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Assessment of Dapagliflozin by Using HPLC- Method Development, Validation and Stability Indicating Studies

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ABSTRACT

This project's main objective is to develop and validate an RP-HPLC technique for dapagliflozin, an anti-diabetic medication that reversibly inhibits the human Sodium-Glucose Co-Transporter (SGLT2). The method should be easy to use, precise, and accurate. Methanol, ethanol, and isopropyl alcohol are among the organic solvents that dissolve it effectively. A p-value of 12.6 is provided. This experiment made use of the Column-C18 (5µm; 4.6×250mm). Acetonitrile and 1% IPA were combined in an 80:20 (v/v) ratio to create the selected mobile phase. 10 µg/ml was the injection volume, and 1.0 ml/min was the flow rate. A duration of retention of 2.9 minutes at 30°C was measured at 224 nm. With a r^2 value of 0.9995, the range of linearity was found to be 5-100 µg/ml. A range of 98-102% was seen for the recovery. The respective limits of detection were 0.62 ɛg/ml and 0.2 ɛg/ml. The stress test results demonstrate that even in the presence of degradants, the method was still able to identify the medication.

Keywords: *Dapagliflozin, Assay, stability studies, validation, ICH Guidelines.*

1. INTRODUCTION

Dapagliflozin1 is an oral, reversible inhibitor of the human sodium-glucose co-transporter 2 (SGLT2), the main transporter in charge of renal glucose reabsorption. It reduces glucose reabsorption and inhibits the Sodium-Glucose Co-Transporter 2, both of which contribute to better glycaemic management in type 2 diabetic patients. The selection of Dapagliflozin as a single medicine for analysis was based on the fact that there were just three articles reported on this drug.

In 2015, Jeyabaskaran et al.2 published the analytical technique for RP-HPLC Dapagliflozin estimation. The stationary phase was Column-Hypersil BDS C18 (250 x 4.6 x 5 µm), and the mobile phase liquids were Acetonitrile: 0.1% OPA buffer (50:50 v/v). The instrument was set to a flow rate of 1.0 millilitres per minute, and the elution time was determined to be 2.226 minutes. With an R2 of 0.9998, the linearity range that was stated was 25–150µg/ml. The Wave length of 245nm was considered, LOD (0.04µg/ml) and LOQ- (9.121µg/ml) was also reported. Mitali *et al.*[3] in 2017 reported similar mobile phase except OPA buffer replaced with phosphate buffer and the ratio of mobile phase was (40:60 v/v). The column employed was C_{18} (4.6mm, 150mm, 5µm). The Flow rate-was set at 1ml/min for which the retention time was reported as 3.160 min at the selected wavelength of 222nm. There was a small change in the linearity range (50-150 µg/ml) with a R^2 = 0.999 than the earlier reported method. The reported LOD, LOQ were - 5.14µg/ml and 15.6µg/ml respectively. Mante *et al.*[4] in 2018

also worked on the same drug with Column-C_{18} with a flow rate of 1ml/min and elution time was found to be 5.163min.

They employed Acetonitrile: 0.1%Triethylamine (pH-5.0) (50:50 v/v) as mobile phase component. The reported linearity range was 10-70 µg/ml with R^2 0.999

The wavelength was two units more than the above mentioned method. The LOD,

2.1µg/ml and 6.39µg/ml were the LOQ values, respectively. Dhanshri et al. 5 developed and validated the HPLC method for identifying dapagliflozin and its impurities in tablet dosage form. To ascertain the chromatographic separation of the medication and its contaminants, I employed a Hypersil BDS C18 column (250 mm × 4.6 mm, 5 µ) with an ultraviolet detector at 245 nm and a gradient program operating at 1 mL/min. Mobile phases A (Buffer pH 6.5) and B (acetonitrile: water 90:10) made up the mobile phase. Afshan et al.6 created an RP-HPLC method to assess Dapagliflozin and Metformin concurrently in bulk and in synthetic combinations. Gajanan et al.7 tried to develop straightforward, accurate, and exact UV-spectrophotometric techniques for dapagliflozin estimation. Priyanka et al. (8) examined the literature and contrasted the estimations of dapagliflozin and metformin HCl in bulk and pharmaceutical dose forms.

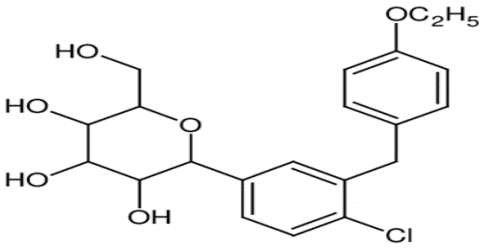

Fig. 1 Chemical structure of Dapagliflozin

2. MATERIAL AND METHODS

Material

The equipment employed includes a digital ultrasonic cleaner (SONICA 2200MH), a hot air oven (INFRA DIGI ISO 9001-2015), an electronic balance (Shimadzu ATY224), ultraviolet visible spectroscopy (Shimadzu UV-1800), high-performance liquid chromatography (HPLC) (Shimadzu LC-20AD), etc.

Chemicals

JSL Health Sciences Private Limited in Hyderabad provided the gift sample of dapagliflozin, together with acetonitrile (HPLC grade, Merck), methanol (HPLC grade, Merck), water for HPLC (Merck), and isopropyl alcohol (HPLC grade, Merck).

Method:

Making a regular stock solution for the UV technique

In order to make the standard stock solution, 10 milligrammes of dapagliflozin were dissolved in 10 millilitres (10 milligrammes per millilitre) of acetonitrile, 1% isopropyl alcohol, and water in a volumetric flask.

The secondary standard stock solution for the UV technique was created by pipetting 1 ml of the stock solution and using a 10 ml volumetric flask to increase the volume to 10 ml (20 stock solution 100µg/ml).

Chromatographic condition in RP-HPLC

A Shimadzu Corporation HPLC system, complete with a reservoir tray, column oven, and detector (PDA), was used for the analysis. The mobile phase is an acetinitrile:1% isopropyl alcohol (80:20, v/v) mixture, while the reverse phase column is 250mm long, with 4.6 internal diameters and 5 µm particle size packing. To create the Dapagliflozin standard solution, 10 milligrammes of the drug was dissolved in 10 millilitres of methanol, and further dilutions were carried out using the mobile phase to reach a concentration of 10µg/ml. A micropore filter with a pore size of 0.45µ was used to filter both the mobile phase and the medication solution. A variety of Dapagliflozin dilutions, spanning from 5 to 100µg/ml, were made. Chromosomes were recorded when the solutions were fed into the system using a 20 µl fixed loop at a flow rate of 1 ml/min, with the effluents being monitored at 224 nm. Eluted at 2.9 minutes was the peak. As a pharmaceutical tablet, Dapagliflozin can now be determined using an expanded version of the technique. The medication was taken in the form of a tablet with a 10 mg strength. 10 milligramme (mg) Dapagliflozin tablets were ground in a glass mortar and then transferred to a 10 millilitre (ml) volumetric flask along with diluents (mobile phase) to bring the total volume to 10 ml. A working standard of 50µg/ml was achieved by performing additional dilutions using the mobile phase. Using micropore filter paper with a pore size of 0.45µ, the solution was filtered. In order to determine the drug concentration in the tablet sample solution, the peak area was compared to the standard. While the API analyses factors including system appropriateness, linearity, LOD, and

LOQ, the tablet powder studies precision, accuracy, robustness, and degradation. The ICH criteria were followed during the validation of the suggested approach.

3. RESULTS AND DISCUSSION

UV Spectrum of the Dapagliflozin

System suitability

Standard solutions that had already been prepared were used to flush the chromatographic equipment. According to the system suitability studies, all measures, including Rt (2.921 min), theoretical plates (2574), tailing factor (1.978), peak area (2677518), and HETP (58.279), were found to be within the designated limits. Table 1 lists the characteristics for system appropriateness, and Figure 2 displays the chromatogram for system suitability.

Table 1: System appropriateness characteristics for the HPLC method

S. No	Peak Area	Retention Time	PlateCount	Tailing
1.	2677518	2.921	2574	1.978
2.	2632025	2.922	2556	1.984
3.	2681339	2.920	2527	1.979
4.	2678912	2.924	2554	1.985
5.	2653120	2.925	2574	1.980
6.	2655349	2.926	2558	1.946
Average	2663043.83	2.92	2557.17	1.26
STDEV	19574.85	0.00	17.26	0.01
% RSD	0.74	0.08	0.67	1.16
Limits	_	_	>2000	<2.0
% RSD	<2.0	<2.0	<2.0	<2.0

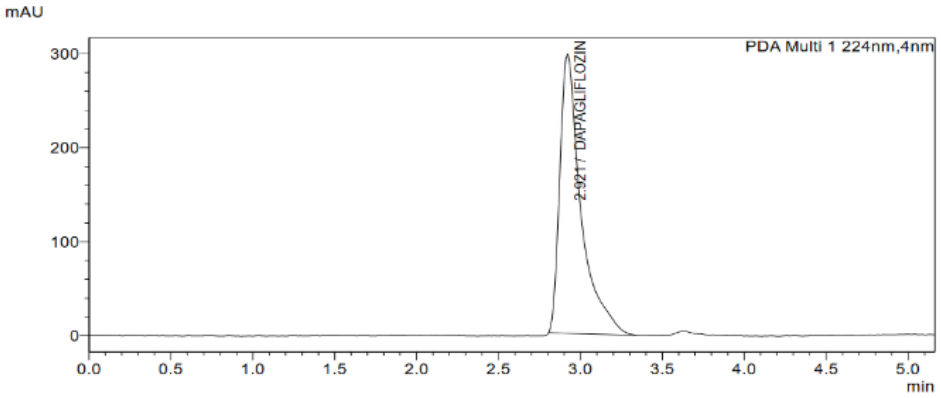

Fig. 2 System suitability chromatograms

Linearity

Dapagliflozin was determined to have a linearity range of 5-100µg/ml. A value of 0.999 was determined for the correlation coefficient. Table 2 displays the data, and Figure 3 displays the linearity curve, which were generated by graphing the peak area against the drug concentration.

Table 2: Linearity of the suggested HPLC technique

S. No	Linearity level	Conc. (µg/ml)	Peak area
1	50%	05.00	0421995
2	75%	27.50	1348597
3	100%	50.00	2677518
4	125%	75.00	3768531
5	150%	100.00	5329735

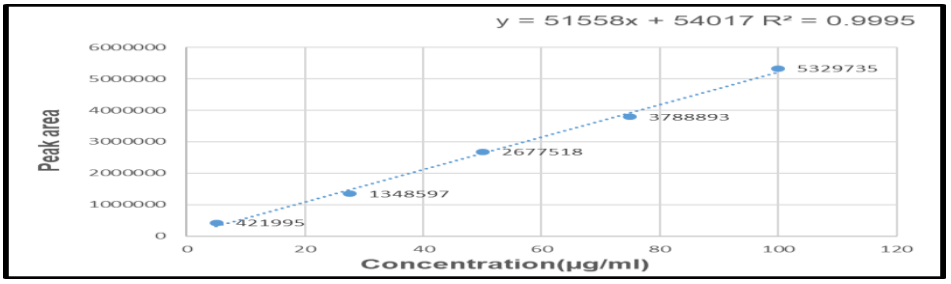

Fig. 3 Linearity graph of Dapagliflozin

Precision

The chromatographic method was shown to be within limits when a 100% concentration was injected and the %RSD was less than 2. Tables 3 and 4 display the values.

Table 3: Inter-day accuracy for the suggested HPLC technique

S. NO	Day-1	Day-2	Day-3
1.	2837946	2884974	2866873
2.	2857773	2898716	2870970
3.	2846599	2891446	2859344
4.	2847642	2853399	2898716
5.	2865564	2827183	2889104
6.	2886802	2826152	2895765
Average	2857054.33	2863645.00	2880128.62
STDEV	17440.69	32551.44	16505.37
% RSD	0.61	1.14	0.57
Limits	% RSD <2.0	% RSD<2.0	% RSD <2.0

Table 4 Intra-day Precision

S.NO	9:00 AM	1:00 PM	5:00 PM
1	2763292	2796715	2780293
2	2791742	2789595	2786451
3	2773902	2777669	2769311
4	2778603	2718140	2752895
5	2781240	2791032	2789930
6	2786426	2784060	2760688
Average	2779200.83	2776201.83	2773261.33
STDEV	9951.56	29172.52	14763.04
% RSD	0.3	1.05	0.53
Limits	% RSD <2.0	% RSD <2.0	% RSD <2.0

Accuracy

By combining a tablet working solution with a standard solution, a series of solutions ranging from 50% to 150% were generated. Analysis of the chromatograms produced by the aforementioned concentrations revealed that the percentage recovery fell within the specified range, namely, 100-102%. Table 5 displays the values that were obtained.

Table 5: Accuracy of the suggested HPLC technique

Accuracy level	Peak area	Conc. taken (ug/ml)	Conc. Added (ug/ml)	Conc. Found (ug/ml)	% Recovery	Mean % Recovery
50%	3930638	50	25	75.18	100.75	100.76
	3930638	50	25	75.18	100.75	
	3930638	50	25	75.18	100.75	
100%	5280350	50	50	101.36	102.73	101.72
	5254177	50	50	100.86	101.72	
	5227747	50	50	100.34	100.69	
150%	6561037	50	75	126.20	101.61	101.86
	6585940	50	75	126.69	102.25	
	6565339	50	75	126.29	101.72	

LOD & LOQ

Figures 4 and 5 summarise the chromatograms, and Table 6 lists the parameters, which include the limit of detection (LOD) of 0.2µg/mL and the limit of quantification (LOQ) of 0.6µg/mL.

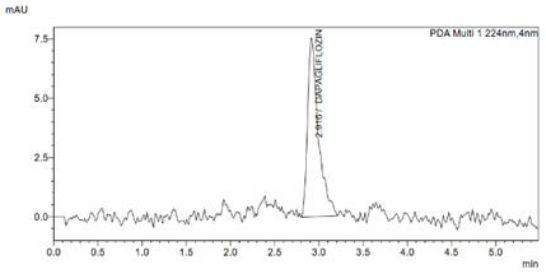

Fig 4 Chromatograms of LOD

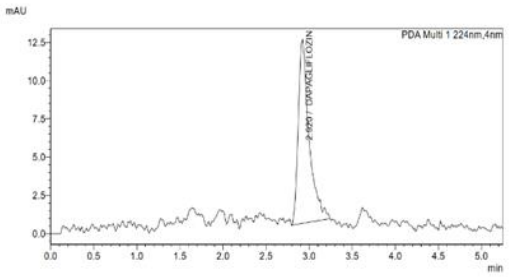

Fig. 5 Chromatograms of LOQ

Table 6: LOD and LOQ for the suggested HPLC technique

Parameters	Slope from Linearity	SD of peak from lowest concentration
	51558	**3186.94**
LOD = 3 x SD/Slope	0.2µg/ml	

LOQ = 10 x SD/Slope	0.6µg/ml

Robustness:

We injected the chromatographic apparatus with a 100% concentration sample solution that we had produced and the values that were observed fall within the acceptable range.

DEGRADATION STUDIES

The following degradation studies were performed.

- Degradation of acids
- Degradation of bases
- Degradation of peroxide
- Degradation by photolysis
- Degradation by UV

When the medication was deteriorated to roughly 10%, the degradation experiments show that the devised method was sensitive enough to perform the analysis of the Dapagliflozin drug even in the presence of the unknown degradants. The results are shown in Table 7.

Table 7: Degradation analyses for the suggested HPLC technique

S. No	Condition	Peak Area	% Assay	% Degradation
1.	Degradation of acids	2487628	92.9	7.1
2.	Degradation of bases	2498617	93.3	6.6
3.	Degradation of peroxide	2425744	90.5	9.4
4.	Photolytic Degradation	2411872	90	9.9
5.	UV Degradation	2488742	92.9	7.0

4. CONCLUSION

The pharmaceutical tablet dosage form of dapagliflozin and its active pharmaceutical ingredient (API) can be quantified with the help of RP-HPLC. Adherence to ICH Q2R1 and ICH Q1A (R2) standards allowed for the method's successful validation. The linearity was in the range of 5µg/ml to 100µg/ml, and we saw theoretical plates well beyond 2000. The fact that the %RSD values for accuracy, precision, and robustness are all less than two indicates that the parameters are within the predefined range. The results showed that the limits of detection (LOD) were 0.2µg/ml and 0.6µg/ml, respectively. Degradation studies were also conducted on the tablet dosage type.

When compared to other solvents mentioned in the literature, the mobile phase consisting of acetonitrile and IPA is more cost-effective. In comparison to the approaches that have been described, the lowest limit for linearity, which is 5 µg/ml, is the least. Therefore, when compared to other approaches, ours has a high level of sensitivity. There will be a marked decrease in analysis time due to the very short retention time of 2.91 minutes. Compared to the published approaches, the LOD and LOQ are superior. For regular analysis of dapagliflozin in API and its pharmaceutical tablet dosage form, this approach is sensitive, affordable, repeatable, and quick.

ASSERTIVE

The facilities needed to conduct this research were provided by the management, principal, and department head of Santhiram College of Pharmacy in Nandyal, Andhra Pradesh, India, for which the authors are very grateful.

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