Development of a Validated Highly Sensitive and Eco-Friendly Approach for the Simultaneous Determination of Dapagliflozin and Gliclazide in Bulk and Tablet Formulation by RP-HPLC Method

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ABSTRACT

A novel, green, rapid, economical, simple, sensitive, robust, specific, and linear DAPA and GLIC method was developed for simultaneous determination in bulk and tablet formulation by the RP-HPLC method with greenness assessment tools. The method was based on the HPLC separation of a drug on reverse phase using Lichrospher 100 RP-18e (250 mm × 4 mm, 5µ) column with isocratic elution with 0.01% formic acid: acetonitrile (30:70, v/v) at room temperature with a flow rate of 0.8 ml/min at the detection wavelength of 219 nm and the chromatographic run time within 5 min for the mixture. The linearity calibration curves were linear over a concentration range of 0.1 – 20 µg/ml for both drugs. The correlation coefficient was found to be 0.9995 for dapagliflozin and 0.9994 for gliclazide, respectively. The methods were validated in terms of accuracy, precision, system suitability, robustness, and specificity of the same solution within the acceptable range of less than 2 % RSD. This method proves highly effective for routine quality control analysis of the examined drugs in the pharmaceutical industry, and less harmful to living begins and ecological footprint.

Keywords: *DAPA, GLIC, RP-HPLC, Green, ICH Q2 (R1).*

1. INTRODUCTION

Dapagliflozin (DAPA), chemically (2S, 3R, 4R, 5S, 6R)-2-[4-Chloro-3-(4-ethoxy benzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3, 4, 5-triol, belongs to the SGLT 2 (sodium-glucose co-transporter 2) inhibitor class of drug[1]. Gliclazide (GLIC), chemically N-(hexahydrocyclopenta[c]pyrrol-2(1H)-ylcarbamoyl)-4-methylbenzenesulfonamide, belongs to the sulfonyl urea receptor 1 (SUR1) class of drug[2]. DAPA and GLIC drug is used for non-insulin-dependent diabetes mellitus (NIDDM), which is also known as type 2 diabetes mellitus shown in Fig. 1 and 2, respectively. In case metformin monotherapy fails, a Fixed-Dose Combination of DAPA (10 mg) and GLIC (30 mg) is used. It is approved by the CDSCO (Central Drugs Standard Control Organization) to grant permission for manufacturing and marketing in October 2024. The literature survey revealed that analytical methods in UV spectrophotometric methods, RP - HPLC methods, and HPTLC methods were reported for DAPA and GLIC individually and in combination with others[3–14]. Due to the vital significance of this combination, more quality control evaluation and validated methods are essential for its quantification. The aim and objective of the proposed method are to the determination of DAPA and GLIC by RP – HPLC method and green assessment tools. It is useful to the environmental and pharmaceutical industry as well as research laboratories to analyze in combination with the marketed formulation.

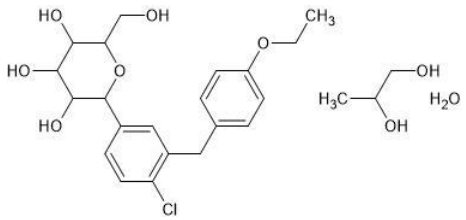

Fig. 1: Dapagliflozin

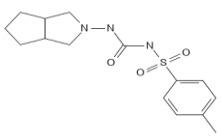

Fig. 2: Gliclazide

2. MATERIALS AND METHODS

Chemical and reagents

Drug samples of DAPA and GLIC were provided as gift samples by Pons Laboratories, Tamil Nadu, India, and Atlas Life Sciences Private Limited, Gujarat, India. A film-coated bilayered tablet manufactured by Eris Lifesciences Limited and purchased from a local pharmacy store. A solvent like water (HPLC grade) and formic acid were from Thermos Fisher Scientific India Ltd, Mumbai, India. Acetonitrile (HPLC grade) was from Loba Chemie Pvt. Ltd., Mumbai, India.

Instrument

HPLC instrument was Shimadzu conspicuousness UPLC (Shimadzu Enterprise, Kyoto, Japan) is prepared with an LC-20AD pump and an SPD-M40A photodiode cluster locator. Chromatograms were recorded and coordinated on a PC introduced with LabSolutions chromatographic computer program, form 5.97 SPI. Expository adjust from Scale-Tee advanced adjust (Scale Tec Mechatronics Pvt. Ltd., Vadodara, India) and Ultra-Sonicator from Leelasonic ultrasonic cleaner (Leelasonic, Mumbai, India).

Preparation of stock solution

Ten milligram of DAPA and GLIC were weighed and exchanged to the particular 10 ml standard jar (blend arrangement). Broken down it with acetonitrile and the volume was made up to the check to urge a concentration of 1000 µg/ml. from each stock solution, 1 ml was weakened to urge a concentration of 100 µg/ml. At last, the working stock arrangement was made up to volume with acetonitrile to induce a concentration within the run of 0.1-20 µg/ml for DAPA and GLIC.

Preparation of the sample solution

Twenty tablets were weighed and finely powdered. The powder mass comparable to 10 mg and 30 mg of DAPA and GLIC was precisely weighed and exchanged to a 100 ml volumetric jar; broken down utilizing acetonitrile and sonicated for 20 min, made up to the volume with acetonitrile. The arrangement was sifted utilizing Whatman (No: 1) channel paper and reasonable aliquots were arranged and utilized within the instrument.

Greenness assessment tools

Analytical Greenness Calculator V.0.5 (Agree).

3. METHOD DEVELOPMENT AND OPTIMIZATION

Due to the critical contrast within the chemical and physical properties of DAPA and GLIC, a few portable stages, pH, portable stage proportion, ionic quality, stream rate, and determination of versatile stage as dissolvable were at first trailed to have both drugs eluted on the same chromatogram. The appropriateness of the column and versatile stage utilized within the optimized strategy has been decided based on selectivity and affectability in terms of crest sharpness, top symmetry, following figure, and determination between two peaks. To ensure the least clamor and to apportion with any undesirable dissolvable crests.

Selection of mobile phase

In the aqueous phase and organic phase, 100 % methanol, water: methanol, trifluoracetic acid: methanol, formic acid: methanol, ammonium acetate: methanol, ammonium acetate: acetonitrile, and glacial acetic acid: acetonitrile was tried, but formic acid: acetonitrile was observed good separation and sharpness.

Selection of pH

In a selection of pH, acetonitrile: ammonium acetate (5.45) and acetonitrile: ammonium acetate (4.5) tried, and obtained a sharpness of the peak for acetonitrile: formic acid (2.7).

Selection of mobile phase ratio

In the mobile phase ratio formic acid: acetonitrile (80:20), (70:30), (65:35), (60:40), (50:50). From that formic acid: acetonitrile (70:30) ratio gets an acceptable resolution.

Selection of ionic strength

The ionic strength of formic acid was tried at 0.01 % and 0.1 %. The two ionic strengths have shown good characteristic peaks, so the lowest ionic strength of 0.01 % formic acid is selected.

Selection of flow rate

The flow rate at 1 ml/min, 0.8 ml/min was initially tried. From that 0.8 ml/min has a symmetrical peak.

4. METHOD VALIDATION

The concurrent assurance of DAPA and GLIC was run in the settled chromatographic condition to be approved as per ICH Q2 (R1) rules.

System suitability

The system suitability parameters concerning theoretical plates, tailing factor, repeatability, and resolution between the drug and nearest peak were assessed by injecting a blank mobile phase followed by six replicates of DAPA and GLIC.

Specificity

The crest virtue of DAPA and GLIC was decided by crest immaculateness thinks about by comparing its UV range at three diverse districts of the spot i.e. top start (S), best summit (M), and best conclusion (E).

Linearity

The linearity of an informative procedure is its capacity (interior a given degree) to urge the test almost, which particularly compares to the concentration of the analyte within the test. Calibration benchmarks were arranged in concentrations extending from 0.1-20 µg/ml.

LOD and LOQ

The LOD and LOQ of DAPA and GLIC were assessed in triplicate for (50 and 100 %) and the recuperation (%) was calculated from the distinction between the sum of analyte shown within the pre-analyzed detailing to that of the standard DAPA and GLIC included to the detailing.

Accuracy

The exactness of the strategy was confirmed by repeatability and intra and inter-day exactness. The intra-day precision of the DAPA and GLIC was examined by infusing a standard arrangement of DAPA and GLIC inside the same day. The inter-day precision of the strategy was confirmed by rehashing the same strategy on distinctive days at the same research facility. The repeatability of the test application was moreover confirmed by infusing six times the standard arrangement of DAPA and GLIC. In all the cases RSD (%) of the top region was calculated.

Robustness

The vigor of the strategy was carried out within a variety of chromatographic conditions such as stream rate, versatile stage proportion, and pH of the buffer component was moreover assessed for determination between DAPA and GLIC top, number of hypothetical plates, and following calculation.

Stability

When the arranged arrangement is uncovered to the sir, the analyte is likely to break down; thus it is vital to conduct stability studies. For soundness thinks about, the arrangement of DAPA and GLIC was put away at fridge temperature. The rate deviation of the reaction was calculated to ponder the steadiness of the arrangement.

Assay of tablet formulation

The created LC strategy was connected for the assurance of DAPA and GLIC substances in tablet definition.

5. GREENNESS ASSESSMENT

Greenness assessments of the developed spectroscopic and chromatographic method were investigated using the greenness assessment tools viz. AGREE and Analytical Eco-Scale.

Analytical Greenness (AGREE)

AGREE is a metric tool in which the input criteria are based on the 12 significant principles of GAC. The 12 input variables were fed into the software and investigated for the greenness of the method.

Analytical Eco-Scale

The expository eco-scale aims to quantify the method's greenness. Penalty Points (PPs) serve as its foundation which has an ideal score of 100. Reagent toxicity, waste, and energy consumption PPs are subtracted from the ideal score of 100 to get the residual point.

The residual point > 75 demonstrates shows prevalent green investigation,

> 50 demonstrates worthy green investigation and

< 50 demonstrates lacking green investigation.

6. RESULTS AND DISCUSSION

Method development and optimization

In all trials of DAPA and GLIC to fix the chromatographic condition, variation of solvents in the aqueous and organic phase shows the poor peak separation of both drugs. The lowest acidity in the aqueous phase does not have good selectivity, peak shape, and retention time. The different mobile phase ratios will not obtain an acceptable resolution and ionic strength has shown good characteristics of the peak, from that ionic strength 0.01 % formic acid it will be useful to the analyst and eco-friendly. The flow rate of 1 ml/min in this chromatographic condition does not give a symmetrical peak.

Fixed chromatographic condition

Stationary phase: Lichrospher 100 RP-18e (250 mm × 4 mm, 5µ), LichroCART®250-4.
Mobile phase: 0.01% formic acid (pH 2.7): acetonitrile
Mobile phase ratio: 30: 70, v/v
Flow rate: 0.8 ml/min
Retention time: 3.1 min (DAPA), and 4.7 min (GLIC)
Detection wavelength: 219 nm
Column temperature: Room temperature

Using the optimized chromatographic conditions, DAPA and GLIC were well separated with acceptable resolution, Fig. 3.

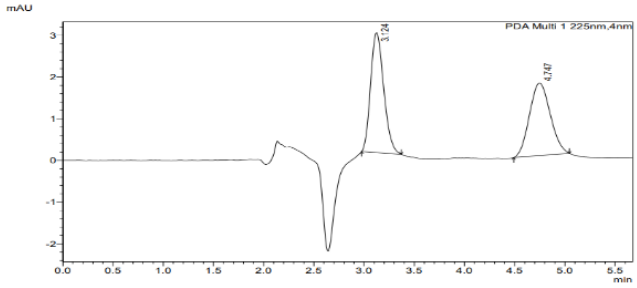

Fig. 3. Chromatogram of the standard mixture of DAPA and GLIC

System suitability

The DAPA and GLIC gave great crest shape inside a run time of 5 minutes. The number of hypothetical plates & the following variables were found to be inside the limits utilizing the created chromatographic conditions. The comes about of framework reasonableness is displayed in Table 1.

Specificity

Beneath specificity, the capacity of the explanatory strategy to degree the analyte reaction of API within the nearness of excipients was examined. The top of the analyte was spectrally unadulterated (top immaculateness file esteem = 0.999950, 0.999972 for DAPA and GLIC) and there were no co-eluting crests and no trouble in crest integration. Hence, the created strategy is particular to the assurance of DAPA and GLIC, Fig. 4 - 7.

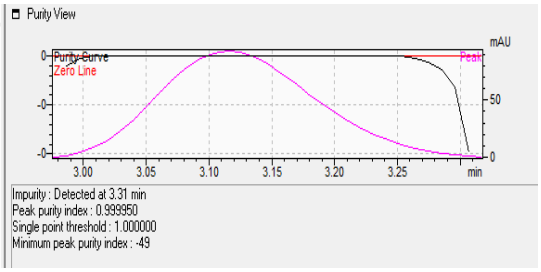

Fig. 4. Peak purity of dapagliflozin

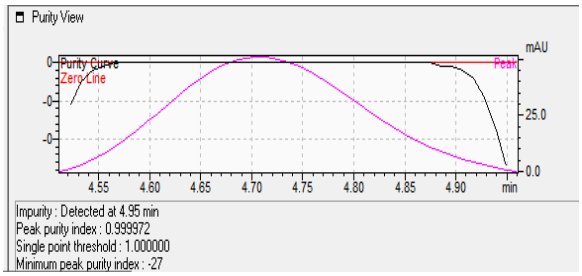

Fig. 5. Peak purity of gliclazide

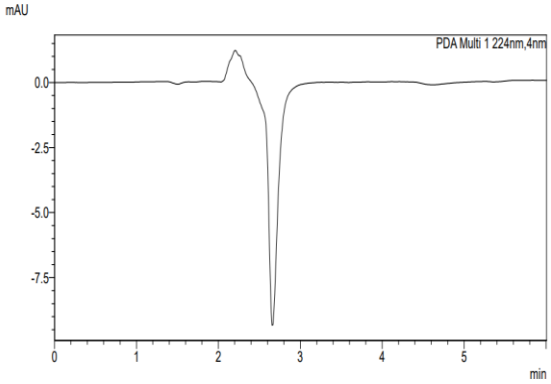

Fig. 6. Blank spectrum of acetonitrile

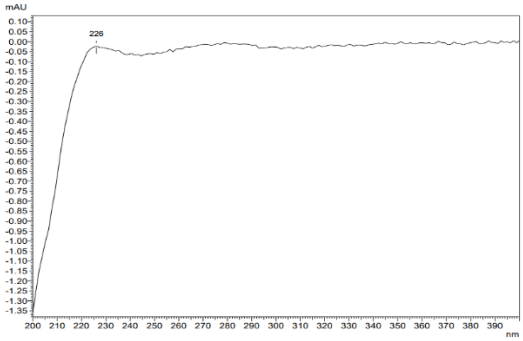

Fig. 7. Spectrum of blank

Linearity

The calibration range for DAPA and GLIC was 0.1 – 20 µg/ml. The relapse condition from the calibration plot for DAPA was y = 703322x + 3265.43 and the relationship coefficient esteem was found to be 0.9995, for GLIC was y = 63251.8x – 4400.63 and the relationship coefficient esteem was found to be 0.9994. The overcomes appeared a great relationship between the top zones and concentrations of DAPA and GLIC.

Accuracy

The recuperation (%) of DAPA and GLIC was found to be inside 2 % RSD, affirming the precision of the created strategy. The recuperation (%) values for DAPA and GLIC are given in Table 2.

Precision

The RSD (%) values for intra-day precision, inter-day precision, and repeatability of the test application for the test methodology were found to be interior 2 % RSD, asserting the exactness of the made technique. The results are shown in Table 3.

LOD and LOQ

LOD and LOQ values for DAPA were found to be 0.06 and 0.21 µg/ml.
LOD and LOQ values for GLIC were found to be 0.07 and 0.23 µg/ml.

Robustness

The vigor of the strategy was decided by utilizing varieties in the chromatographic condition such as stream rate, versatile stage proportion, and ionic quality were calculated as % RSD, Table 1.

Stability

The comes about of arrangement solidness appeared that the arrangement put away beneath fridge temperature conditions at a concentration of 5 µg/ml was unfaltering for up to 6h., Table 4.

Assay of marketed formulation

The test arrangement was infused, the chromatogram was recorded and the evaluation was done utilizing the relapse condition, Fig. 8. The comes about of the definition considers are appeared in Table 2.

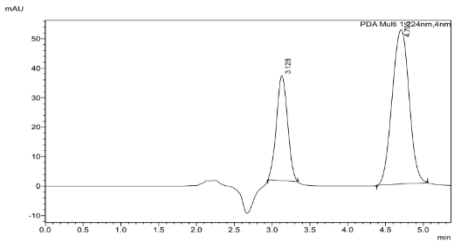

Fig. 8. Chromatogram of formulation

Table 1. System suitability and robustness study

Condition		Retention time (% RSD)		Tailing factor		Theoretical plate		Resolution
		DAPA	GLIC	DAPA	GLIC	DAPA	GLIC	
System suitability		0.20	0.18	1.11	1.12	2583	2354	5.09
Robustness	0.7 ml/min	0.58	0.66	1.27	1.22	3143	3157	5.24
	0.9 ml/min	0.82	0.86	1.13	1.16	2326	2555	4.65
	29: 71	0.55	0.40	1.22	1.13	2596	2962	4.84
	31:69	0.74	0.62	1.20	1.17	2659	2791	5.13

Mean of six determinations*

Table 2. Results of accuracy and assay

Drug	Accuracy				Assay			
	50 %		100 %		Amount of drug (mg/tablet)		% Label claim	% RSD
	% Recovery	% RSD	% Recovery	% RSD	Labeled	Estimated		
DAPA	101	0.48	99	0.66	5	5.11	102	0.19
GLIC	99	1.57	98	0.93	15	15.21	101	0.51

Mean of six determinations*

Table. 3. Precision study

Precision studies	Analyte	µg/ml	% RSD	Analyte	µg/ml	% RSD
Intra-day precision	DAPA	0.1	0.76	GLIC	0.1	0.72
		2	0.66		2	0.59
		8	1.45		8	1.33
Inter-day precision		0.1	1.76		0.1	1.40
		2	0.10		2	0.12
		8	0.37		8	0.75
Repeatability		6	0.52		6	0.79

Mean of six determinations*

Table 4. Stability study

Time (hours)	DAPA (peak area)	GLIC (peak area)
1	249194	293781
2	247336	291044
3	243313	289294
4	240125	287929
5	238348	285781
6	**237623**	**283011**

7. GREENNESS ASSESSMENT

The greenness profile of the created strategy was assessed in terms of 12 parameters of GAC like health, environment, energy, amount of waste, and safety using tools like Analytical eco-Scale, and AGREE.

Analytical Greenness (AGREE)

AGREE calculator was based on the 12 significant principles. The developed method was found to be moderately green using the AGREE greenness calculator tool (AGREE score: 0.62), shown in Fig. 9.

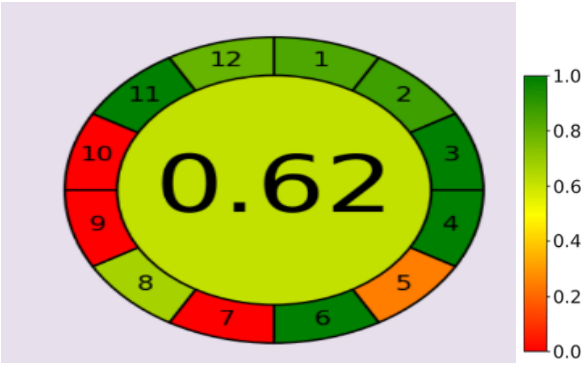

Fig. 9. AGREE score

Analytical Eco-Scale

In this tool Penalty Points (PPs) were calculated based on the four criteria amount of reagents used, energy consumption, occupational hazard, and waste. The residual PPs were calculated by subtracting the total PPs from the ideal value of 100, Table 5.

Table 5. Analytical Eco-Scale

S. No	Parameters	Penalty points
1.	Acetonitrile	8
2.	Formic acid	4
4.	Energy waste	1
5.	Waste	3
6.	Occupational hazard	8
Total		24

Analytical eco-scale score = 100-24(penalty points)
= 76

8. CONCLUSION

This method demonstrates exceptional sensitivity, specificity, linearity, and robustness, making it suitable for schedule investigation of both drugs either exclusively or in combination details. It is validated as per ICH guidelines Q2 (R1). Furthermore, its greenness assessment underscores its environmental friendliness, promoting health safety, energy efficiency, and waste reduction. This combined efficacy and eco-friendliness position it as a valuable tool for pharmaceutical analysis and contributes to sustainable practices in the field.

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