International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 2 Mar-Apr 2022, pp: 112-120 www.ijprajournal.com ISSN: 2456-4494

RP-HPLCAssay Method Development and Validation of Dapagliflozin in TabletDosage Form

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Submitted: 01-03-2022 Accepted: 13-03-2022

ABSTRACT: A Simple, fast, precise and accurate method was developed and validated for assay of Dapagliflozin in tablet dosage form.Theseparation was done using a NourynKromasil C18column(4.6x250mm,5µ particle size) with mobile phaseas phosphate buffer pH 2.9, acetonitrile intheproportionof 60:40(%v/v), flowratewas1.2mL/min,column temperature 30°C, sampler temperature 15°C, having injection volume 20µL anddetection at 224 nm. Retention time of Dapagliflozin found about 7.5 min. In validation study precision showed results with RSD less than 2.0%, the accuracy found within the range of 98% to 102%, the method found linear in the range of 10 µg/ml to 60 µg/ml (Coefficient correlation =0. 999).

KEYWORDS: Dapagliflozin, RP HPLC, Validation.

I. INTRODUCTION

Dapagliflozin is the first in a novel class of glucose-lowering agents known as sodium-glucose co-transporter-2 (SGLT2) inhibitors and is used in the treatment of patients with type 2 diabetes. [1]

Inhibitor of renal sodium-glucose cotransporter 2, allows an insulin-independent approach to improve type 2 diabetes hyperglycemia.[2] Dapagliflozin propanediol monohydrate is a hydrate that consists of dapagliflozin compounded with (S)-propylene glycol and hydrate in a (1:1:1) ratio.[3]

Chemically Dapagliflozin is (1S)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) Methyl]phenyl]-D-glucitol.[4]TheDapagliflozinisawhite to off white,crystallinepowder which issoluble in Ethanol,Methanol, dimethyl sulphoxide and dimethyl formamide. (4). It has molecular formula $C_{24}H_{33}ClO_8$ with molecular weight 408.98.[5] IUPAC name of Dapagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol.[6]Gliflozin is a new class of anti-diabetic drugs, these inhibits subtype 2 of sodium-glucose transport proteins (SLGT-2 protein). Various new gliflozin drugs are under clinical trials. USFDA has approved some new gliflozin drugs like dapagliflozin, canagliflozin, empagliflozin, tofogliflozin, ertugliflozin, ipragliflozin, romogliflozinetabonate, sotagliflozin.[7]

Dapagliflozin acts by inhibiting SLGT-2 proteins which are responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine.[8]

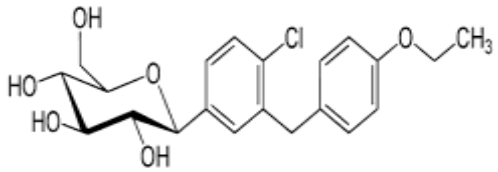

Fig.1 Structure of Dapagliflozin[9]

II. EXPERIMENTAL WORK

Materials and Methods

Chemicals and Reagents

Potassium dihydrogen orthophosphate,Methanol, Acetonitrile, Mili Q Water, Orthophosphoric Acid. Dapagliflozin Propanediol Monohydrate Working Standard, Diabiz Tablets 10 mg (Blue Cross laboratories Pvt. Ltd.)

Instruments

HPLC(Waters),UV Spectrophotometer(Shimadzu), pH Meter (Labindia), Analytical Balance (Sartorius), Sonicator (PCi Analytics), Magnetic Stirrer (REMI), Centrifuge (REMI).

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Column
NourynKromasilC18column(4.6x250mm,5µ particle size).

Filters
Whatman No. 1, Whatman No. 41, 0.45µ Nylon syringe filter.

Method Development

DeterminationofλmaxofDapagliflozin Propanediol Monohydrate
The 50µg/ml standardsolution of Dapagliflozin wasscanned in the scaleof 200-400 nm and λ max observed at224nm in Methanol. (Refer Fig.3).

PreparationofBuffersolution
Dissolve 6.8gmofPotassium dihydrogen orthophosphate in 1000 ml inMilli-Q water. Adjust the pH was adjusted to2.9 ±0.02with Orthophosphoric acid. Filter through 0.45µm Nylon Membrane filter.

Mobilephase
Prepare a mixture of BufferpH 2.9 and acetonitrile inthe proportion 60:40 v/v, respectively. Mix welland sonicate for 15 minutes.

Diluents
Prepare a mixture of BufferpH 2.9, acetonitrile and methanol in the proportion 30:20:50 %v/v.

PreparationofStandard Solution
Weigh accurately about 29.7 mg of Dapagliflozin Propanediol Monohydrate working standard eq. to 25mg of Dapagliflozin into a 50 ml volumetric flask add to it 30 ml of diluent and sonicate to dissolve the contents completely. Make up the volume upto the mark with diluent. Dilute 5ml of above solution to 50ml with diluent. (Concentration: Dapagliflozin 50 µg/ml)

Sample Preparation
Weightwentytablets and determine theaverageweight. Weigh and transfer intact 5 tablets into 250 ml volumetric flask (equivalent to 50 mg of Dapagliflozin), add about 150 ml of diluent and sonicate for 20 min with intermittent shaking so as to dissolve contents completely. Make up the volume upto the mark with diluent and mix well. Filter the solution through Whatman No. 1 filter paper.

Placebo Preparation
Weigh accurately and transfer placebo eq. to 50 mg of Dapagliflozin into 250 ml volumetric flask, add about 150 ml of diluent and sonicate for 20 min with intermittent shaking so as to dissolve contents completely. Make up the volume upto the mark with diluent and mix well. Filter the solution through Whatman No. 1 filter paper.

Optimised Chromatographic Conditions
The separation and analysis of Dapagliflozin was carried out on Kromasil C18 column (4.6 x 250 mm, 5µ) at 30°C column temperature and15°C sampler temperature at flow rate of 1.2 ml/min. The analyte was monitored at UV detection of 224 nm.

The injection volume kept 20 µl. Standard and sample solution was prepared by above procedure and the chromatograms were recorded. The retention time of dapagliflozin was found to be about 7.5 min.

Method Validation[10]
Validation of proposed method was carried out ass per ICH Q2 (R) 1 guideline. Parameters such as Specificity, Precision, Linearity and Range, Accuracy, Robustness, Solution Stability and Filter Study were performed.

Specificity
Specificity was performed by injecting each of diluent, placebo solution, standard solution given chromatographic conditions utilising PDA detector and chromatograms were checked for interference.

Precision
The precision was performed by repeatability and intermediate precision (ruggedness). The repeatability was checked by injecting six sample sets and intermediate precision was done by different analyst in different day and different column. The percent relative standard deviation of % assay was calculated.

Linearity and Range
The linearity was performed in the concentration range of 10µg/ml to 60µ/ml. The co-relation coefficient was should not less than 0.999.

Accuracy
Accuracy of the method determined in terms of recovery at 3 different levels that is; 50%,

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100% & 150% of increments in the Placebo, this is done by adding Dapagliflozin APIin to placebo. Then recovery placebo should inject & % recovery for respective recovery samples should calculate. The % Recovery for each increment of standard addition is calculate, it should be in between 98 % to 102%.

Robustness

Robustness of method was verified by deliberately varying instrumental conditions by flow rate (±0.2ml/min) and wavelength (±2nm). The % RSD of Precision samples and robustness samples should not more than 2.0

Solution Stability

Sample solution containing Dapagliflozin in diluent and it should inject in a well-equilibrated chromatographic system at the time intervals of 0.00 hrs and 24 hrs respectively. The Difference of % assay between initial sample and 24 Hrs sample should not more than 2.0

Filter Study

Filter study was performed to check suitability of different filters with filter specified in method. The Difference of % assay between filter specified in method and other filters/centrifuge should not more than 2.0

III. RESULTS AND DISCUSSION

Method Development

The estimation of Dapagliflozin was carried out by RP HPLC method. The method was optimised at wavelength 224 nm on UV absorption of Dapagliflozin. On the basis of several trials taken on various columns, different mobile phase compositions finally above optimised chromatographic conditions were achieved.

Method Validation

Specificity

The Specificity of the method was tested by comparing chromatograms of diluent, placebo solution and standard solution. No interference of any peak observed at the RT of Dapagliflozin. (See Fig. No.4, 5 & 6).

Precision

System Suitability

A standard solution of Dapagliflozin working standard was prepared and injected 5 times into HPLC system. The system suitability parameters were evaluated from standard chromatograms obtained. The RSD of peak areas from 5 replicate injections was less than 2.0%. Tailing factor was less than 2.0 and theoretical plate count found to more than 2000. (Refer Table No.1)

Parameter	Limit	Result
% RSD of Area	NMT 2%	0.26
Tailing Factor	NMT 2	1.04
Theoretical plates	NLT 2000	9289

Table No.1. System suitability

Repeatability

Six homogeneous samples were prepared and injected into chromatographic system. The RSD of % assay of 6 samples was less than 2.0%. (Refer Table No.2 and Fig No 4, 5 & 7)

Sample No.	Dapagliflozin Assay
1	100.46
2	100.79
3	100.47
4	100.46
5	99.42
6	100.79
Avg	**100.40**
SD	**0.50**
% RSD	**0.50**

Table No.2. Repeatability

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Intermediate Precision (Ruggedness)

Intermediate precision was done by different analyst in different day and different HPLC system.

Six samples Prepared and injected by different analyst in different HPLC system. %RSD of assay of all 12 samples (6 from repeatability and 6 from intermediate precision) found to less than 2.0(Refer Table No.3)

Sample		**Dapagliflozin (% Assay)**
Method Precision Sample	1	100.46
	2	100.79
	3	100.47
	4	100.46
	5	99.42
	6	100.79
Intermediate Precision Sample	1	100.29
	2	100.38
	3	100.07
	4	100.25
	5	99.76
	6	99.94
Average		100.26
SD		0.40
%RSD		0.40

Table No.3. Intermediate Precision

Linearity

The linearity graph was plotted by taking concentration on X-Axis and Peak area on Y-Axis.

The calibration curve showed linearity in the range of 10 µg/ml to 60 µg/ml for Dapagliflozin API with correlation coefficient of 1.0(Refer Table No.4 and Fig. 2).

Linearity Level %	**Conc. (µg/ml)**	**Dapagliflozin Avg Area**
20	10	505468
40	20	939748
60	30	1404239
80	40	1858474
100	50	2356735
120	60	2841539

Table No.4. Linearity

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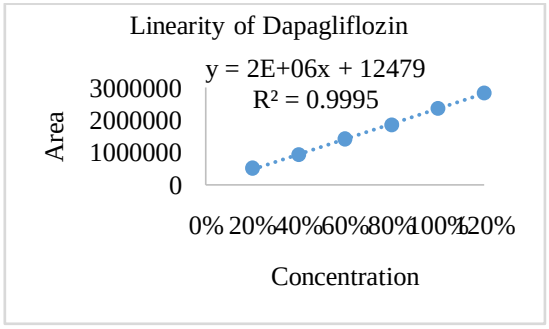

Fig.2 Linearity Graph

Accuracy

Accuracy for Dapagliflozin was performed at 3 levels i.e. 50%, 100% and 150%. Each level injected triplicate into chromatographic system. From the area of each level % recovery was calculated. The % recovery found to be in between 98% to 102% with relative standard deviation less than 2%. (Refer Table No.5)

Dapagliflozin Recovery				
Level (%)	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Statistical Analysis**
50	26.56	26.92	101.36	Avg=101.58 SD=0.29 %RSD=0.29
	26.13	26.63	101.91	
	26.39	26.78	101.48	
100	51.00	51.81	101.59	Avg=101.35 SD=0.22 %RSD=0.22
	51.34	52.00	101.29	
	51.59	52.19	101.16	
150	76.46	77.82	101.78	Avg=101.57 SD=0.44 %RSD=0.44
	75.96	76.77	101.07	
	77.31	78.76	101.88	

Table No.5. Accuracy

Robustness

In robustness small changes done in flow rate (1.2±0.2 ml/min) and in wavelength (224±2 nm). Duplicate samples analysed and calculated for each altered condition. The % RSD of 6 Precision samples and 2 robustness samples (n=8) for each condition was found to be less than 2.0
(Refer Table No.6)

Dapagliflozin		
Parameter	**Assay (n=8)**	**%RSD (n=8)**
Wavelength Plus 226 nm	100.56	0.53
Wavelength Minus 222 nm	100.37	0.43
Flow Plus 1.4 ml/min	100.39	0.51
Flow Minus 1.0 ml/min	100.20	0.59

Table No.6. Robustness

Solution Stability

Sample solution injected in a chromatographic system at the time intervals Initial sampleandafter 24 hrs. The Difference of % assay between initial sample and 24 Hrs sample found to be less than 2.0
(Refer Table No.7)

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Dapagliflozin	
Time (in Hrs)	**% Assay**
Initial Sample	100.46
24 Hrs Sample	99.78
Difference	0.68

TableNo.7. Solution stability

Filter Study

Same sample solution filtered through different filters/ centrifuged and injected into chromatographic conditions to check suitability of filters/ centrifuge. The Difference of % assay between Whatman No. 1 filter and Whatman No. 41, 0.45µ Nylon syringe filter, centrifuge found less than 2.0%. Hence %assay does not affect by using above filters.(Refer Table No.8)

Dapagliflozin		
Type of filter	**% Assay**	**Difference**
Whatman No.1	100.04	--
Whatman No.41	98.75	1.29
0.45µ Nylon	98.81	1.23
Centrifuge	100.16	0.12

Table No.8. Filter study

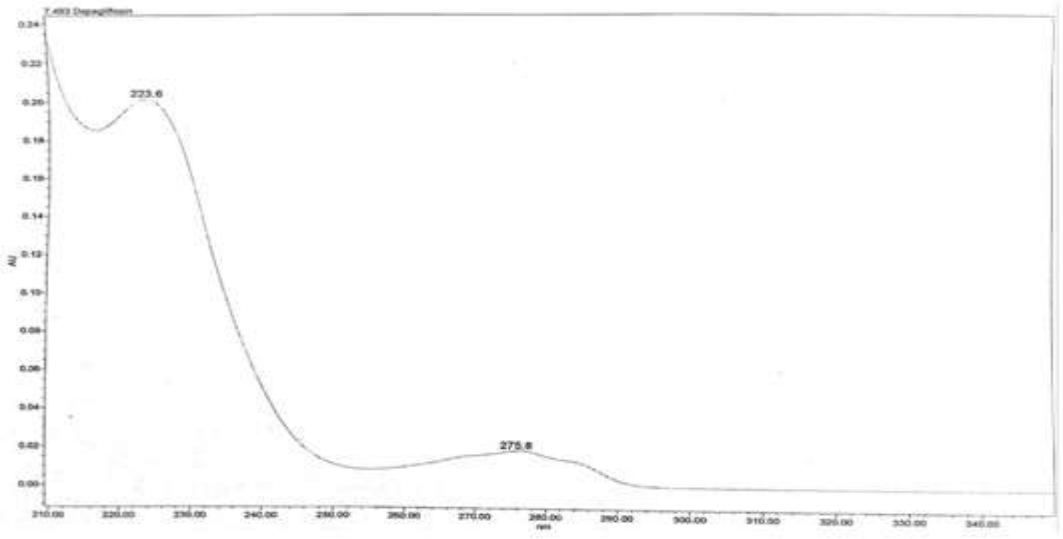

Fig.3. UV Spectra of Dapagliflozin

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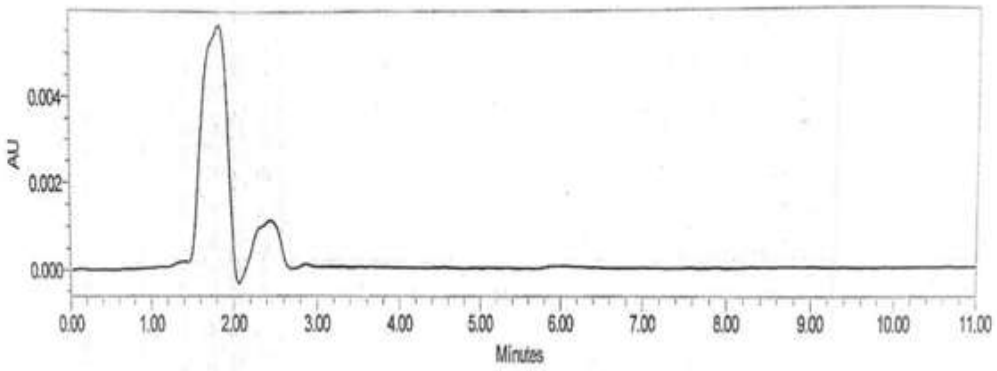

Fig. 4. Blank Chromatogram

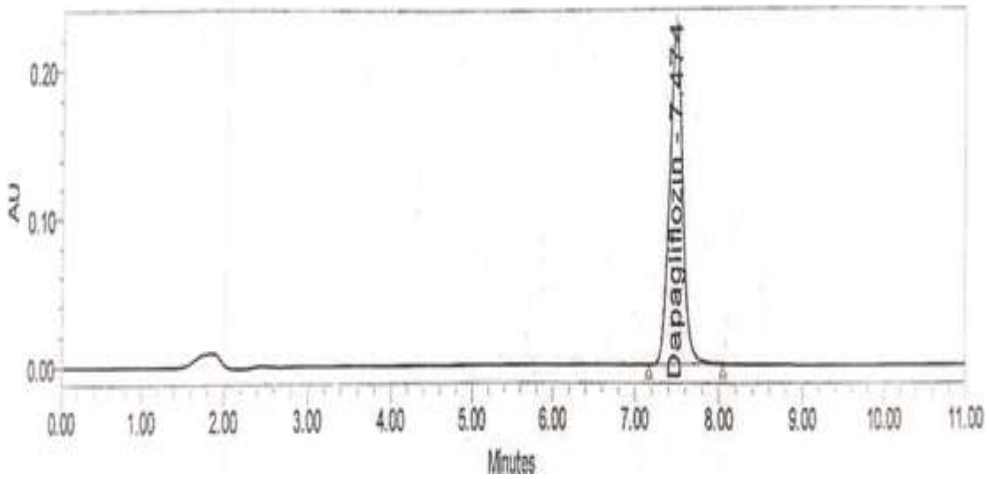

Fig.5. Standard Chromatogram

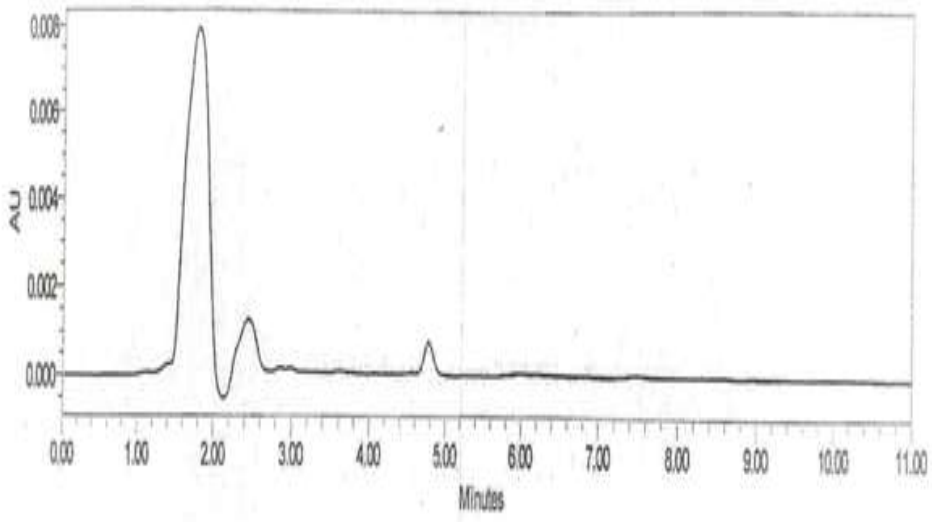

Fig.6. Placebo Chromatogram

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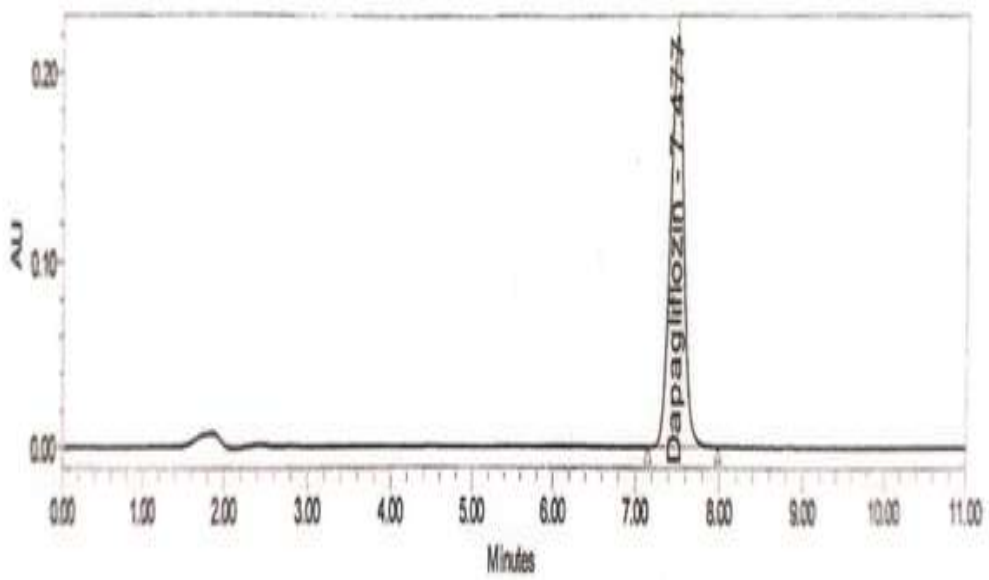

Fig .7. Sample Chromatogram

IV. CONCLUSION

A new, simple, accurate, rapid and precise reverse phase high performance liquid chromatographic method has been developed for estimation of Dapagliflozin in tablet dosage form. The developed method was validated as per ICH guidelines. The developed method has excellent accuracy, precision and reproducibility. According to results
of developed method is suitable for Assay of Dapagliflozin in tablet Dosage form.

Some of The Advanages From The Above Results

1. Simple, reproducible and robust analytical method.
2. Cost saving and time saving procedure.
3. Method can be used for routine analysis of Dapagliflozin Tablets.
4. Validated analytical method as per ICH guideline.

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