ORIGINAL RESEARCH

ANALYTICAL METHOD DEVELOPMENT AND STATISTICAL VALIDATION OF DAPAGLIFLOZIN IN TABLET DOSAGE FORM AND BULK DRUG

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ABSTRACT

A simple, specific, sensitive, precise and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the estimation of Dapagliflozin in tablet formulation and bulk drug. The method was achieved on Zorbax Eclipse Plus C8 column, (150 x 4.6mm) using buffer (pH 7.6) Tris : Methanol (60:40 v/v) as a mobile phase at a flow rate of 1ml/min by employing UV detection at 224 nm. The retention time for dapagliflozin was found to be 1.467 min.The method was validated as per ICH guidelines.The limit of detection (LOD) and limit of quantification(LOQ) was found to be 0.207 and 0.693 µg/ml respectively. The HPLC method developed may be recommended for the routine determination of Dapagliflozin in bulk drug and pharmaceutical formulations.

KEYWORDS: Dapagliflozin (DAPA), Reverse phase high performance liquid chromatography (RP-HPLC) ,International Conference on Harmonization(ICH).

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INTRODUCTION

Dapagliflozin is a orally effective anti-diabetic drug. It is chemically (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol.It is a inhibitor of subtype-2 of Sodium glucose cotransporter. It is soluble in water and organic solvents with PK_a value of 12.57.Since it leads to heavy glycosuria, it can lead to rapid weight loss.[1] The extensive literature survey carried out reveals out that not much work has been done on this particular drug for its determination in bulk and pharmaceutical dosage form using HPLC and UV techniques. Method Validation was carried out according to ICH guidelines.

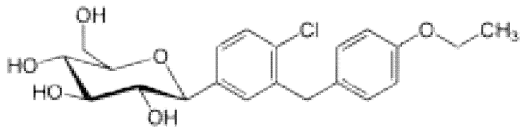


Fig. 1 : Structure of Dapagliflozin [1]

MATERIALS AND METHODS:

Instrumentation

HPLC: Agilent LC Compact-1120 system equipped with EZ chrome Elite software, UV variable wavelength detector, rheodyne injector, injection volume: 20µL, Column: Zorbax Eclipse Plus C8 Enable (150 × 4.6 mm) 5 µm, flow rate: 1.0 mL/min, detection wavelength 224 nm, final optimized chromatographic conditions are Tris : Methanol pH 7.6 (60:40 v/v) as mobile phase.

Materials

Pharmaceutical formulation tablet of Dapagliflozin (label claim containing 5mg of dapagliflozin) was used in HPLC analysis.

Preparation of Stock Solutions:

Standard stock solution of 1000µg/mL of DAPA was prepared by dissolving 10 mg of DAPA in 10 mL of Methanol.

Preparation of Working Standard Solutions

From Standard Stock Solution 1 mL of the solution is taken and further diluted to 10 mL with methanol to get mixed standard solution containing 100µg/mL of DAPA.

Preparation of Sample Solution:

Aliquot equivalent to 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 mL of Working Standard Solution were transferred into 10 mL volumetric flasks and finally diluted upto the mark with methanol.

METHOD DEVELOPMENT

Wavelength Selection:

Selectivity of HPLC method that uses UV detector depends on proper selection of wavelength. A wavelength which gives a good response for the drugs to be detected is to be selected. Appropriate dilutions were made for the drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400nm. The component shows reasonably good response at 224nm.

VALIDATION PARAMETERS

Accuracy

The accuracy is the closeness of the measured value to the true value for the sample. The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentrations levels the specified range (i.e, three concentrations and three replicates of each concentration). Accuracy was tested (% Recovery and % RSD of individual measurements) by analyzing samples at least in triplicate, at each level (80,100 and 120 % of label claim) is recommended. For each determination fresh samples were prepared and assay value is calculated. Recovery was calculated from regression equation obtained in linearity study.

Figure 2: Absorption Maxima of Dapagliflozin

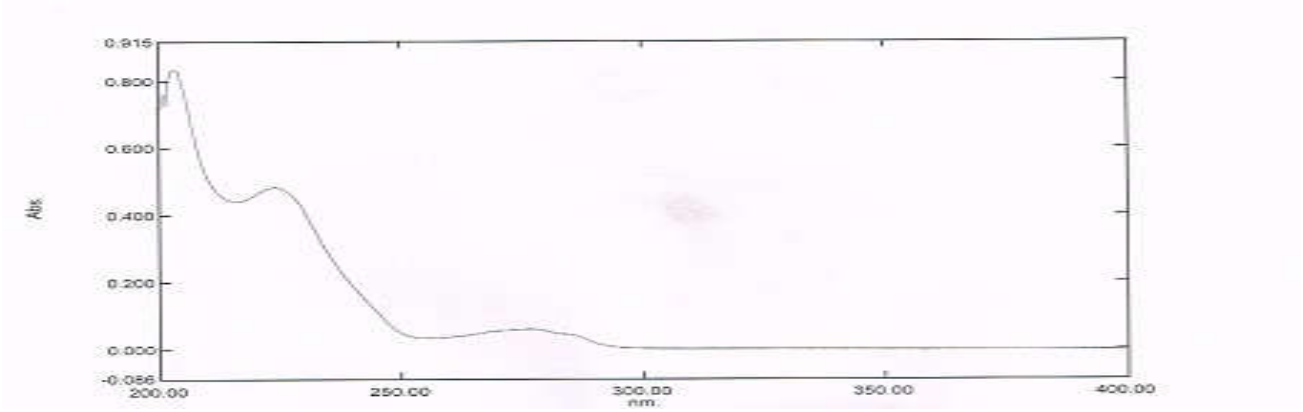


Table : Acceptance criteria for accuracy

% Active/Impurity Content	Acceptable Mean Recovery
≥ 10	98-102%
≥ 1	90-110%
0.1-1	80-120%
< 1	75-125%

Figure 3: Chromatogram of 80 % accuracy.

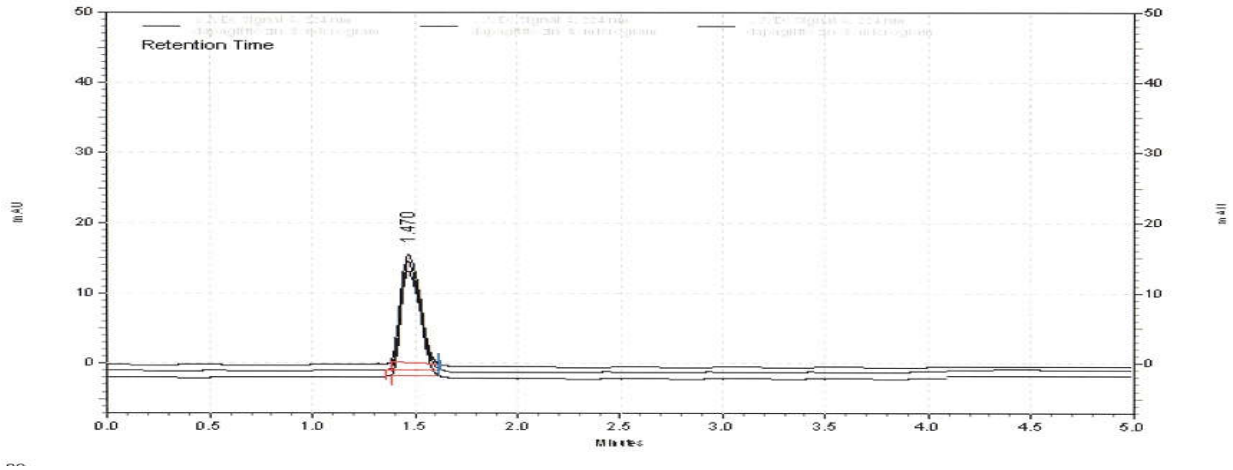


Figure 4: Chromatogram of 100 % accuracy.

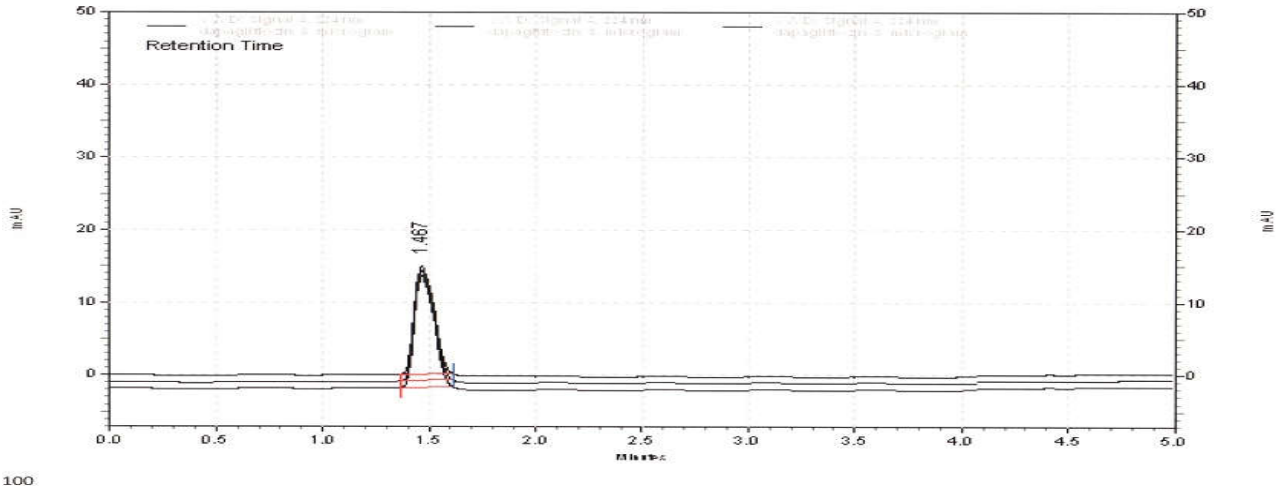
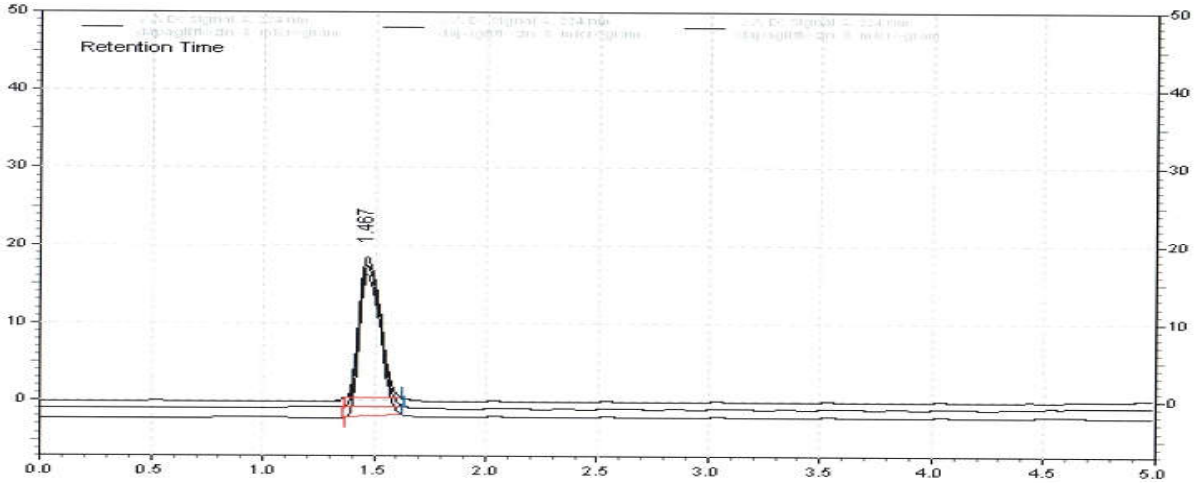


Figure 6: Chromatogram of 120% accuracy.



Precision:

The precision of an analytical procedure expresses the closeness of agreement between series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical method is usually expressed as the standard deviation, relative standard deviation or coefficient of variations of a series of measurements. The ICH documents recommend the repeatability should be assessed using a minimum of nine determinations covering specified range of procedure.. The % RSD for precision should not be more than 2%. The inter day (between 2 days) and intra day (at the same days: morning and evening) precision were carried out . The variation of results were calculated and %RSD was determined.

Intra day:

Figure 7: Chromatogram showing Intraday precision (At morning)

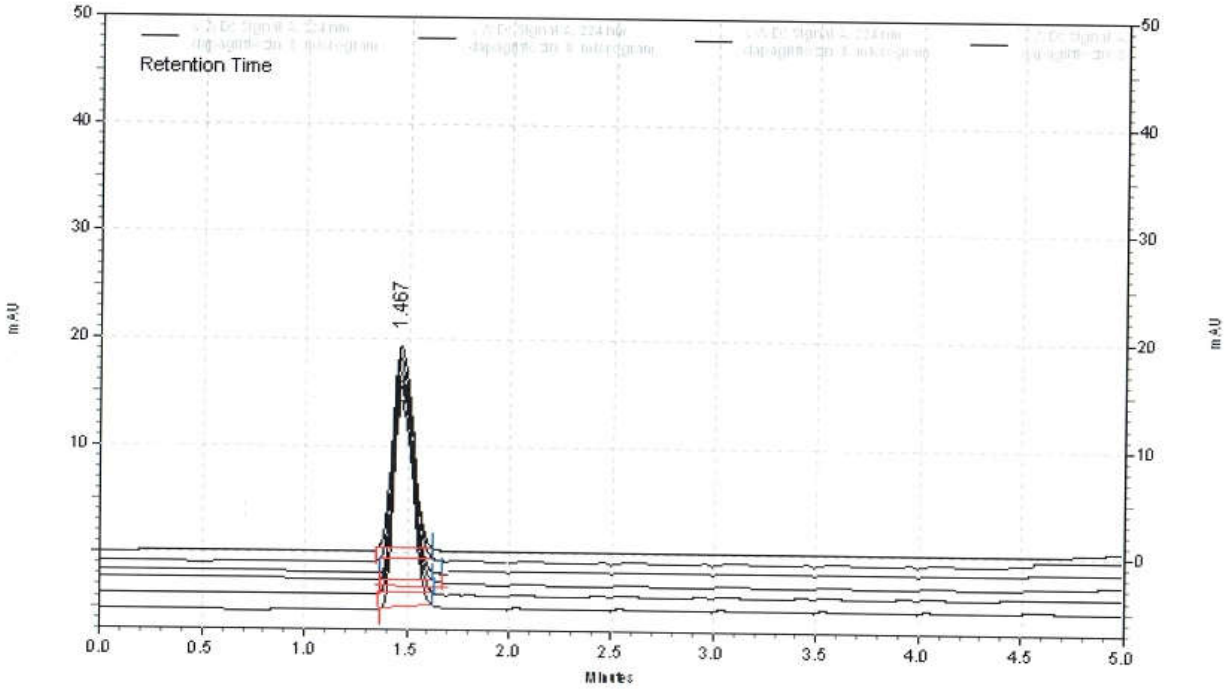


Figure 8 : Chromatogram showing Intraday precision (At evening)

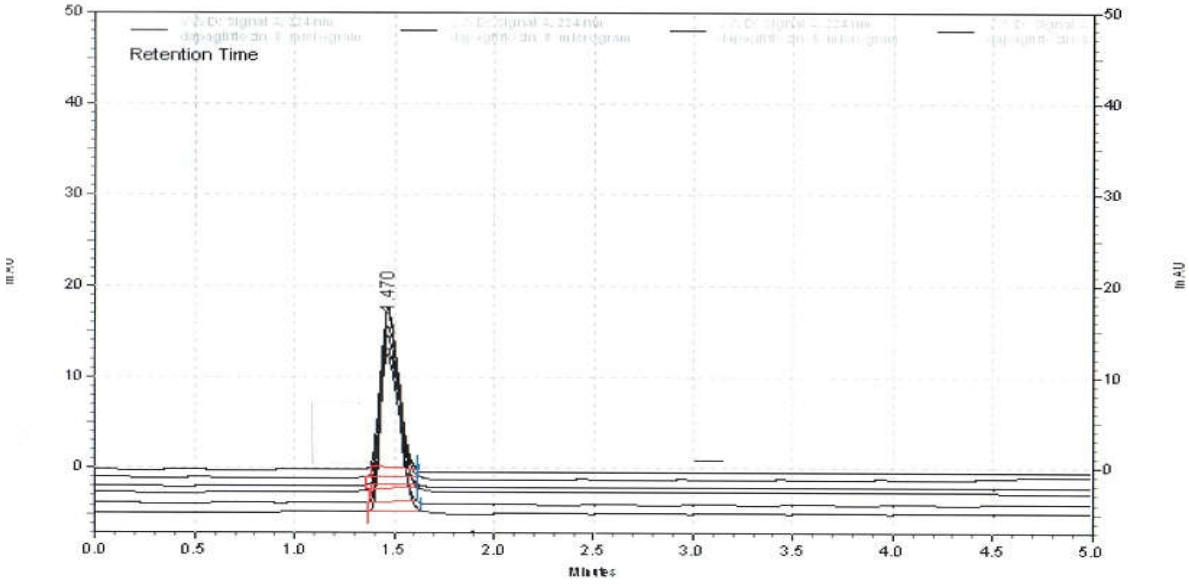


Figure 9: Chromatogram showing interday precision (Day-1)

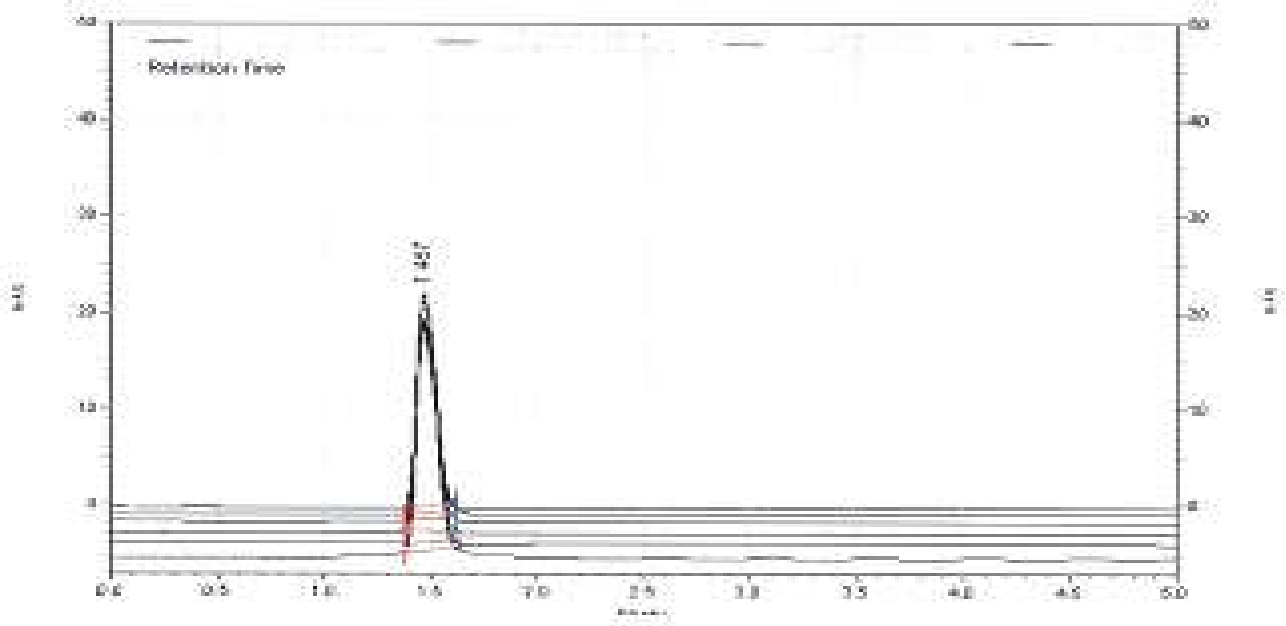
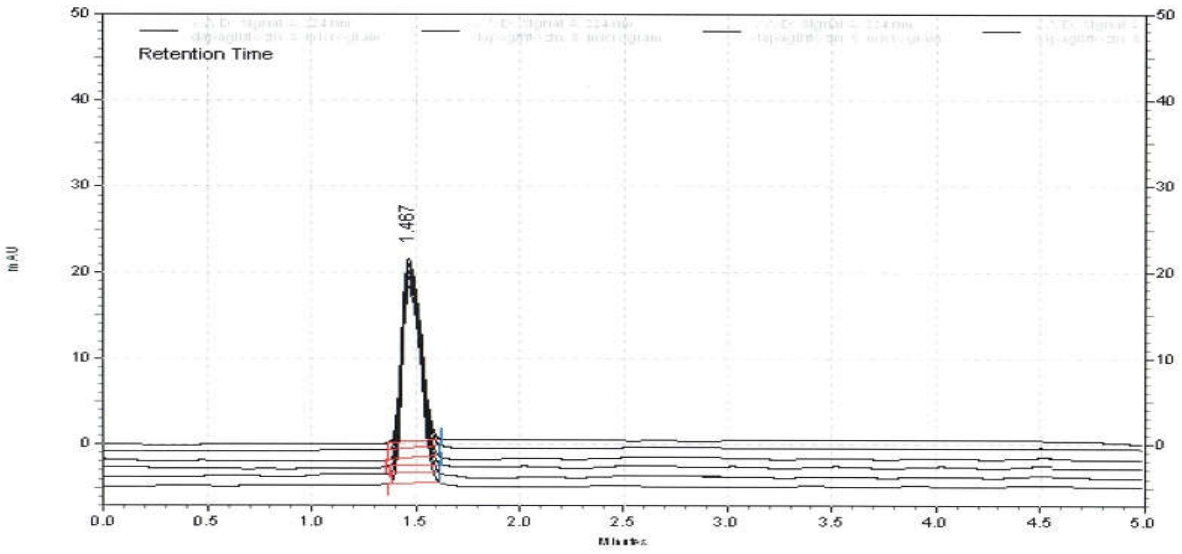


Figure 10: Chromatogram showing interday precision (Day - 2)



Linearity :

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to concentration of analyte in samples. The range of an analytical is the intervals between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated which it has been demonstrated that the analytical procedure has a suitable level of precision accuracy and linearity.Correlation coefficient should be not less than 0.999.

Figure11: Chromatogram of Dapagliflozin at concentration range 1-32 µ/ml.

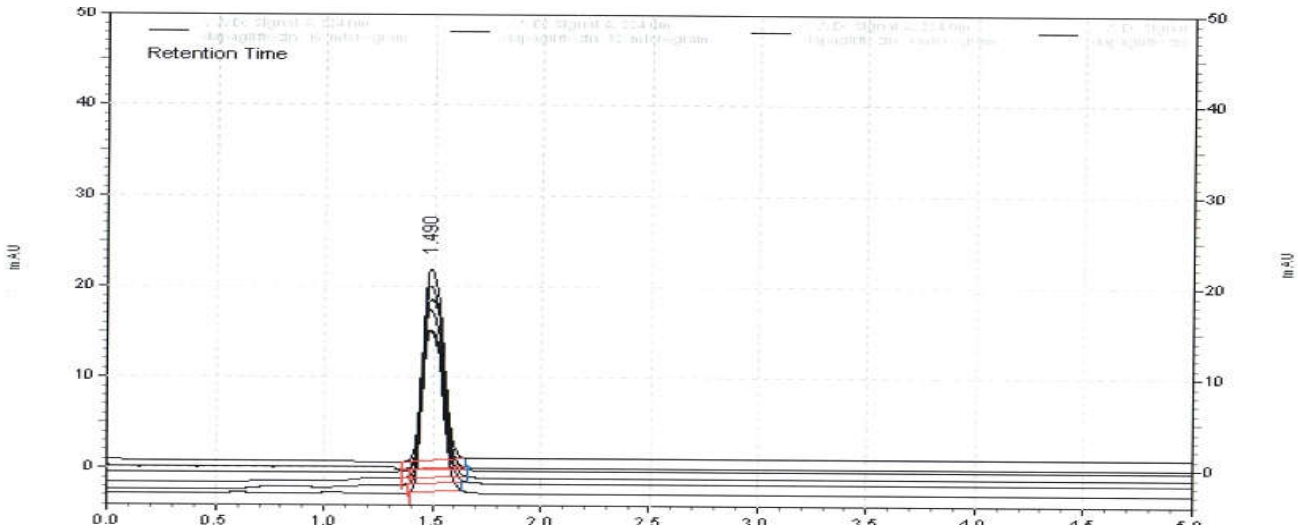
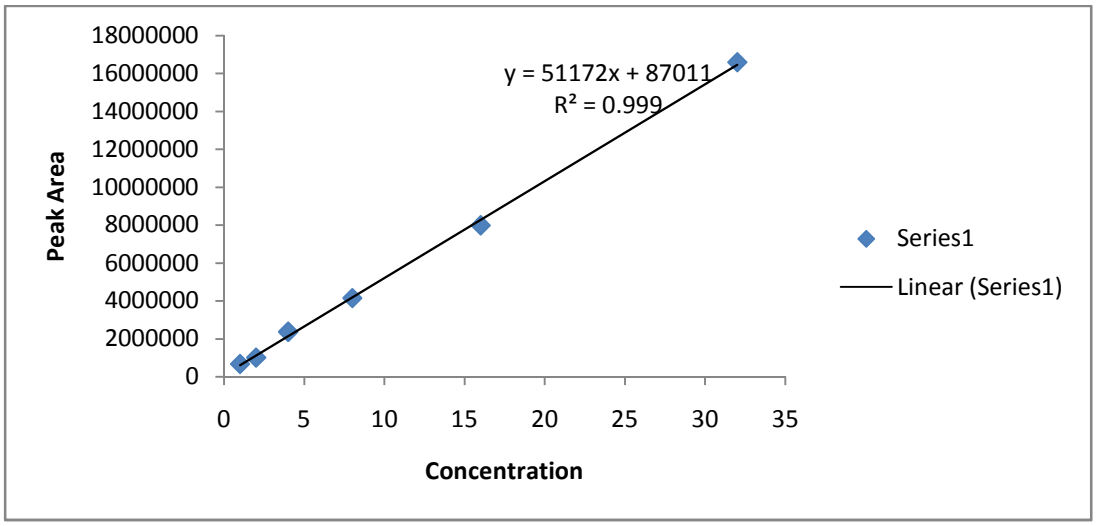


Figure12 : Linearity curve for estimation of Dapagliflozin:



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Detection Limit

It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities as an exact value. The detection limit is usually expressed as the concentration of analyte (percentage parts per million) in the sample. S/N Ratio value should be 3 for LOD solution.

The LOD may be expressed as:

LOD = 3.3 σ / S

Where, σ = Standard deviation of Intercepts of calibration curves

S = Mean of slopes of the calibration curves.

The slope S may be estimated from the calibration curve of the analyte.

Quantification Limit (QL)

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Quantification limit is expressed as the concentration of analyt (e.g- % ppm) in the sample. S/N Ratio value should be 10 for LOQ solution.

The LOQ may be expressed as:

LOQ = 10 σ / S

Where, σ = Standard deviation of Intercepts of calibration curves

S = Mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

Ruggedness

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. Degree of representative of test results is then determined as a function of the assay variable. The % RSD for Ruggedness should not be more than 2%. By analysis of aliquots from homogenous lots, in different laboratories by different analyst, using operational and environmental conditions that may differ but are still within the specified parameter of the assay variable.

Robustness

Robustness of an analytical method is measure of its capacity to remain unaffected small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System Suitability

System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole

SYSTEM SUITABILITY PARAMETERS:

Table 4: System Suitability Parameters and Recommendations:

Capacity Factors(k')	The peak should be well-resolved from other peaks and the void volume, generally k"<2.0
Repeatability	RSD </= 2% for N >/= 5 is desirable.
Relative retention	Not essential as long as the resolution is stated.
Resolution(Rs)	Rs of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.
Tailing Factor(T)	T of </= 2
Theoretical plates(N)	In general should be > 2000

System suitability tests are most often applied to analytical instrumentation. System suitability tests are integral part of the gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for analysis.
The following information furnishes the parameters used to calculate the system performance values for the separation of two chromatographic components.

Relative rétention (selectivity):

α = (t2-ta) / (t1-ta)

Where, α = Relative retention.
t1 = Retention time of the first peak measured from point of injection.
t2 = Retention time of the second peak measured from point of injection.
ta = Retention time of an inert peak not retained by the column, measured from point of injection.

Theoretical plates:

n = 16 (t / w) 2

Where, n = Theoretical plates.
t = Retention time of the component.
w = Width of the base of the component peak using tangent method.

Capacity factor:

K1 = (t2 / ta) – 1

Where K1 = Capacity factor.

Resolution:

R = 2 (t2 - t1) / (w2 + w1)

Where R = Resolution between a peak of interest (peak 2) and the peak preceding it (Peak 1).
W2 = Width of the base of component peak 2.
W1 = Width of the base of component peak 1.

Peak asymmetry:

T = W0.05 / 2f

Where, T = Peak asymmetry or tailing factor.
W0.05 = Distance from the leading edge to the tailing edge of the peak, measured
at a point 5 % of the peak height from the baseline.
f = Distance from the peak maximum to the leading edge of the peak.

Plates per meter:

N = n/ L

Where, N = Plates per meter.
L = Column length, in meters.
HETP = L / n

Linear fit:

A linear calibration fit determines the best line (linear regression) for a series of calibration points. A minimum of two calibration points is required to determine a linear fit. The equation for calibrating the uncorrected amount is:

[Y = a X + b]

Where, Y = Component area or height.
a = Slop of the calibration line.
X = Uncorrected amount.
b = Y- axis intercept of the calibration line.[2]

RESULT AND DISCUSSION

Optimization of mobile phase strength
Retention time for DAPA was found To be 1.467 min.

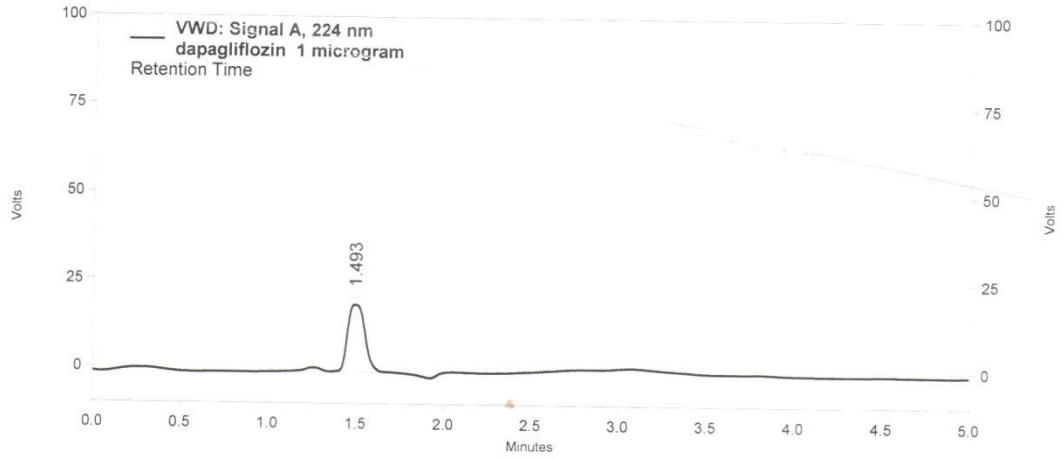

Figure 13: Chromatogram of standard DAPA.

The method that was developed and optimized in HPLC was considered for method validation. The analytical method validation was carried out in accordance with ICH guidelines. The results are discussed in the following section.

Accuracy:

The accuracy of an analytical method is measure of the closeness of test results obtained to the true value.

Table 5: Accuracy results

s.n	Level of percentag e recovery	Amount Present (mg/table)	Amount standard drug added	Area response	mean	Standard deviation	Relative standard deviation	Total amount recove red mg	% recovery
1.	80%	5	4	4078653 4132278 4154379	4121770	38941.87	0.944	4.97	99.49
2.	100%	5	5	4234555 4165733 4178484	4192924	36612.87	0.873	5.06	101.2
3.	120%	5	6	4134675 4144339 4098744	4139253	42980.72	1.038	4.99	99.9

Precision results:

The precision of the method was also ensured by injecting six individual preparations of DAPA solution. Upon repetitive injections at quantification limit, the peak properties like retention time, area were not influenced. Results have shown negligible variation in measured responses which revealed that the method was repeatable with RSD below 2 %.

Table 6: Intraday Precision

S.N.	Injection	Morning Peak Area	Afternoon Peak Area
1	Injection 1	4142737	4135682
2	Injection 2	4193662	4243679
3	Injection 3	4206623	4124567
4	Injection 4	4098812	4147845
5	Injection 5	4160091	4078433
6	Injection 6	4104564	4098454
Average		4151082	4138110
SD		44597.62	57554.9
% RSD		1.074	1.390

Table 7: Interday Precision

S.N.	Injection	Day 1 Peak Area	Day 2 Peak Area
1	Injection 1	4087432	4098765
2	Injection 2	4155734	415234
3	Injection 3	4206447	4234567
4	Injection 4	41424453	4169834
5	Injection 5	4173349	4087345
6	Injection 6	4209876	4143564
Average		4159549	4148572
SD		47686.09	53126
% RSD		1.146	1.280

Linearity

Linearity was studied by preparing standard solutions at 6 different concentrations. The linearity for DAPA were determined in the range of 1-32 µg/mL respectively. Linearity was assessed in terms of slope, intercept and correlation coefficient.

Table 8: Linearity Results

Sl.No	**Concentration (µg/ml)**	**Peak Area**
1.	1	669583
2.	2	1007370
3.	4	2363369
4.	8	4142737
5.	16	7985995
6.	32	16591367
Linear Coefficients	**0.999**	

System suitability

System suitability testing is an integral part of chromatographic method. The tests are based to ensure that the equipment, analytical operations, electronics and samples to be analyzed make an integral system and it can be calculated as such:

Table 9: Result of System Suitability

S.N.	Parameters	DAPA
1	Theoretical Plates/Column	4787
2	Capacity Factor	0.00224
3	Assymetry	1.27842
4	S/N (6 sigma)	14.433686

Limit of Quantification (LOQ) and Limit of Detection (LOD):

Table 10: Result of LOD and LOQ

Parameter	DAPA
LOD	0.207 ug/ml
LOQ	0.693 ug/ml

CONCLUSION

In addition to positive requirements for analytical methods, the striking advantage of all the developed method is that they are economical, cheap, precise. The proposed RP-HPLC method were suitable technique for the determination of Dapagliflozin. In the present investigation, we have developed a simple, sensitive, precise and accurate RP-HPLC method for the quantitative estimation of Dapagliflozin in bulk and pharmaceutical formulations. The HPLC method developed may be recommended for the routine determination of Dapagliflozin in bulk drug and pharmaceutical formulations.

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