Development and Validation of QbD Assisted HPLC Method for Dapagliflozin and Gliclazide in Bulk and Dosage Form.

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ABSTRACT

This work describes the development and validation of a simple, rapid, precise and accurate RP-HPLC Method for simultaneous estimation of Dapagliflozin & Gliclazide in bulk and tablet dosage form by Quality by Design (QbD) approach. The systematic approach for method development was based on a risk-based framework using Fishbone (Ishikawa) diagram to identify CMPs and CQAs. Chromatographic conditions (percentage of mobile phase, pH, flow rate and column temperature) were optimized using a Central Composite Design (CCD).

A C18 (250 mm × 4.6 mm, 5 µm) column with a mobile phase of Acetonitrile and 0.1% Orthophosphoric acid buffer] ratio of [60:40 v/v] at flow rate of 1.0 mL/min were used for chromatographic separation.Footnote, Detection by UV detector at 229 nm. Retention time for Dapagliflozin and Gliclazide were 3.94 min and 6.4 min respectively.

The method has been validated for various parameters such as linearity, accuracy, precision (intra-day and inter-day), specificity, LOD and LOQ etc. according to ICH Q2(R1) guidelines. For Dapagliflozin and Gliclazide solutions, linearity was demonstrated over the range of 2-10 µg/mL with correlation coefficients (r^2) of ≥ 0.999. Mean percentage recoveries as presented in Table 6 indicated the retrieved values for the three compounds fell within 98 to 102% and thus attest their accuracy from further recovery studies. This method showed acceptable precision as %RSD values were less than 2.0%. Based on the results of validation studies, developed QbD assisted RP-HPLC method is suitable for routine quality control analysis of Dapagliflozin and Gliclazide in bulk as well as pharmaceutical formulations.

KEYWORDS: Dapagliflozin, Gliclazide, RP-HPLC, Quality by Design (QbD), ICH Q2(R1), Method Validation

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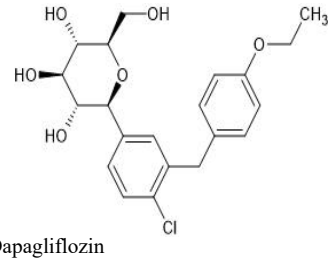
INTRODUCTION

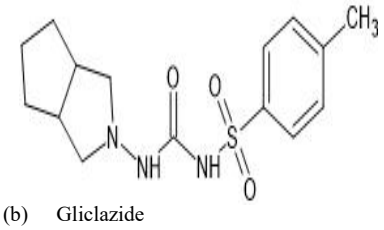
Type 2 Diabetes Mellitus has become one of the most important public health issues, with more than 537 million adults worldwide affected in 2021 [IDF Atlas, 2021]. [1] The rationale for using antidiabetic agents with complementary mechanisms of action in the same patient under limestone is a mainstay modern pharmacotherapy. These two agents are used right now together increasingly as they provide a mechanism of action that is complementary.

Dapagliflozin is an SGLT2 inhibitor (chemical name 2S,3R,4R,5S,6R)-2-{4-Chloro-3-(4-Ethoxyphenyl)

methylmethyl}] -6-(hydroxymethyl) oxane-3. Its glucose-lowering action is the result of SGLT2 inhibition in the proximal convoluted tubule, which increases urinary glucose excretion independently of insulin.[2] Gliclazide, a second-generation sulfonylurea (chemically designated as 1-(3,3a,4,5,6,6a-hexahydro-1Hcyclopenta[c]pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea) stimulates pancreatic β-cell insulin secretion and has antioxidant properties. [3,4]



(a) Dapagliflozin



(b) Gliclazide

Fig. 1: Chemical structure of Dapagliflozin and Gliclazide

From the literature, a survey indicates that different analytical techniques has been reported for the individual assay of Dapagliflozin and Gliclazide such as UV spectrophotometric methods [5–10], HPLC [11] LC-MS/MS along with stability-indicating methods. [3,5,6,8,9] But there are limited reports for the simultaneous HPLC estimation of drugs in combination and more few with a Quality by Design (QbD) framework. QbD robust analytical method development ensures reliability based on systematic optimization (DoE). [10,11,14]

Keeping the above in mind current research was proposed with an aim to develop and validate a QbD-based RP-HPLC method for the simultaneous estimation of Dapagliflozin and Gliclazide from bulk drug substance and tablet formulations in accordance to ICH Q2(R1) parameters. [11,12,13]

MATERIAL AND METHODS

Materials

Dapagliflozin was a gift sample from Glenmark Pharmaceuticals Ltd. Mumbai. Gliclazide was obtained from SM Pharma & Chemicals, Mumbai. HPLC-grade water was supplied by Merck, HPLC grade acetonitrile was procured from Qualigens, and water was obtained from Merck (India) Ltd. orthophosphoric acid, was purchased from Loba Chemie Pvt. Ltd, Mumbai. Cyblex D30 (Dapagliflozin 10mg & Gliclazide 30 mg) commercially available tablet formulations were purchased from a local pharmacy.

Instrumentation

The development was conducted using Thermoscientific HPLC system Vanquish series with a UV-Vis detector, an manual injector, and Vanquish Quaternary pump a column oven. For data acquisition and processing, Chromeleon 7.3.2.10759 software was used. Weights were carried out on digital balance SR Electronics, Mumbai, India and for the preparation of samples by using ultrasonicator CD 4820, Digital ultra sonic cleaner, Mumbai, India.

Selection of solvents:

Acetonitrile : Water 50:50 v/v proportion of solvent was selected on the basis of solubility study for dissolving the DAPA and GLICLA.

Preparation of 0.1 % Orthophosphoric acid buffer:

A volume of 1ml of Orthophosphoric acid was transferred into a 1000ml of volumetric flask . The volume was adjusted up to the mark with HPLC grade water and mixed thoroughly to obtain 0.1% orthophosphoric acid buffer.

Preparation of Mobile Phase:

The mobile phase was prepared by mixing 60% volume of Acetonitrile and 40% of volume of 0.1% Orthophosphoric acid buffer. The mixture was sonicated for 20-25 min to ensure proper mixing.

Preparation of Diluents (Acetonitrile :Water 50:50%v/v)

The diluent was prepared by mixing 250ml of Acetonitrile and 250ml of HPLC grade water in 500ml volumetric flask . The resulting solution was sonicated for 15 min to obtain a homogeneous mixture.

Preparation of Standard Stock Solution of DAPA and GLICLA:

A stock solution containing 1000 µg/mL of Dapagliflozin and Gliclazide reference standards were separately prepared from each accurately weighed 10 mg transferred into separate 10 mL volumetric flasks, dissolved in diluent ACN:Water (50:50 v/v proportion) and diluted to volume. The working standard solutions of Dapagliflozin and Gliclazide separately were prepared with the diluent, by appropriate dilution of the stock solutions ranging from 2-10 µg/mL.

Preparation of Sample Solution

From the standard stock solution of Dapagliflozin and Gliclazide, 1ml of aliquot was pipetted

accurately and then transferred into a 10ml volumetric flask. Diluted with diluent to mark to prepare valid sample solution of dapagliflozin and gliclazide at a copy concentration of 100 µg/ml.

Tablet Stock solution

Twenty tablets from this formulation (Cyblex D30) were weighed and ground to a fine powder. Equivalent of 10 mg Dapagliflozin and Gliclazide 30mg weight accurately was transferred in a volumetric flask 100 mL, dissolved in60mL diluent by ultrasonicated for 15 minutes then made up to volume with the same diluent, mixed well and filtered through what man filter. Dilution of filtrate to the working concentration

Selection of Analytical Wavelength:

UV spectra of Dapagliflozin and Gliclazide were prepared separately after that over lay. Common wavelength from overlain spectra for simultaneous estimation of both the drugs were determined. A 229 nm Isobetic point was observed. Hence, 229 nm was chosen as the analytical wavelength for HPLC further analysis.

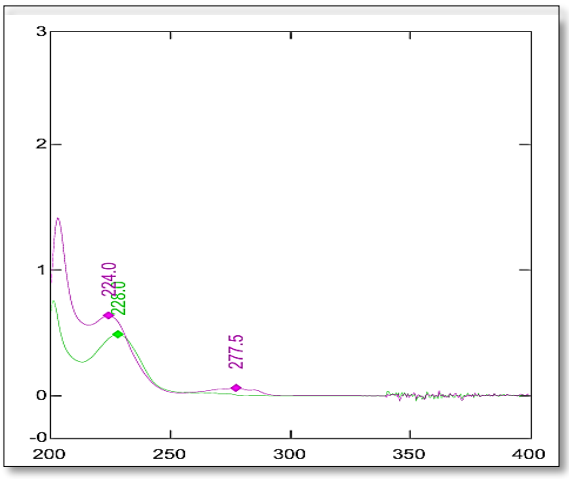


Fig.2: Overlay spectra of Dapagliflozin and Gliclazide

QbD Approach for Method Development

The QbD framework was applied systematically as follows:

Analytical Target profile (ATP): An ATP was implemented to develop stability indicating, specific and precise RP-HPLC method for simultaneous quantitative estimation of Dapagliflozin and Gliclazide with Rs≥ 2.0, tailing factor (≤ 2.0), theoretical plates (≥ 2000) ≤15 min of runtime.

Risk Assessment: Since data is available upto October 2023, using maxis enabled a cause and effect Ishikawa fishbone diagram to assess the potential critical method parameters (CMPs) influencing critical quality attributes (CQAs). These CMPs were identified as mobile phase composition (percentage of ACN), flow rate and column temperature. [11,12,13,14].

Selection of Critical Method Parameters

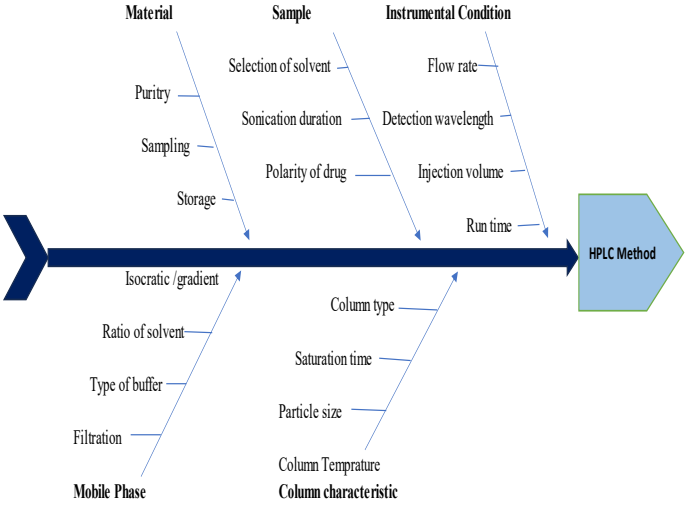


Fig.3: Risk assessment using Fishbone diagram

Instrumental condition:

Flow rate: The flow rate was selected as a critical parameter because it influences retention time and peak area. Variation in flow rate can affect chromatographic efficiency and may lead to inadequate separation of analytes.

Mobile phase:

Mobile phase composition was selected because it significantly affects retention behaviour and resolution of analytes. Changes in the ratio of organic components (Acetonitrile) alter the elution pattern and peak characteristics.

Column characteristic:

Column temperature was selected because it affects the viscosity of mobile phase , analyte retention and column efficiency. Change in column temperature can influence the interaction between analytes and the stationary phase, resulting in variations in retention time and chromatographic resolution. Hence column temperature was considered a critical parameter.

Based on the risk assesment factors having a significant impact on the CQA were shortlisted for further investigation.

Design of Experiments (DoE): A Central Composite Design (CCD) was applied using Design Expert ®13 software (Trial Version) to systematically study the effect of three critical factors on the CQAs (retention time, resolution, and theoretical plates). [3,10,14] The factor levels studied are shown in Table 1.

Table 1: CCD Factor Levels for QbD Optimization

Factor	Name	Units	Coded Low (-1)	Coded High (+)
A	ACN	%	55	65
B	Flow Rate	Min/ml	0.8	1.2
C	Temp.	ºC	25	35

Table 2: Central Composite Design structure

Std	Run	Factor 1	Factor 2	Factor 3
		A: CAN	B:Flow rate	C: Temperature
		%	Min/ml	ºC
5	1	55	1	25
14	2	60	1	30
1	3	55	0.8	30
3	4	55	1.2	30
11	5	60	0.8	35
13	6	60	1	30
4	7	65	1.2	30
2	8	65	0.8	30
7	9	55	1	35
6	10	65	1	25
12	11	60	1.2	35
10	12	60	1.2	25
9	13	60	0.8	25
8	14	65	1	35

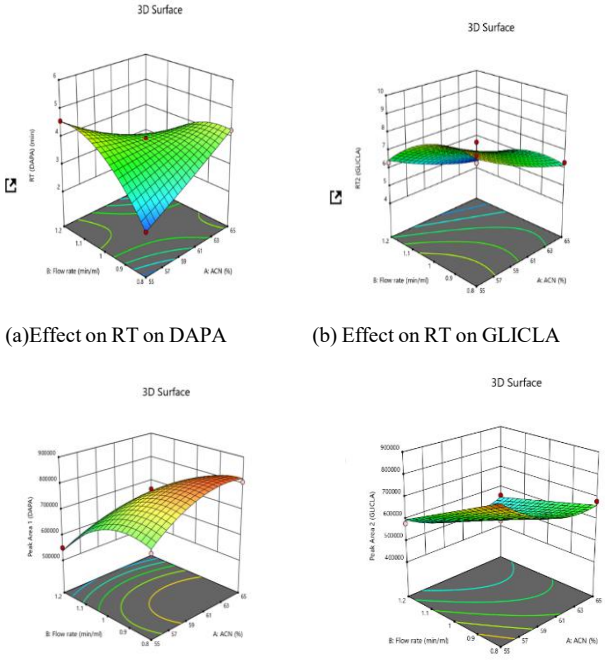
Total 14 experimental runs were generated by the design with Central composite design

Optimized Chromatographic Conditions

The response surface method (RSM) on the basis of DoE optimization and desirability function analysis revealed the final chromatographic conditions to be as Column: C18, 250 mm × 4.6 mm, 5 µm Mobile phase: Acetonitrile : Buffer solution containing 0.1% Orthophosphoric acid in ratio of 60:40 v/v; Flow rate (1.0 mL/min); Detection wavelength(229 nm); Column temperature: (30°C) injection volume (20 µL), Run time (10 min).

Table 3 : optimization parameter of response variable

Model F-value	6219.43	15.08	18.04	7.48
Model P- value	0.0001	0.0095	0.0068	0.03451
R^2	0.9999	0.9714	0.9759	0.9439
Adjusted R^2	0.9998	0.9069	0.9218	0.8178
Predicted R^2	0.9989	0.7702	0.6311	0.1066
Adequate precision	288.82	14.04	11.55	10.7349



(a)Effect on RT on DAPA (b) Effect on RT on GLICLA

(c) Effect on Peak area on DAPA (d) Effect on Peak area on GLICLA

Fig. 4: Effect of Independent variables

Method Validation

Validation of the developed method was performed according to ICH Q2(R1) related parameters:

Specificity[5,6,15]

In order to test the specificity of the developed method, a blank solvent was injected into the chromatographic system with no drug substance to ensure that there is no potential interference from any blank peak present with main analyte.

Single doses of Dapagliflozin and Gliclazide were separately prepared at conc. This was recorded and the relevant peaks were identified at 100µg/ml. Finally, the absence of co-eluting or interfering peaks at the retention times of analytes ensured method specificity.

System suitability[15,16]

The system suitability of the developed HPLC method was evaluated by making six injections from a homogeneous sample solution. Each chromatographic response from each injections of the samples were analyzed to check and determine the consistency and performance of our analytical method.The following parameters were determined as part of system suitability:

i.Retention Time

ii.Theoretical Plates

iii. Tailing Factor

Linearity[3,7,8,15]

To check the linearity of the method, standard solutions of Dapagliflozin and Gliclazide were prepared in five different concentrations (2–10 µg/mL) and injected into the chromatographic system.

Precision [3,8,15]

The precision of the developed method was evaluated by preparing six independent sample solutions at a concentration of 8µg/mL according to the standard operating procedure. The prepared samples that were analyzed under uniform chromatographic conditions.

Accuracy[3,7,15]

Percentage recovery study was performed at three concentration levels of the developed method corresponding to 80% 100% and 120% from their nominal value. Known amounts of drugs were spiked into CCD samples that had already been analyzed previously, and the amount of drug recovered was used to determine how accurately the method could quantify a known concentration.

Robustness[10,14,15]

The contribution of minor variations in Critical chromatographic parameters was assessed on system suitability and method performance to study its robustness.

The following parameters were modified:

In this study, the variation in mobile phase flow rate was investigated by increasing and decreasing the flow rate of mobile phase from the optimized value ±0.1 mL/min and observing its effect on retention time and peak shape.

Detection Wavelength: The detection wavelength was varied by ±2nm to the selected wavelength and its effect peak area and retention time was checked and recorded.

RESULTS AND DISCUSSION

Method Optimization by QbD

The CCD analysis of the DoE produced a total of 14 experimental runs. The ANOVA analysis performed on the model confirmed that it is significant (p 4 To illustrate the relationship between factors for each CQA, three-dimensional (3D) response surface plots and contour plots were created. Confirmation of the optimum conditions steps: desirability function approach provided an optimal solution with a composite desirability . [10-14]

Specificity:

Study of specificity also demonstrated that the blank, solvent or excipients did not interfere at the retention time of analytes. The peaks have high resolution and sharpness suggesting that the developed HPLC technique is specific and a suitable tool for drug analysis in pharmaceutical formulation.

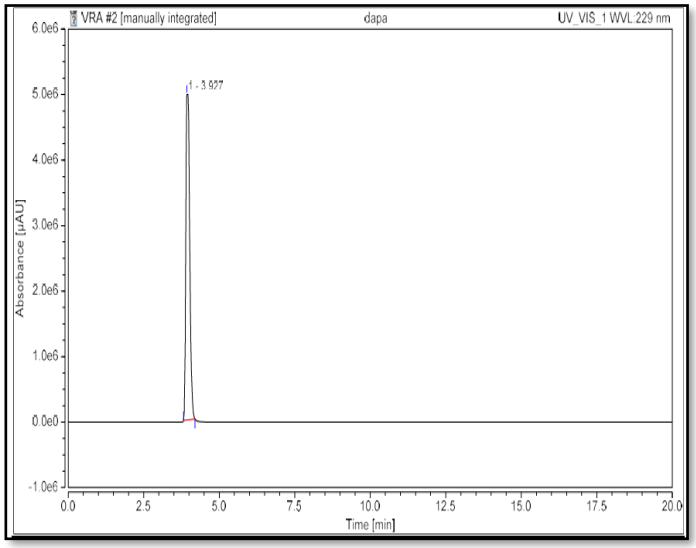


Fig.5 : HPLC Chromatogram of Dapagliflozin

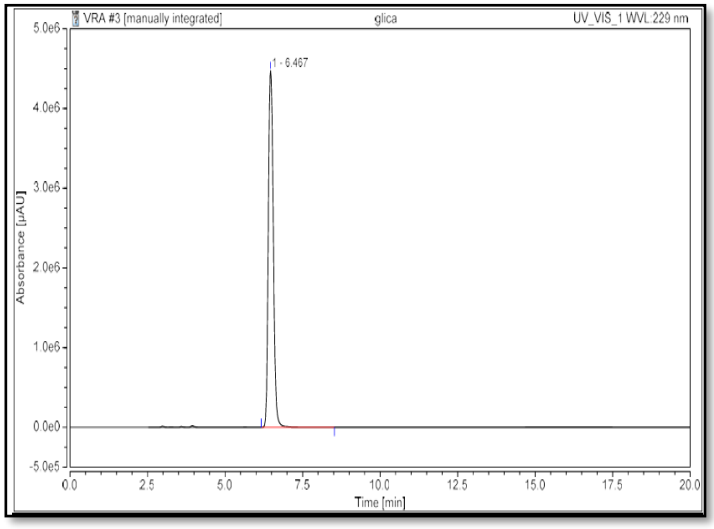


Fig.6 : HPLC Chromatogram of Gliclazide

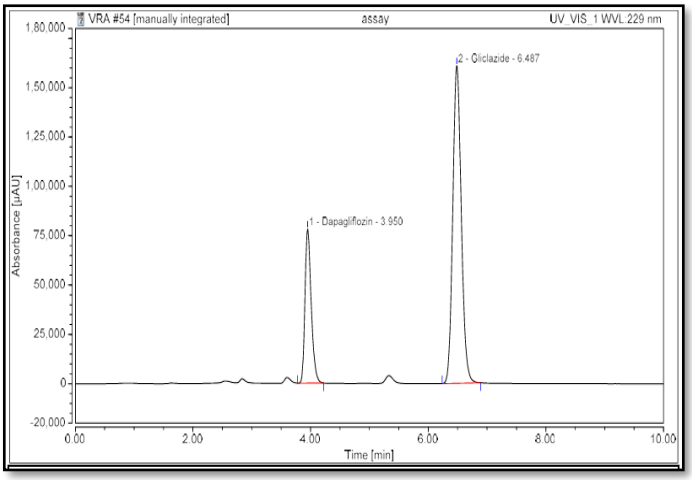


Fig.7 : HPLC Chromatogram of Tablet sample solution

System Suitability

The system suitability parameters measured under optimized conditions such as theoretical plates (N) for Dapagliflozin 7188 and Gliclazide 10129, tailing factor 1.36 for DAPA & 1.16for GLICLA ≤2.0, showed acceptable limits where Rs ≥2.0 was the initial resolution between two peaks confirming adequate separation of peaks from both analytes. The retention times of Dapagliflozin and Gliclazide were 3.940 ± 0.05 min and 6.473 ± 0.05min respectively.

Table 4: System Suitability

Parameters	Dapagliflozin	Gliclazide
Retention Time	3.940	6.473
Theoretical plates	7188	10129
Tailing factor	1.36	1.16

Plotting of Calibration curve (Linearity)

Linearity of the developed HPLC method was performed using 2-10µg/ml calibration standards of Dapagliflozin analyzed at 229nm. Calibration curve was plotted between concentration and area. The correlation coefficient of the linearity was found to be 0.9964.

Table 5: Calibration curve of Dapagliflozin

Conc. (µg/ml)	Area (µAU*sec)
2	153152
4	294014
6	432907
8	565007
10	748244

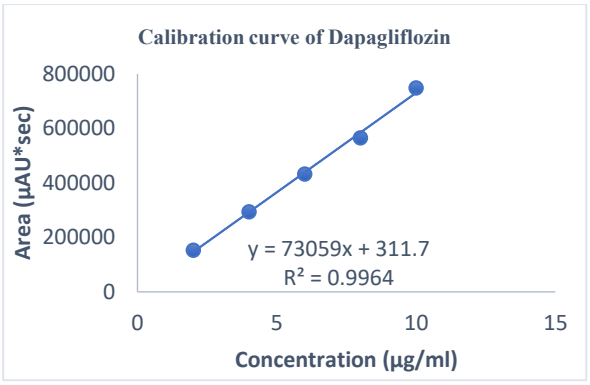


Fig.8: Calibration curve of Dapagliflozin

Linearity of the developed HPLC method was performed using 2-10µg/ml calibration standards of Gliclazide analyzed at 229nm. Calibration curve was plotted between concentration and area. The correlation coefficient of the linearity was found to be 0.9981.

Table 6 : Linearity for Gliclazide

Conc. (µg/ml)	Area (µAU*sec)
2	120598
4	229701
6	352655
8	462690
10	7603410

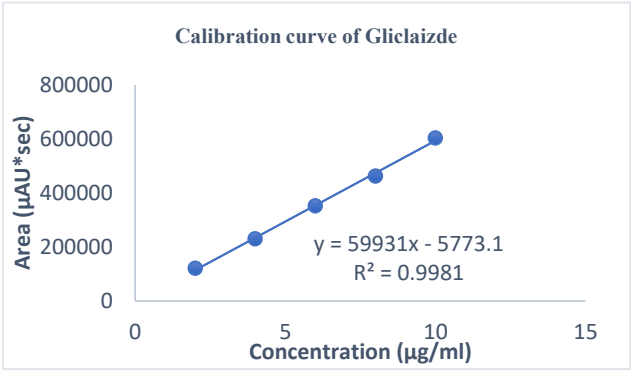


Fig.9: Calibration curve of Gliclazide

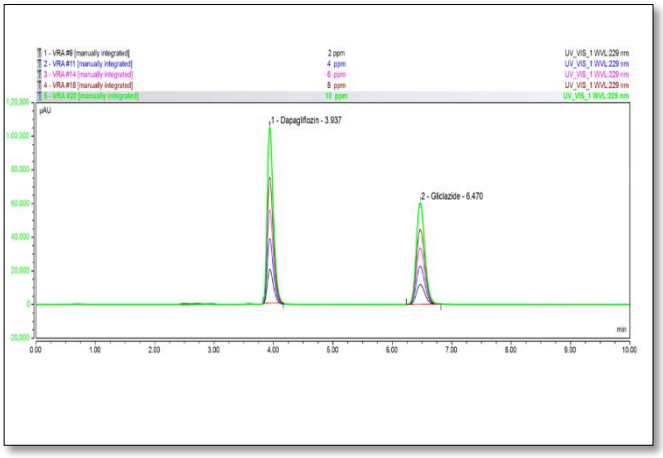


Fig 10 : Overlay chromatogram of Linearity

Precision

Repeatability

Table 7: Repeatability of DAPA and GLICLA

Sr.No.	Conc. (µg/ml)	Area(µAU*sec)	
		DAPA	GLICLA
1.	6	564446	455437
2.		561289	452453
3.		560362	453026
4.		560879	450726
5.		563590	458473
6.		558036	458642
Mean		561433.7	454642
± SD		2312.7	3136.2
%RSD		0.4119	0.6898

A repeatability study of Dapagliflozin and Gliclazide were carried out based on the peak area of 6µg/ml solution; six repetitions at 229nm. The retrieved area was already observed to be very similar with minimal deviation of standard deviation 2312.796 and %RSD for Dapa and for Glicla is 3136.2 and %RSD 0.6898 accordingly.

Intermediate Precision

Intermediate Precision Intermediate precision was determined by analyzing Dapagliflozin and Gliclazide at 8 µg/ml, over three consecutive days. The %RSD values for Dapagliflozin was 0.149, 0.380 and 0.457% respectively. For Gliclazide the %RSD values 0.122%,0.311% and 0.954% respectively. The %RSD values for both drugs were within the acceptable limit of 2% as per ICH Q2 (R2) guidelines.

Table :8 Intermediate Precision of Dapagliflozin

Conc. (µg/ml)	Area (µAU*sec)			Mean	(±) SD	%RSD
	Day 1	Day2	Day3			
8	567520	564446	560879	568503	852.31	0.149
8	568953	561289	563590	562032	2141.06	0.380
8	569036	560362	566035	563501	2579.14	0.457

Table 9: Intermediate Precision of Gliclazide

Conc. (µg/ml)	Area (µAU*sec)			Mean	(±) SD	%RSD
	Day1	Day2	Day3			
8	462290	455137	450726	461690	564.26	0.122
8	461610	452453	458473	453538	1413.53	0.311
8	461170	453026	458036	455745	4352.07	0.954

LOD and LOQ parameters

Limit of detection and limit of quantification were determined by standard deviation method of response and slope of calibration curve. LOD and LOQ were found to be 0.725µg/ml and 2.20µg/ml for Dapagliflozin and 0.615µg/ml and 1.86µg/ml for Gliclazide respectively.

Table 10: LOD and LOQ parameters

Sr.No.	Parameter	Dapagliflozin	Gliclazide
1.	LOD (µg/ml)	0.725	0.615
2.	LOQ(µg/ml)	2.20	1.86

Accuracy

Standard deviation was determined by performing recovery studies by standard addition method at three spiking levels i.e. 80%,100% and 120% in the pre analysed sample (10 µg/ml). At 10µg/ml the percentage recovery were calculates to found be 101.9%,101.1%(w/w)& 98%(w/w) at 80%,100% and 120%. % recovery of Dapagliflozin at both levels of concentration was found to be in the limits (98% - 102%) as mentioned in ICH Q2(R2).

Table 11 : Recovery study of DAPA and GLICLA

Drug	Level	Conc. (µg/ml)	Amt. Spiked (µg/ml)	Total Amt. (µg/ml)	Mean Area (µAU*sec)	Amt. Found (µg/ml)	% Recovery (w/w)
DAPA	80%	10	8	18	1050982	18.34	101.9
	100%	10	10	20	1158726	20.23	101.1
	120%	10	12	22	1244491	21.72	98
GLICLA	80%	30	24	54	2910130	54.14	100.20
	100%	30	30	60	3227089	60.04	100.00
	120%	30	36	66	3489307	64.92	98.37

Standard deviation was determined by performing recovery studies by standard addition method at three spiking levels i.e. 80%,100% and 120% in the pre analysed sample (30 µg/ml). At 30µg/ml the percentage recovery were calculates to found be 100.20%,100.00% (w/w) & 98.37%(w/w) at 80%,100% and 120%. %recovery of Gliclazide at both levels of concentration was found to be in the limits (98% - 102%) as mentioned in ICH Q2(R2).

ROBUSTNESS

Small intentional changes (± 0.1 ml/min in flow rate and ±2nmin detection wavelength) were implemented to assess the robustness of the HPLC method that was developed. Varied Flowrate 1.0 ml/min-2 : When flow was changed to end the area, peak area of Dapagliflozin and Gliclazide.

Table 12: Robustness parameter for DAPA and GLICLA

Conc. (µg/ml)	Area of DAPA (µAU*sec)		Area of GLICLA (µAU*sec)	
	Flow rate 0.9ml/min	Flow rate 1.1ml/min	Flow rate 0.9ml/min	Flow rate 1.1ml/min
8	622088	515014	504618	415279
	631443	515183	510836	416121
	634036	51544	510669	413955
Mean	629189	515212.67	508707.7	415118.3
(±) SD	6284.82	215.04	3542.73	1091.90
% RSD	0.9988	0.0417	0.6964	0.2630

Table 13:Robustness study Change in wavelength

Conc. (µg/ml)	Area (µAU*sec) DAPA		Area (µAU*sec) GLICLA	
	At 227nm	At 231nm	At 227nm	At 231nm
8	591719	471418	3121434	311933
	592107	472041	321192	311742
	591111	472138	320959	311728
Mean	591645.7	471865.7	321195	311701
SD	502.03	390.712	237.51	254.98
%RSD	0.0848	0.0525	0.0739	0.0818

Table 14: Summary of Validation Parameters as per ICH Q2(R1)

Parameters	Dapagliflozin	Gliclazide
λmax (nm)	224nm	228nm
Detection wavelength (λ)	229nm	229nm
Beers law limit(µg/ml)	2-10µg/ml	2-10µg/ml
Retention Time	3.937	6.470
Correlation coefficient (r)	0.9964	0.9981
Regression equation (y=mx+c)	y= 73059x+311.7	y = 59931x-5773.1
Slope (s)	73059	59931
Intercept (c)	311.7	5773.1
Mean Standard deviation of response(σ)	16045.7	11163.00
LOD (µg/ml)	0.725	0.615
LOQ (µg/ml)	2.20	1.86

5. CONCLUSION

In the present work, a new simple, fast, specific and robust QbD RP-HPLC method has been developed and validated for simultaneous estimation of Dapagliflozin and Gliclazide in bulk and tablet dosage forms. Using risk assessment and Design of Experiments for method development this foundation can be productively used in a systematic way to develop complementary methods that are reproducible and robust. The optimized HPLC method is achieved through Design of Experiments (DoE) which indicates flow rate and mobile phase composition as critical parameters influencing peak area and retention time. Obtained conditions for optimization (flow 1.1 mL/min, 60:40 Acetonitrile:0.1%Orthophosphoric acid buffer) resulted in sharply separated peaks with the reproducibility of retention. The robustness of the models was confirmed using statistical analysis with a high R^2 and significant ANOVA. The validated HPLC method in the present study was confirmed as specific, precise and accurate (99.25%–100.42% recovery) and linear over a range of 2–10 µg/mL for all parameters. The method complies with all the validation requirements of ICH-Q2 (R1) and can be used for routine quality control analyses of these antidiabetic agents in pharmaceutical manufacturing and research laboratories.

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