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Research Article

Analytical Method Development and Validation of Dapagliflozin By RP-HPLC Method In Tablet Dosage Form

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ABSTRACT

The main aim of the present research work is to develop a sensitive, precise and accurate HPLC (High-Performance Liquid Chromatography) procedure for the selective estimation of Dapagliflozin. An isocratic separation of Dapagliflozin through column used was a Inertsil ODS-3V with dimensions of 150 mm length and 4.6 mm inner diameter, packed with 5µm particle utilizing mobile phase composition of Acetonitrile and Water, with a proportion of 50% Acetonitrile and 50% water (v/v). The detection of the analyte was processed at the maximum wavelength of 223 nm and with 1 ml/min flow of the mobile phase. In the developed reversed-phase high-performance liquid chromatography (RP-HPLC) method, the analytes were separated using an isocratic program. The separation was performed on an HPLC system data acquisition and analysis was handled using EZ-Chrome Elite Software. The results of the analysis were verified for linearity, accuracy, precision, robustness, limit of detection, and limit of quantification in the developed method. Five variable concentration levels of 1, 5, 10, 12.50 and 15.0 µg/ml were used for the estimation of recovery and linearity. The %RSD was also under 2%, demonstrating the great degree of precision of the suggested approach.

INTRODUCTION

Dapagliflozin is mainly used to treat type 2 diabetes. Dapagliflozin is a selective inhibitor of the reneal sodium-glucose cotransportor-2(SGLT-2) & has an anti-hyperglysemic effect by reducing renal glucose reabsorption, leading to increased urinary glucose excretion. Dapagliflozin helps to improve glycemic control by inhibiting glucose reabsorption in the proximal tubule of the nephron and causing glycosuria. Dapagliflozin has been investigated either as a monotherapy or as an adjunct treatment with insulin or other oral hypoglycemic agents. Dapagliflozin inhibits the sodium-glucose cotransporter 2(SGLT2) which is primarily located in the proximal tubule of the nephron. SGLT2 facilitates 90% of glucose reabsorption in the kidneys and so its inhibition allows for glucose to be excreted in the urine. This

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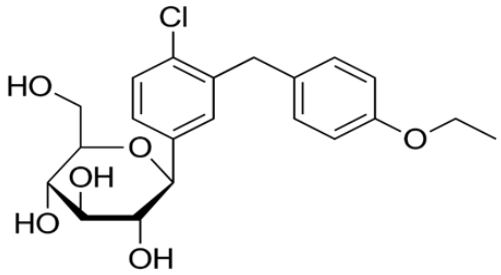
excretion allows for better glycemic control and potentially weight loss in patients with type 2 diabetes mellitus. Dapagliflozin is used together with proper diet and exercise to treat type 2 diabetes. It works in the kidneys to prevent absorption of glucose (blood sugar). This helps lower the blood sugar level. Dapagliflozin does not help patients who have insulin-dependent or type 1 diabetes.



Structure of Dapagliflozin

Material & Method:

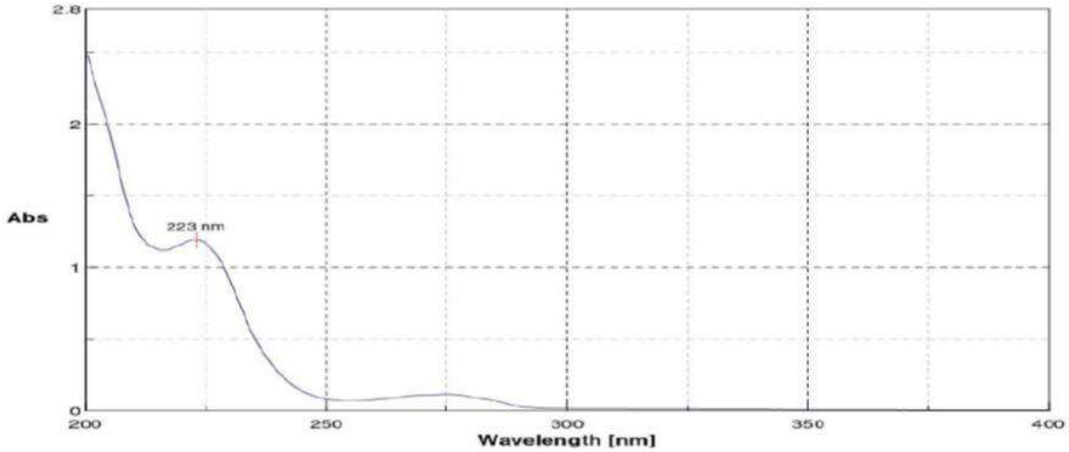
1. Methanol procured from Merck HPLC grade.
2. Acetonitrile procured from Merck HPLC grade.
3. Water procured from Siddhi Lab HPLC grade.
4. Methanol, Acetonitrile procured from Qualigens HPLC grade

INSTRUMENTS:

HPLC system, specifically a Agilent HPLC Binary Gradient System with model number 1260 Infinity by Agilent. The pump is identified as DEAX02386, and the detector as UV detector. The column utilized is a Inertsil ODS-3V, measuring 150 mm X 4.6 mm with a particle size of 5 µm. The software employed for operation is EZ Chrome Elite. The analytical balance utilized is an Sartorious High Precision Balance, model CPA 225D, with a maximum capacity of 210 grams and a minimum readability of 0.001 grams. The pH meter employed is a digital pH meter manufactured by LabIndia. For sonication purposes, a Bio-technic Ultra Sonicator with a capacity of 13.5 litres is used. Additionally, two types of filters are employed: a Nylon membrane with a pore size of 0.45 µm and a PVDF membrane also with a pore size of 0.45 µm.

Selection of analytical wavelength

Methanol as a blank and Dapagliflozin standard solution (20 ppm) was scanned from 400 nm to 200 nm. Absorption maxima was determined for drug. Dapagliflozin showed maximum absorbance at 223 nm shown in results.



UV spectrum of Dapagliflozin

METHOD DEVELOPMENT BY RP – HPLC

Preparation of standard stock solution:

Dapagliflozin standard stock solution was prepared by transferring 20 mg Dapagliflozin into a 20 mL clean and dried volumetric flask added about 15 mL of Methanol to dissolve it completely and made volume up to the mark with methanol.

(1000 ppm). Further diluted 1 ml of stock solution to 10 mL with Methanol. (100 ppm).

Chromatographic Conditions:

Detector: U.V. Detector Column: Inertsil ODS-3V Column Dimension: (150 mm X 4.6 mm i.d.) 5µm Column Oven temperature: 35°C, Injection Volume: 20 µl Wavelength: 223 nm, Mobile phase: Acetonitrile : Water (50:50) Flow Rate: 1.0 ml/min.

Sample preparation of Marketed test sample:

Weighed 20 tablets and determine the average weight. Then transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material equivalent to 10 mg of Dapagliflozin and transferred to clean and dried 20 mL of volumetric flask. Added 15 mL of Methnol, sonicated for 10 minutes with intermittent shaking. After 10 minutes allow to cool he solution to room temperature and made volume up to the mark with Methanol. Filtered the solution through suitable 0.45 µ Nylon syringe filter discarding first 3-5 mL of filtrate. Further diluted 0.4 ml of filtered stock solution to 20 ml with mobile phase (10 mcg of dapagliflozin). Injected the resultant solution and chromatograms were recorded and results are recorded.

System suitability

System suitability is a Pharmacopeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injection of standard drug solution and the results are recorded.

Linearity & Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. 5 levels of Linearity was performed from 10%, 50%, 100%, 125%, 150% of working concentration.

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

Detection limit: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy .As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Accuracy (% Recovery):

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value of the value found, Accuracy will be conducted in the range from 50 %, 100%, 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Precision

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on tablet test sample.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by

small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

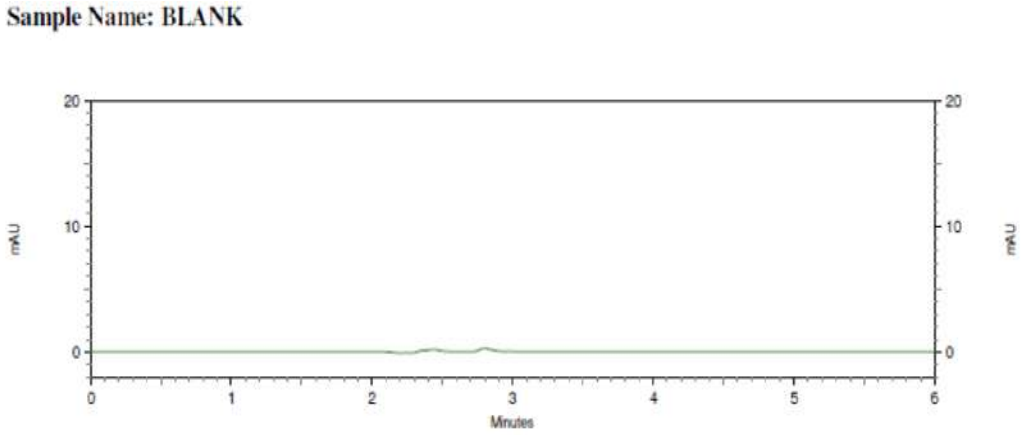
Blank and Standard solution were injected under different chromatographic conditions as shown below.

a) Changes in flow rate by ±10%. (± 0.1ml/min)
b) Change in column oven temperature. (± 2ºC)
c) Change in wavelength (± 3 nm)

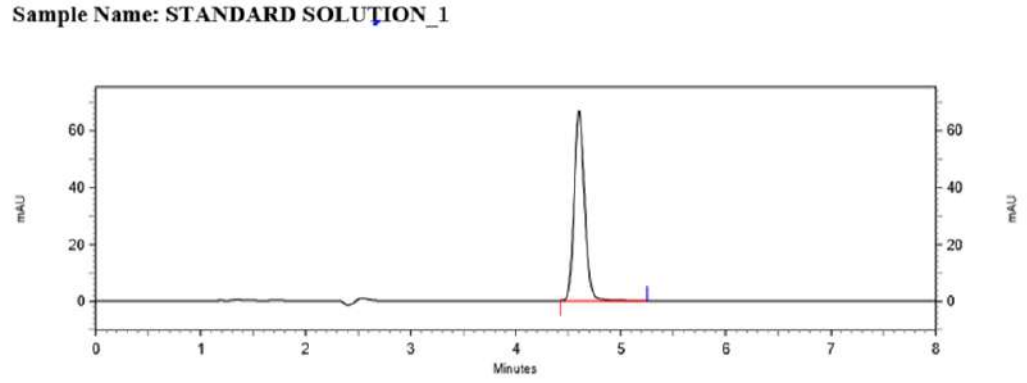
RESULT & DISCUSSION:

Optimized Chromatographic Conditions

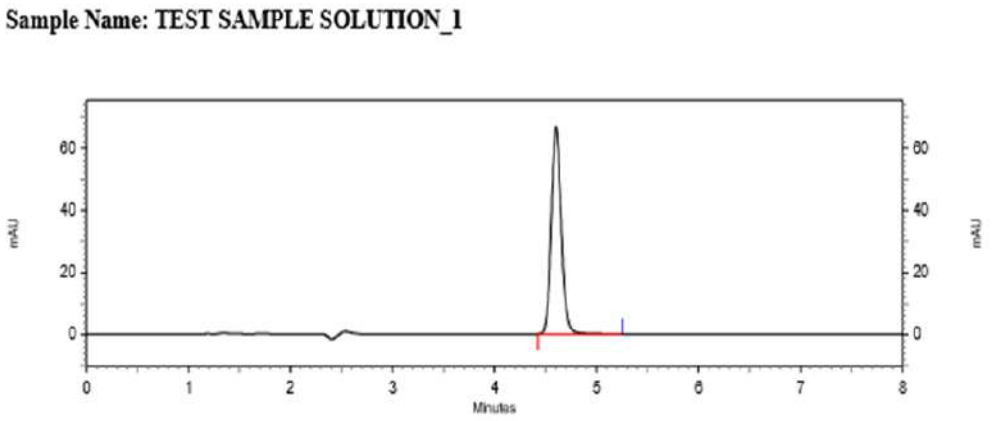
Parameter	Description
Mode	Isocratic
Column Name	Inertial ODS-3V, 150 mm X 4.6 mm, 5 µm
Detector	UV Detector
Injection Volume	20 µl
Wavelength	223 nm
Column Oven temp	35ºC
Mobile Phase	Acetonitrile: Water (50: 50 % v/v)
Flow Rate	1.0 ml/min
Diluent	Methanol
Run time	8 Minutes



Typical chromatogram of Blank solution.



Typical chromatogram Standard solution 1 of system suitability solution.



Typical chromatogram of Test sample solution.

Results For System Suitability Test Of Dapagliflozin

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	7813751	1.11	11526
2	Standard_2	7815429	1.11	11532
3	Standard_3	7886529	1.11	11546
4	Standard_4	7821406	1.11	11546
5	Standard_5	7856859	1.12	11515
Mean		**7838795**	**1.11**	**11533**
STD Dev		**31938.97**		
% RSD		**0.41**		

Analysis of Marketed Test samples (Assay)

Diabiz 10 mg Tablet:

Weight of 20 tablets = 2.9120 gm

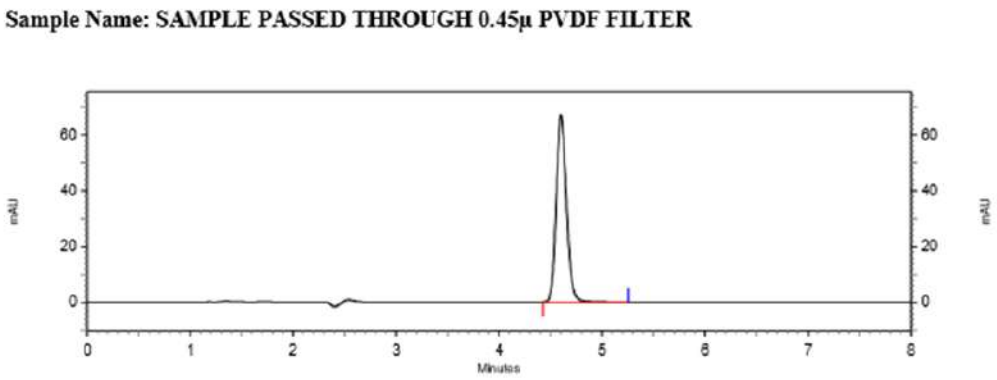
Average weight of tablet = 2.9120 / 20 = 0.1456 gm = 145.6 mg

Assay results of Diabiz 10 mg Tablet:

Sample	Area	% Assay	Mean Assay
Sample 1	7742128	98.16	98.00
Sample 2	7732568	97.84	

Filtration Study:

Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample. Performed on tablet test sample. Both filters PVDF and Nylon passes the criteria for filter study, hence both filters can be used. We used PVDF filter because it showed less absolute difference as compare to Nylon filter.



Typical chromatogram of sample filtered through 0.45µ PVDF filter.

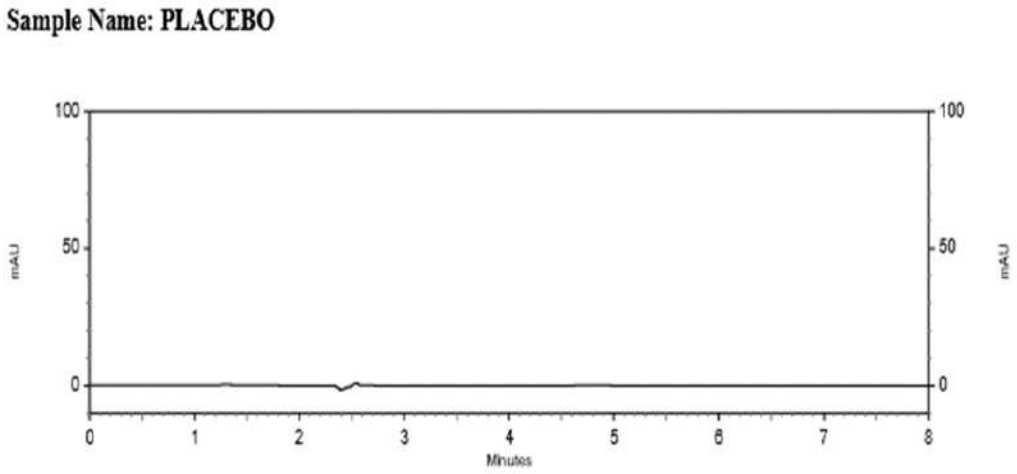
Results Of Filter Study

Sample description	Area	% Absolute difference
Unfiltered	7748568	NA
0.45 µ PVDF filter	7731529	0.22
0.45 µ Nylon filter	7705425	0.56

Specificity:

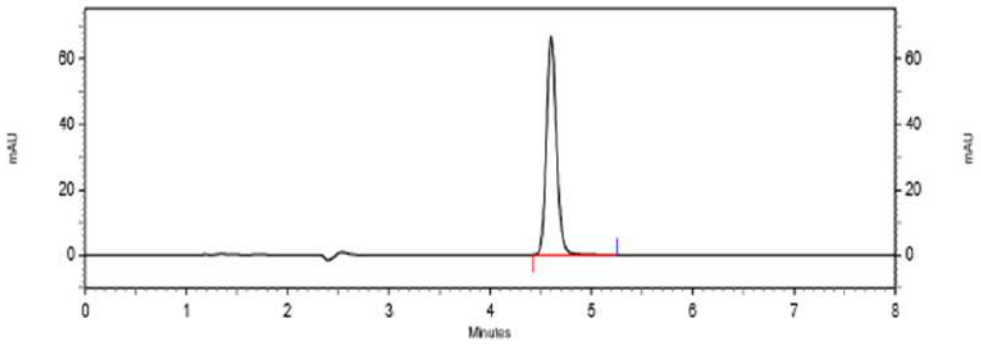
Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present.

Blank, standard solution prepared and injected to check peak purity. Blank and placebo was not having interference at R.T. of Dapagliflozin. Peak purity for Standard as well as test solution was well within limits. Hence developed chromatographic method passes the criteria for specificity.



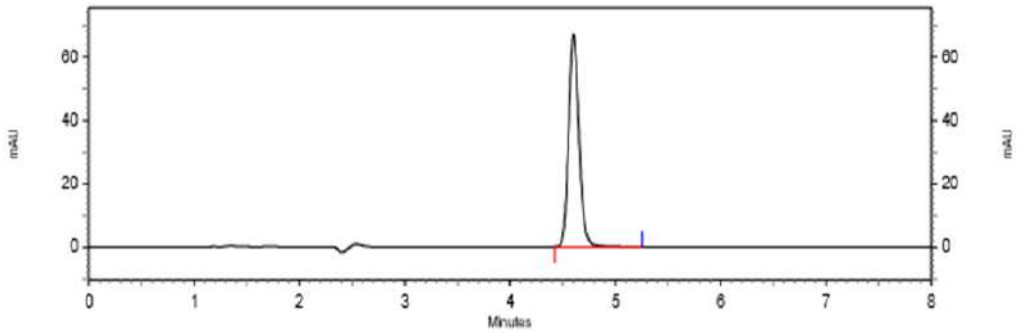
Typical chromatogram of Peak purity of Placebo solution

Sample Name: STANDARD SOLUTION



Typical chromatogram of Peak purity of Standard solution.

Sample Name: TEST SAMPLE SOLUTION



Typical chromatogram of Peak purity of Test sample solution.

Results Of Specificity

Description	Observation
Blank	No interference at R.T. of Linezolid due to blank
Placebo	No interference at R.T. of Linezolid due to placebo
Standard solution	Peak purity was 0.999
Test Solution	Peak purity was 0.998

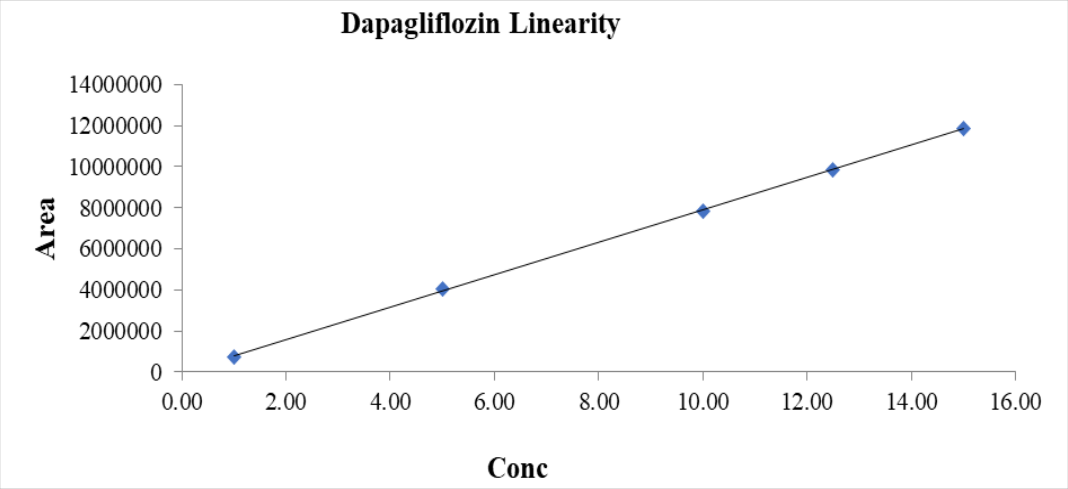
Linearity and Range

Linearity of an analytical method is its ability to elicit test results that are proportional to the concentration of analyte in samples within a given range.From the calibration curve it was concluded that the Dapagliflozin shows linear response in the range of 1.00-15.00 µg /ml. The Regression value was found well within the limit.

Results Of Hplc Linearity Data For Dapagliflozin:

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	1.00	725864	728986	0.488
		732859		
		728235		

50%	5.00	4035263	4038438	0.078
		4038524		
		4041526		
100%	10.00	7835424	7843473	0.109
		7852428		
		7842568		
125%	12.50	9835624	9843521	0.086
		9842521		
		9852418		
150%	15.00	11852006	11853499	0.071
		11862526		
		11845965		



Detection limit (LOD):

LOD = 3.3 σ / S

LOD = 3.3 x 61409.43 / 789778.93

LOD = 0.257 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 3.3 x 61409.43 / 789778.93

LOQ = 0.778 µg/mL

Accuracy (Recovery):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added. Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate.

Result And Statistical Data Of Accuracy Of Dapagliflozin

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	4048235	5.16	5.10	101.18	100.19	0.988
	4086493	5.21	5.20	100.19		

	3886859	4.96	5.00	99.20		
100	7892328	10.07	10.20	98.73	99.74	1.084
	8039548	10.26	10.30	99.61		
	8065874	10.29	10.20	100.88		
150	11815849	15.07	15.20	99.14	99.87	0.805
	11923056	15.21	15.10	100.73		
	11645859	14.86	14.90	99.73		

Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Precision was performed on Test sample.

Repeatability:

The preparation of sample solutions involved creating six separate samples to ensure repeatability and consistency in the experimental procedure.

Intermediate precision:

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared.

Result Of Intra- Day And Inter- Day Precision For Dapagliflozin:

	Sample	**Test Sample (mg)**	**Area**	**% Assay**
Repeatability	Sample 1	145.2	7685429	98.31
	Sample 2	145.7	7720125	98.42
	Sample 3	146.2	7658465	97.30
	Sample 4	145.4	7585964	96.91
	Sample 5	145.8	7765284	98.93
	Sample 6	145.5	7652624	97.69
	Mean			**98.51**
	STD DEV			**0.7589**
	% RSD			**0.7775**
Intermediate precision (Inter-Day)	Sample 1	145.9	7720156	98.28
	Sample 2	144.8	7551454	96.87
	Sample 3	145.6	7676548	97.93
	Sample 4	144.5	7764238	99.80
	Sample 5	147.2	7805496	98.49
	Sample 6	146.2	7690254	97.70
	Mean			**98.18**
	STD DEV			**0.9736**
	% RSD			**0.992**
Repeatability Plus Inter-day	**Mean**			**98.053**
	STD DEV			**0.8426**
	% RSD			**0.859**

Robustness

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

Following changes made under Robustness:

- Change in Wavelength
- Change in flow rate
- Change in column oven temperature

Result Of Robustness Study:

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (261 NM)	4.60	7880690	1.12	10864
Wavelength by -3 NM (255 NM)	4.60	7468981	1.14	10454
Flow rate by +10% (1.1mL/min)	4.16	7142637	1.15	9343
Flow rate by -10% (0.9mL/min)	5.16	8832648	1.17	9168
Column oven temp by +2ºC (37 ºC)	4.56	7853219	1.10	11842
Column oven temp by -2ºC (33 ºC)	4.63	7785237	1.13	10883

CONCLUSION

A successful attempt to determine Dapagliflozin in tablet dosage form using high performance liquid chromatography was made in the current study activity. The development of an appropriate, suitable, and easy-to-use RP-HPLC method was the focus of the current work. Several methods have been published for determining Dapagliflozin in bulk drugs or in pharmaceutical dosage forms, according to a literature review. Thus, a novel, sensitive, and appropriate reversed-phase high performance liquid chromatography method was created and validated in the current study for the measurement of Dapagliflozin in bulk drug and pharmaceutical dosage form. In the developed reversed-phase high-performance liquid chromatography (RP-HPLC) method, the analytes were separated using an isocratic program. The mobile phase consisted of a mixture of Acetonitrile and water, with a proportion of 50% Acetonitrile and 50% water (v/v). The separation was performed on an HPLC system equipped with a UV-visible detector, and data acquisition and analysis were handled using EZ-Chrome Elite Software. The chromatographic column used was a Inertsil ODS 3V with dimensions of 150 mm length and 4.6 mm inner diameter, packed with 5µm particle size. The flow rate of the mobile phase was set at 1.0 ml/min, and the detection of the analytes occurred at a wavelength of 223nm.

REFERENCE

1. Sharma B.K. Instrumental Methods Of Chemical Analysis, Introduction To Analytical Chemistry.; Goel Publishing House, Meerat, 19th Edition, 2000, P 1-4, 200-203.
2. Kasture A.V., Wadodkar S.G., Mahadik K.R., More H.N. Pharmaceutical Analysis (Instrumental Methods).; Nirali Prakashan, Vol 2, 1998, P 1-3.
3. Sethi P.D. High Performance liquid chromatography.; CBS Publishers, New Delhi, 1st Edition, 2001, P 3-72.
4. Manasa S, Dhanalakshmi K, Reddy NG, Sreenivasa S. Method development and validation of dapagliflozin in API by RP-HPLC and UV-spectroscopy. Int J Pharm Sci Drug Res. 2014;6(3):250.
5. Shakir Basha S, Sravanthi P, Development and Validation of Dapagliflozin By RP-HPLC Method and it's Forced Degradation Studies,

Asian Journal Of Pharmaceutical Science & Research,2017.

6. Debata J, Kumar S, Jha SK, Khan A. A New RP-HPLC method development and validation of dapagliflozin in bulk and tablet dosage form. Int J Drug Dev Res. 2017;9(2):48-51.
7. P. Sathya Sowmya, V.Siva Krishna, Analytical Method Development and Validation of Dapagliflozin and Linagliptin Tablets By RP-HPLC, YMER, 2023.
8. G.M.Kadam, A.L.Puyad,T.M. Kalyankar, RP-HPLC Method Development And Validation For The Estimation of Saxagliptine And Dapagliflozin In Pharmaceutical Dosage Form,JETIR,2023
9. P Aakash, P Bhumi , R Urvi , P Ronak , P Jaymin, Stability Indicatiing RP-HPLC Method Development And Validation For Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Teneligliptine in Tablet Dosage Form,IJCRT,March2023
10. Madhavi S, Rani AP. Development and validation of a method for simultaneous determination of dapagliflozin and saxagliptin in a formulation by RP-UPLC. World J Pharma Res. 2017 Aug 11;6(12):904-16.
11. Mante GV, Hemke AT, Umekar MJ. RP-HPLC Method for Estimation of Dapagliflozin from its Tablet. International Journal of ChemTech Research. 2018;11(01):242-8.
12. wikipedia.org/wiki/Dapagliflozin Sanagapati, M., Lakshmi, D. K., Reddy, N. G., & Sreenivasa, S. (2014). Development and validation of stability-indicating RP-HPLC method for determination of Dapagliflozin. Journal of Advanced Pharmacy Education & Research, 4(3).
13. Karuna PC, China E, and Basaveswara Rao MV., Unique UV spectrophotometric method for reckoning of Dapagliflozin in bulk and pharmaceutical dosage forms, J. Chem. Pharm. Res., 2015, 7(9), 45-49.
14. List FJ, Woo V, Morales E, Tang W, Fiedorek FT. Sodium-glucose cotransport inhibition with dapagliflozin in Type 2 diabetes. Diabetes care 2009;32(4):650-7.

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