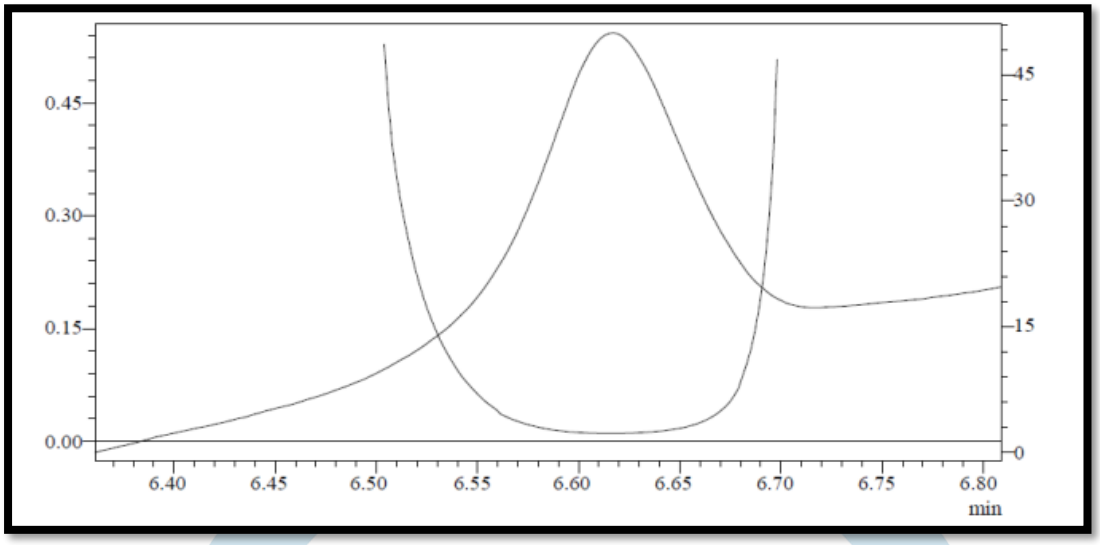


Figure No.8- Peak purity of GLIM (Standard)

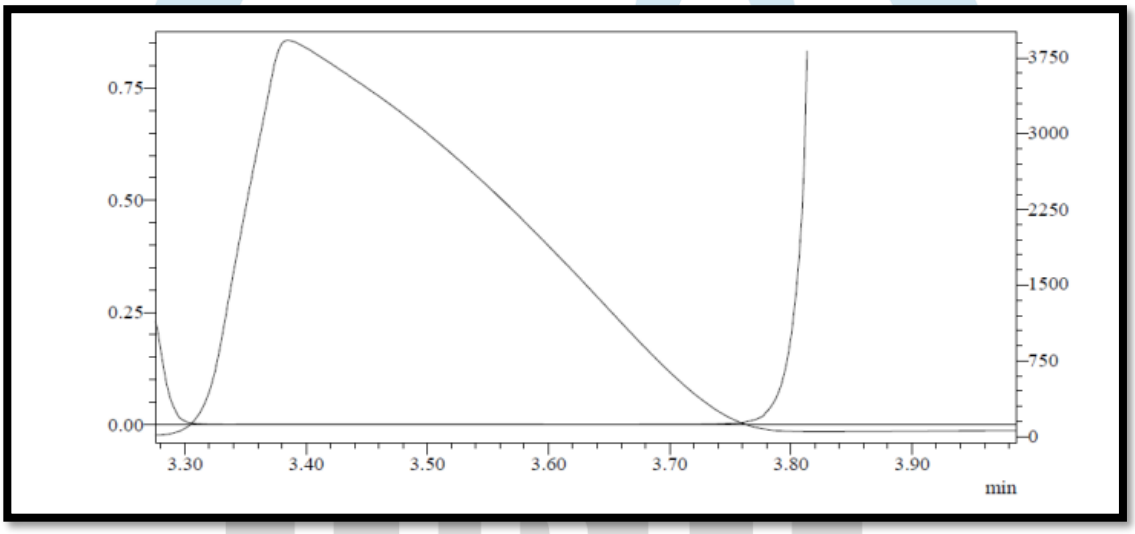


Figure No.9- Peak purity of METF (Standard)

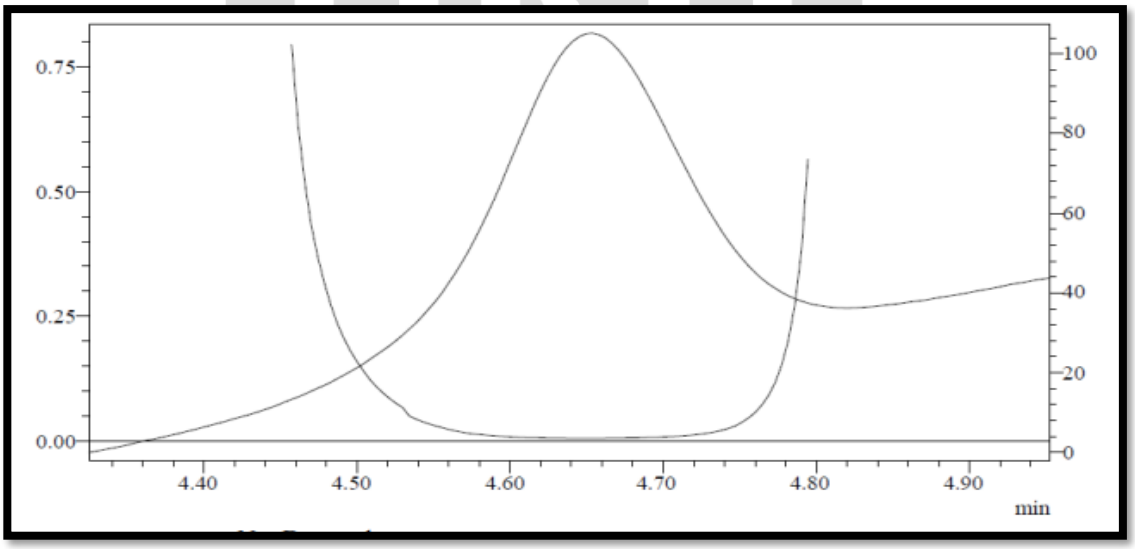


Figure No.10- Peak purity of DAPA (Sample)

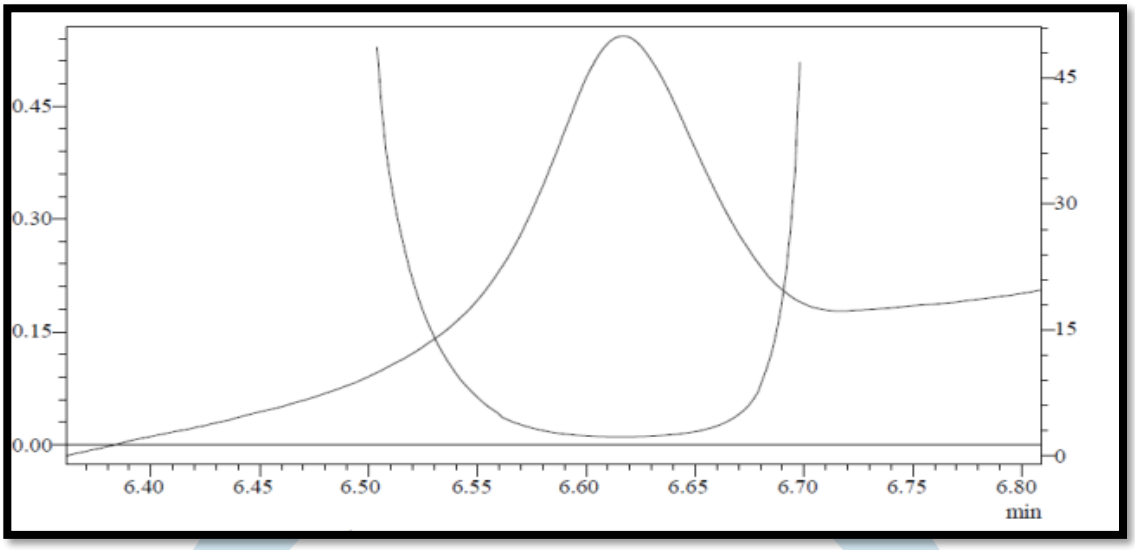


Figure No.11- Peak purity of GLIM (Sample)

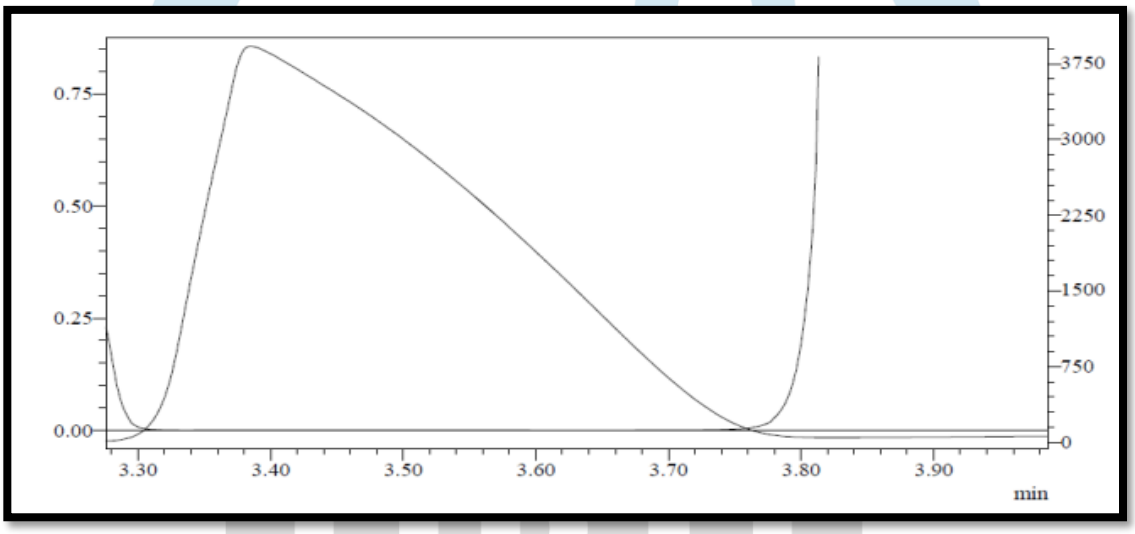


Figure No.12- Peak purity of METF (Sample)

Table No.2- Peak Purity Data of DAPA, GLIM and METF

Drug	Identification	Total Peak purity	Single point Threshold
DAPA	**Sample**	1.000000	0.978614
	Standard	1.000000	0.975412
GLIM	**Sample**	1.000000	0.986587
	Standard	1.000000	0.985247
METF	**Sample**	1.000000	0.978596
	Standard	1.000000	0.985471

1 Linearity and Range

Linearity was assessed by preparing five standard solutions of DAPA, GLIM, and METF (n=3) and analyzing them across different concentration ranges. The method demonstrated linearity within the following ranges:

- **DAPA:** 2.5–7.5 µg/mL with a correlation coefficient (R^2) of 0.9993.
- **GLIM:** 1–3 µg/mL with a correlation coefficient (R^2) of 0.9991.
- **METF:** 250–750 µg/mL with a correlation coefficient (R^2) of 0.9993.

The regression line equations for DAPA, GLIM, and METF were determined as:

- **DAPA:** y = 224618x + 16834
- **GLIM:** y = 122663x – 4097.8
- **METF:** y = 31042x – 382094

The linearity results for DAPA, GLIM, and METF are presented in Tables 3, 4 and 5 respectively. The calibration curves for each analyte are shown in Figures 13, 14 and 15, Additionally, the overlay of the linearity plots is provided in Figures 16 and 17.

Table No.3- Linearity Data for DAPA

Sr. No.	Concentration (µg/ml)	Mean Area (n=3)	%RSD
1	2.50	589235	0.21
2	3.75	845161	0.18
3	5.00	1132486	0.17
4	6.25	1434110	0.15
5	7.50	1698622	0.11

Table No.4- Linearity Data for GLIM

Sr. No.	Concentration (µg/ml)	Mean Area (n=3)	%RSD
1	1.00	159588	0.67
2	1.50	237001	0.46
3	2.00	311974	0.33
4	2.50	389593	0.27
5	3.00	454665	0.11

Table No.5- Linearity Data for METF

Sr. No.	Concentration (µg/ml)	Mean Area (n=3)	%RSD
1	250.00	16017507	1.28
2	375.00	22321718	1.06
3	500.00	28296434	0.99
4	625.00	33743208	0.49
5	750.00	39481308	0.41

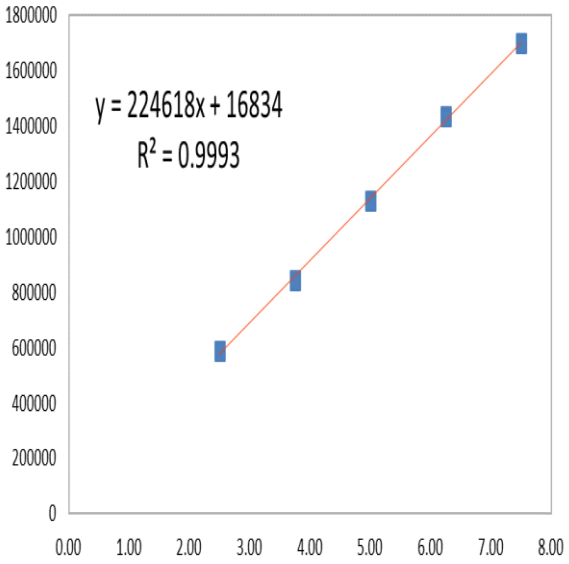


Figure No.13- Calibration Curve of DAPA

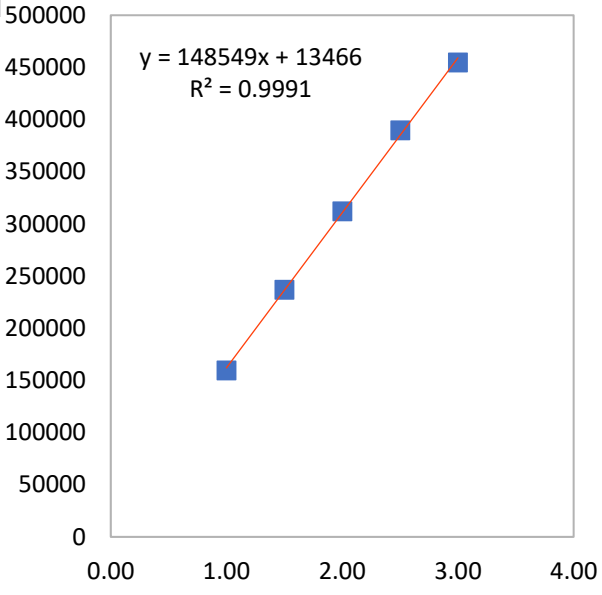


Figure No.14- Calibration Curve of GLIM

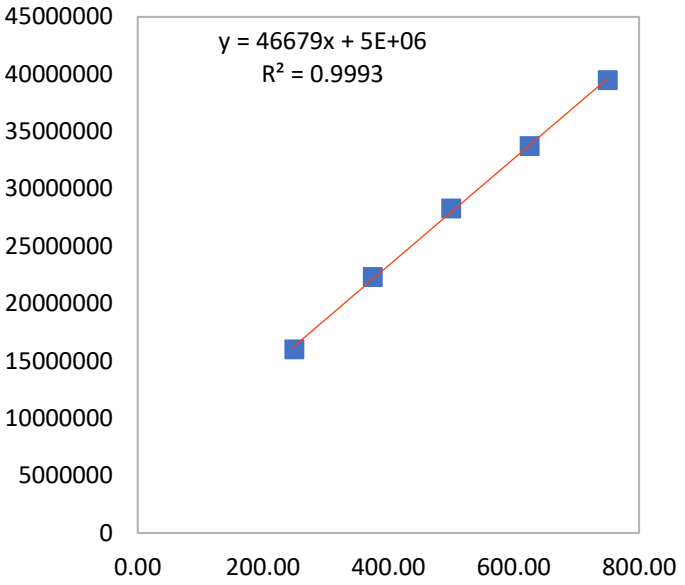


Figure No.15- Calibration Curve of METF

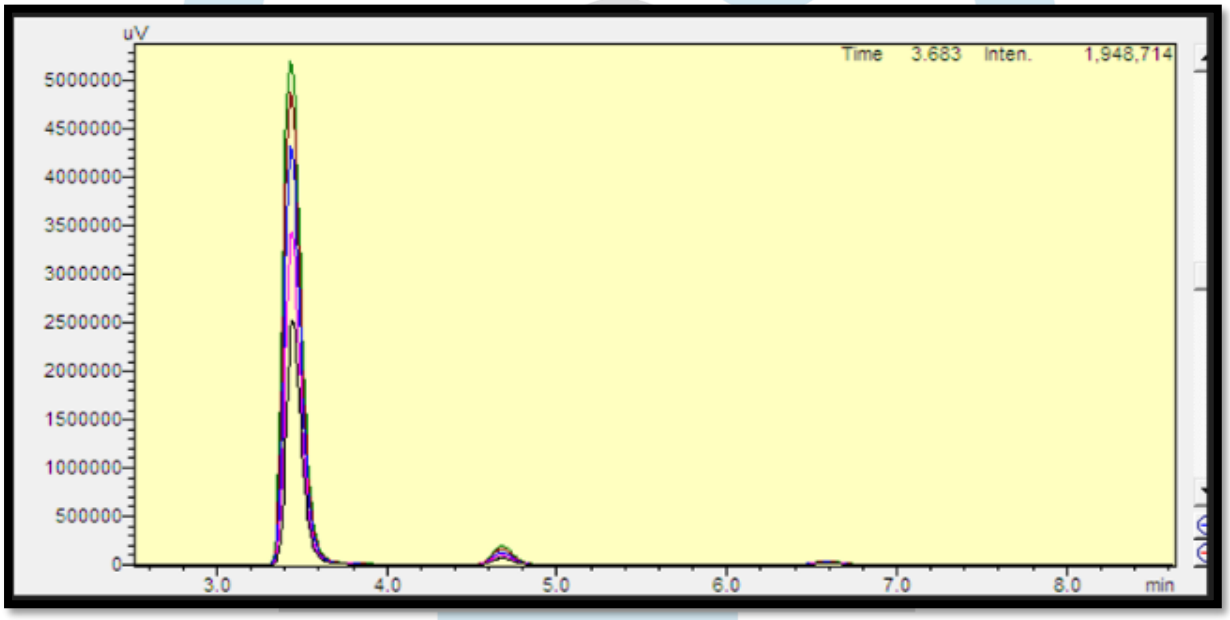


Figure No.16- Overlay Chromatogram of DAPA, GLIM and METF

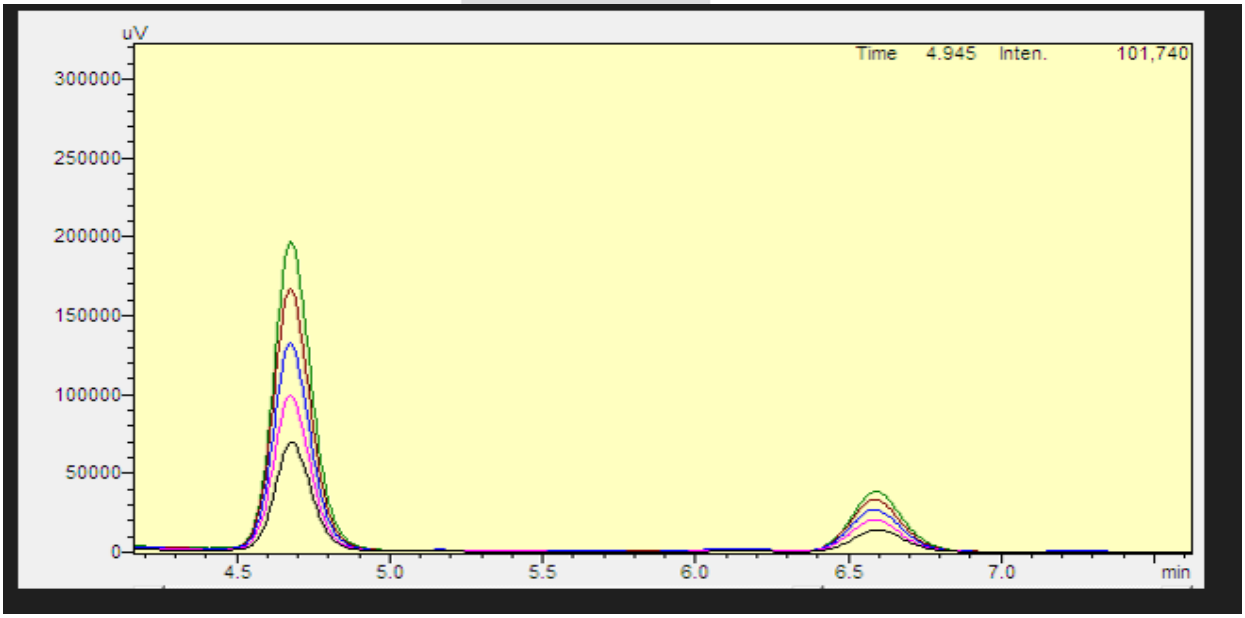


Figure No.17- Zoom Chromatogram of GLIM and DAPA

Precision

Repeatability

The repeatability data (n=6) are presented in Table 6. The percentage relative standard deviations (%RSD) for the analytes were found to be: **DAPA:** 0.12%, **GLIM:** 0.17% and **METF:** 0.16%. These results indicate good repeatability and precision of the method for the simultaneous estimation of DAPA, GLIM, and METF.

Table No.6- Repeatability Data of DAPA, GLIM and METF

Conc of DAPA	Peak area	Conc. of GLIM	Peak area	Conc. of METF	Peak area
5 µg/ml	1127244	2 µg/ml	321112	500 µg/ml	29145516
	1127694		321658		29172539
	1126924		321432		29137514
	1125893		320984		29181021
	1127689		321805		29155036
	1126963		321368		29159998
Mean	**1127068**	**Mean**	321445	**Mean**	29158916
SD	666.4	**SD**	547.45	**SD**	29158.92
% RSD	0.12	**% RSD**	0.17	**% RSD**	0.10

Intraday Precision

Three concentrations of DAPA (2.5, 5, and 7.5 µg/mL), GLIM (1, 2, and 3 µg/mL), and METF (250, 500, and 750 µg/mL) were analyzed on the same day (n=3) to assess intraday precision. The data for intraday precision are shown in Table 7. This analysis helps to evaluate the method's consistency and precision within a single day

Table No.7- Intra Day Precision Data of DAPA, GLIM and METF

Drug	Concentration (µg/ml)	Mean peak area ± SD (n=3)	% RSD
DAPA	2.5	571546 ± 3409.4	0.60
	5	1672124 ± 27388.2	1.64
	7.5	1723657 ± 3447.31	0.20
GLIM	1	166789.6 ± 657.45	0.39
	2	312456 ± 4567.8	1.46
	3	564789 ± 2456.87	0.44
METF	250	16287961 ± 78967.1	0.49
	500	28988956 ± 453454.1	1.57
	750	37548965 ± 82507.37	0.22

Inter Day Precision

Three concentration (2.5, 5 and 7.5 µg/ml) of DAPA, (1, 2, and 3 µg/ml) of GLIM and (250, 500, and 750 µg/ml) of METF were analysed on 3 Consecutive days (n=3). The Inter day precision data were shown in Table 8.

Table No.8- Inter Day Precision Data of DAPA, GLIM and METF

Drug	Concentration (µg/ml)	Mean peak area ± SD (n=3)	% RSD
DAPA	2.5	591652 ± 2049.09	0.35
	5	1144114 ± 10995.0	0.96
	7.5	1704502 ± 2981.1	0.17
GLIM	1	165246.3 ± 706.22	0.43
	2	317774 ± 5548.3	1.75
	3	454231 ± 2763.77	0.61
METF	250	16145586 ± 81104.1	0.50

	500	29961425 ± 441579.1	1.47
	750	39629540 ± 28828.87	0.07

Limit of Detection and Limit of Quantitation

Using slope and SD of Y-intercept, the determined values of LOD and LOQ were evaluated were shown in Table 9.

Table No.9- Limit of Detection and Limit of Quantitation data for DAPA, GLIM and METF

Parameters	LOD (µg/ml)	LOQ (µg/ml)
DAPA	0.44	1.34
GLIM	0.04	0.14
METF	18.6	56.6

Accuracy

The accuracy of the method was evaluated using a recovery study, where known amounts of the standard were added at three levels 50%, 100%, and 150%. The results showed that the percentage recovery for all three drugs was within the acceptable range of 98% to 102%. This confirms that the method is both accurate and reliable for measuring DAPA, GLIM, and METF.

Table No.10- Accuracy Data of DAPA

Name of drug	Conc Level (%)	Added (ml)	Amount Added (mg)	Amount Recovered (mg)	%Mean Recovery	% Mean Recovery	% RSD
DAPA	50 %	0.500	0.025	0.025	100.8	100.9	0.24
	50 %	0.500	0.025	0.025	101.2		
	50 %	0.500	0.025	0.025	100.8		
	100%	1.000	0.050	0.051	101.0	102.0	0.06
	100%	1.000	0.050	0.051	101.0		
	100%	1.000	0.050	0.051	101.0		
	150 %	1.500	0.075	0.075	100.4	100.5	0.25
	150 %	1.500	0.075	0.075	100.5		
	150 %	1.500	0.075	0.076	100.7		

Table No.11- Accuracy Data of GLIM

Name of drug	Conc Level (%)	Added (ml)	Amount Added (mg)	Amount Recovered (mg)	%Mean Recovery	% Mean Recovery	% RSD
GLIM	50 %	0.500	0.010	0.010	102.0	101.3	1.54
	50 %	0.500	0.010	0.010	100.0		
	50 %	0.500	0.010	0.010	101.0		
	100%	1.000	0.020	0.020	100.5	100.3	0.34
	100%	1.000	0.020	0.020	100.0		
	100%	1.000	0.020	0.020	100.5		
	150 %	1.500	0.030	0.030	98.3	98.5	0.09
	150 %	1.500	0.030	0.030	98.3		
	150 %	1.500	0.030	0.030	98.3		

Table No.12- Accuracy Data of METF

Name of drug	Conc Level (%)	Added (ml)	Amount Added (mg)	Amount Recovered (mg)	%Mean Recovery	% Mean Recovery	% RSD
METF	50 %	0.500	2.500	2.503	100.1	100.8	0.76
	50 %	0.500	2.500	2.523	100.9		
	50 %	0.500	2.500	2.539	101.5		
	100%	1.000	5.000	5.025	100.5	100.8	0.34
	100%	1.000	5.000	5.043	100.9		
	100%	1.000	5.000	5.056	101.1		
	150 %	1.500	7.500	7.557	100.8	101.9	0.96
	150 %	1.500	7.500	7.683	102.4		
	150 %	1.500	7.500	7.684	102.5		

Robustness

The Robustness may observed by changing the Temperature of column (± 5°C), Flow rate (10 % ml/min), pH (0.2 ± 0.1) and Organic Phase (± 2) % RSD for DAPA, GLIM and METF was less than 2 %. The Robustness of DAPA, GLIM and METF was shown in Table 11, 12 and 13.

Table No.13- Robustness Data of DAPA

Sr. No.	Area at Temp. -5°C	Area at Temp. +5°C	Area at Flow Rate -10%	Area at Flow Rate +10%	Area at Organic Phase -2%	Area at Organic Phase +2%
1	1137408	1137996	1262162	1031620	1138160	1129081
2	1137448	1137013	1262665	1032518	1138860	1132686
3	1138794	1136914	1263190	1031917	1139438	1132648
Mean	1137883	1137308	1262672	1032018	1138819	1131472
% RSD	0.12	0.17	0.23	0.27	0.18	0.29
Theoret-ical Plates	6217	6385	6786	5947	6360	6313
Tailing Factor	1.15	1.19	1.18	1.17	1.17	1.21

Table No.14- Robustness Data of GLIM

Sr. No.	Area at Temp. -5°C	Area at Temp. +5°C	Area at Flow Rate -10%	Area at Flow Rate +10%	Area at Organic Phase -2%	Area at Organic Phase +2%
1	324522	326047	360466	293209	324357	345530
2	325685	324843	360300	293268	323404	345543
3	325198	324492	360610	292897	323042	346988
Mean	325135	325127	360459	293125	323601	346020
% RSD	0.24	0.33	0.12	0.17	0.27	0.26
Theoretical Plates	6639	6292	6830	7056	6172	6533
Tailing Factor	1.17	1.15	1.15	1.15	1.22	0.96

Table No.15- Robustness Data of METF

Sr. No.	Area at Temp. -5° C	Area at Temp. +5°C	Area at Flow Rate -10%	Area at Flow Rate +10%	Area at Organic Phase -2%	Area at Organic Phase +2%
1	29421912	29464619	32624494	26776327	29504611	29283202
2	29443432	29432549	32632491	26774861	29520802	29335822
3	29482812	29453523	32656698	26776040	29533902	29358793
Mean	29449385	29450230	32637894	26775743	29519772	29325939
% RSD	0.12	0.16	0.18	0.27	0.34	0.44
Theoretical Plates	5461	5453	5901	5204	5430	5676
Tailing	1.47	1.47	1.49	1.47	1.49	1.5

Factor						

Observation: The typical variations studied under this parameters were temperature, flow rate and organic phase ratio. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust.

Assay

Applicability of the proposed method was tested by analyzing the formulation. Results as % Assay is shown in Table 14.

Table No.16- Assay

Drug	Label Claim	Mean Amount Found (n=3)	Assay (%) Mean ± SD (n=3), % RSD
DAPA	5 mg	5. mg	98.0 ± 0.82, 0.83
GLIM	2 mg	2.04 mg	98.3 ± 0.22, 0.23
METF	500 mg	501 mg	99.7 ± 0.24, 0.25

The assay outcomes were comparable to each drug's labelled value in the synthetic mixture.These results indicates that the developed method was specific, accurate, precise, simple, sensitive, robust and rapid.

Summary:

Table 17: Summary of RP-HPLC Validation Parameters of DAPA, GLIM and METF

Parameters	DAPA	GLIM	METF
Linearity Range (µg/ml)	2.5-7.5	1 - 3	250 – 750
Regression equation, y = mx + c	y = 224618x + 16834	y = 122663x + 4097.8	y = 31042x + 382094
Correlation Coefficient (R^2)	0.9993	0.9991	0.9993
Detection limit (µg/ml)	0.44	0.04	18.6
Quantitation limit (µg/ml)	1.34	0.14	56.6
Repeatability (% RSD, n = 6)	0.12	0.17	0.10
Intra-day (n = 3), % RSD	0.20 – 1.64	0.39– 1.46	0.22– 1.57
Inter-day (n = 3), % RSD	0.17- 0.96	0.43– 1.75	0.07 - 1.47
Accuracy (%recovery % RSD (n= 3)	100.5-101.0, 0.06-0.24	98.5-101.3, 0.09-1.54	100.8-101.9, 0.34-0.96
Robustness	% Deviation was found to be less than 2		
el amount found (mg), % label claim ± SD	5.1, 98.0 ± 0.82	2.04, 98.3 ± 0.22	501, 99.7 ± 0.25

Conclusion:

A RP-HPLC method for the simultaneous estimation of Dapagliflozin Propanediol Monohydrate, Glimepiride and Metformin Hydrochloride in tablet dosage form was developed and validated as per ICH guidelines. The method was accurate and precise. The developed method demonstrated linearity in the concentration ranges of 5–15 µg/mL for Dapagliflozin Propanediol Monohydrate, 1–3 µg/mL for Glimepiride, and 500–1500 µg/mL for Metformin Hydrochloride. The correlation coefficients (r^2) were found to be 0.9993, 0.9991, and 0.9989, respectively, indicating excellent linearity. Precision studies showed that the percentage relative standard deviation (% RSD) was below 2%, confirming method repeatability. Robustness evaluation also yielded % RSD values below 2%, demonstrating

method reliability under small variations in conditions. Accuracy was assessed through a recovery study, ensuring the method's suitability for quantitative analysis.

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