"DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ESTIMATION METHOD OF DAPAGLIFLOZIN IN IT'S TABLET DOSAGE FORM"

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ABSTRACT: *In these study stability-indicating RP-HPLC method is developed for estimation of Dapagliflozin in tablet dosage form. A Simple, rapid, economical, precise and accurate Reverse Phase Method High Performance Liquid Chromatography (RP – HPLC) Method for Estimation of Dapagliflozin in tablet dosage form has been developed. The method employed, Zorbax Eclips XDB C_{18} (150mm x 4.6mm), 5µm i.d column in isocratic mode, with mobile phase of Buffer: ACN: Methanol in the ratio (60:37:03%v/v/v). The flow rate was 1.0ml/min and detection wavelength 220 nm. Retention time of Dapagliflozin was found to be 5.99 min and 1.63 min respectively. Forced degradation study of Dapagliflozin was performed by RP-HPLC method which includes Acid, Base, and Oxidative, Photo and Thermal degradation and their result of degradation were found within limit.*

Key Words: *Dapagliflozin, RP-HPLC, Forced Degradation, Development and Validation*

Introduction:

Dapagliflozin is chemically (2~{s},3~{r},4~{r},5~{s},6~{r})-2-[4-chloro-3-[(4-ethoxyphenyl) methyl]phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol used in the treatment of type-2 diabetes patients.

The molecular formula of Dapagliflozin is $C_{21}H_{25}ClO_6$ with the molecular weight of 408.875 g/mol. Dapagliflozin is not official in Indian United States and European Pharmacopoeia. Literature survey reveals that only few analytical methods are reported for analysis of drug. There is no stability indicating analytical method was reported for estimation of Dapagliflozin from Dapagliflozin tablet dosage form.

Therefore, it was thought of interest to develop precise, accurate, sensitive, selective chromatographic method for estimation of Dapagliflozin in it's tablet dosage form which will provide valuable information that can be used to assess the inherent stability of the drug under various stressed conditions, eventually to improve formulation and manufacturing process. The aim of work was to carry out RP-HPLC method development and validation for estimation of Dapagliflozin.

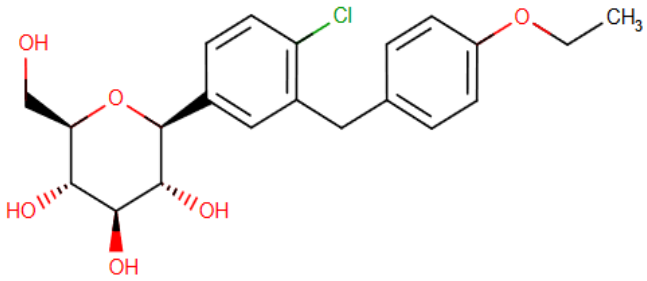


figure 1: chemical structure of Dapagliflozin

MATERIALS AND METHODS: In the present research work Acetonitrile, HCL and NaOH were used of Merck Life science, Methanol and Ortho phosphoric acid were used of Spectrochem Pvt. Ltd. The sample of Dapagliflozin API and Tablets were kindly gifted by ZYDUS CADILA HEALTHCARE, Moraiya, Ahmedabad.

Equipment

The analysis was performed on HPLC Agilent technologies 1200 series, Isocratic mode, Photodiode array detector, injector of 100 µL loop volume. Zorbax Eclips XDB C_{18} (150mm*4.6mm) 5µm column which is maintained at 30 º C temperature. Chromeleon software was used for data collecting and processing.

Preparation of buffer:

Dissolve about 1.0 mL of Orthophosphoric acid into 1000 mL Milli-Q water and mix well. Filter through 0.45 µm- membrane filter paper.

Preparation of mobile phase:

Prepare the mixture of Buffer: Acetonitrile: Methanol in the volume ratio of (60:37:03% v/v/v).

Diluent:

Water and Methanol is used in the ratio of 50:50 % v/v as diluent.

Preparation of standard stock solution (24ppm):

Transfer an accurately weighed quantity of 96 mg of Dapagliflozin API to a 200 mL volumetric flask, add about 100mL of diluent and sonicate to dissolve. Make up the volume up to the mark with diluent and mix dilute 5.0mL of above solution to 100mL with diluent and mix.

2.6 As such sample solution (24ppm):

[Label claim: 5 mg]

Weigh accurately 20 tablets and calculate the average weight. Weight 10 intact tablets and transfer into 500 mL of volumetric flask. Add 300 mL of diluent and sonicate with occasional shaking for 45 mins with shaking every 3 mins for a period of 30 seconds. Make volume up to mark with diluent and mix. Dilute 3.0 mL of supernatant solution to 25.0 mL with diluent and mix.

Filter the solution through 0.45 µm Millipore PVDF filter; collect the filtrate by discarding first 5 mL of filtrate.

I. Chromatographic conditions:

Zorbax Eclips XDB C_{18} (150mm*4.6mm) 5µm column was used as stationary phase. Mixture of OPA Buffer: Acetonitrile: Methanol in the volume ratio of (60:37:03% v/v/v). The mobile phase was pumped at 1.0 ml/min. The eluent was monitored at 220 nm. The injection volumes of sample and standard were 25 µL (micro liter). Total run time is 10 min.

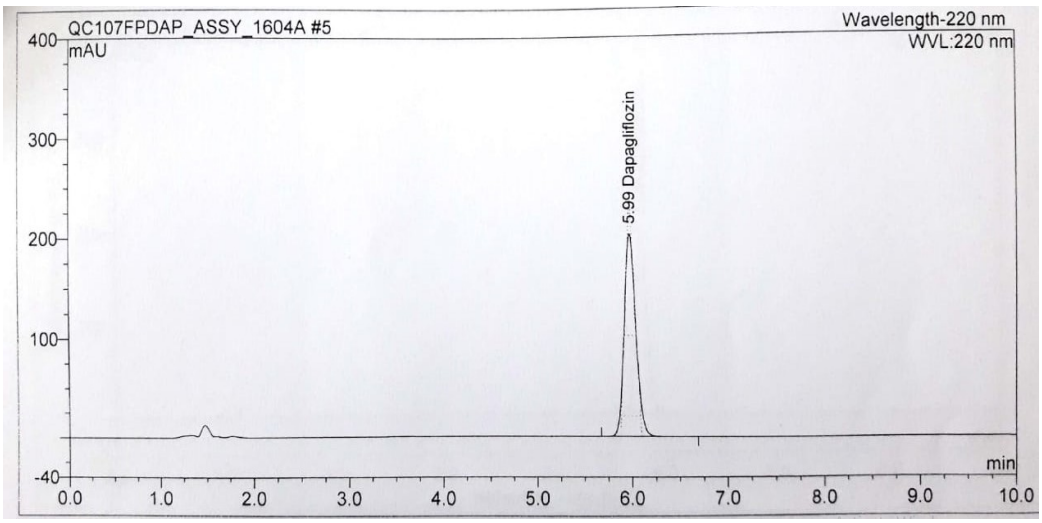


figure 2: chromatogram of dapagliflozin

The developed method was validated for linearity, precision, accuracy, robustness and is applied for forced degradation studies as per ICH guidelines.

RESULT AND DISCUSSION:

Method Development:

ICH prescribed stress conditions such as acid, alkali, oxidative, thermal and photolytic stresses were carried out.

Acid Degradation:

Sample preparation

20 mL reconstituted solution transfer into 100 mL volumetric flask, Add approx. 50 mL of diluent and sonicate for 10 mins, add 5 mL of 1 N HCl and heat for 4 hours on boiling water bath at 110 ^{0}C for acid hydrolysis. Then the solution was neutralized with 1N NaOH and made volume up to mark with diluent. Then pipette out 5 mL of solution in 50 mL volumetric flask and make volume up to mark with diluent.

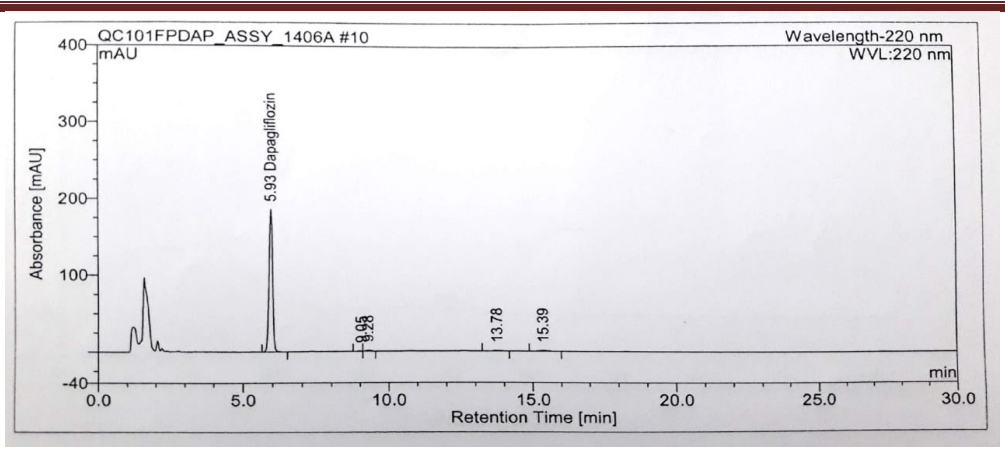


figure 3: acid degradation of dapagliflozin

Alkali Degradation:

Sample preparation

20 mL reconstituted solution transfer into 100 mL volumetric flask, Add approx. 50 mL of diluent and sonicate for 10 mins, add 5 mL of 2N NaOH and heat for 3 hours, on Room Temperature for Alkali hydrolysis. Then the solution was neutralized with 2N HCl and made volume up to mark with diluent. Then pipette out 5 mL of solution in 50 mL volumetric flask and make volume up to mark with diluent.

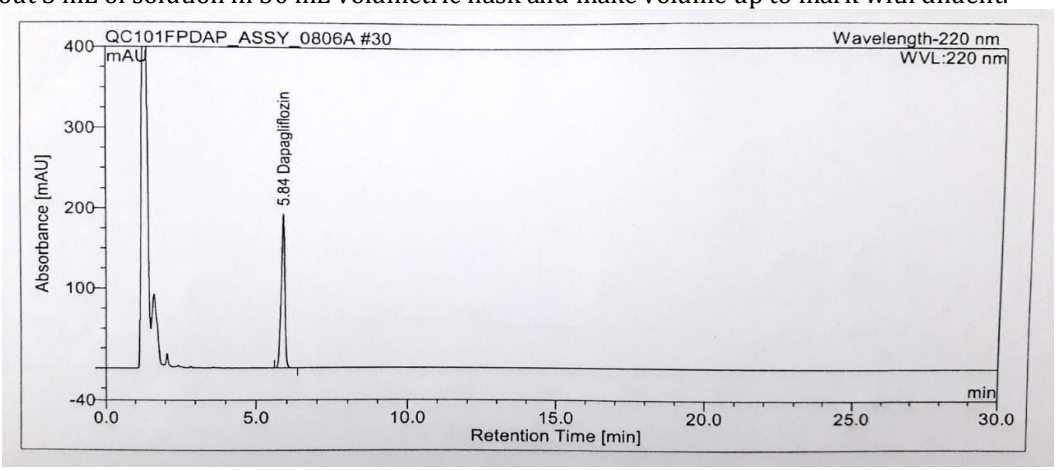


figure 4: alkali degradation of dapagliflozin

Oxidative Degradation:

Sample preparation

20 mL reconstituted solution transfer into 100 mL volumetric flask, Add approx. 50 mL of diluent and sonicate for 10 mins, add 2 mL of 10% H_2O_2 and heat for 2 hours, on Boiling water bath. Then pipette out 5 mL of solution in 50 mL volumetric flask and make volume up to mark with diluent.

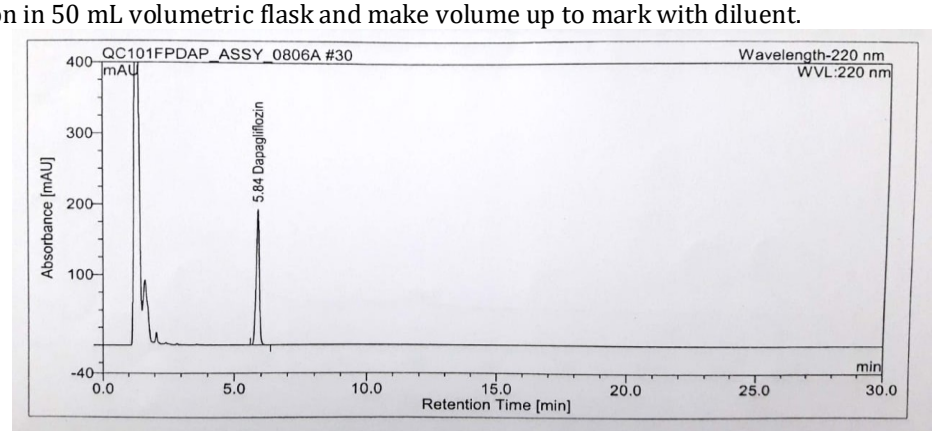


figure 5: oxidative degradation of dapagliflozin

Thermal Degradation:

Sample preparation

1 vial kept at 60^{0}C for 5 days, reconstitute vial with 19 mL water and transfer in 100 mL volumetric flask, make up the volume with diluent. Pipette out 5 mL of solution in 50 mL volumetric flask and make volume up to the mark with diluent.

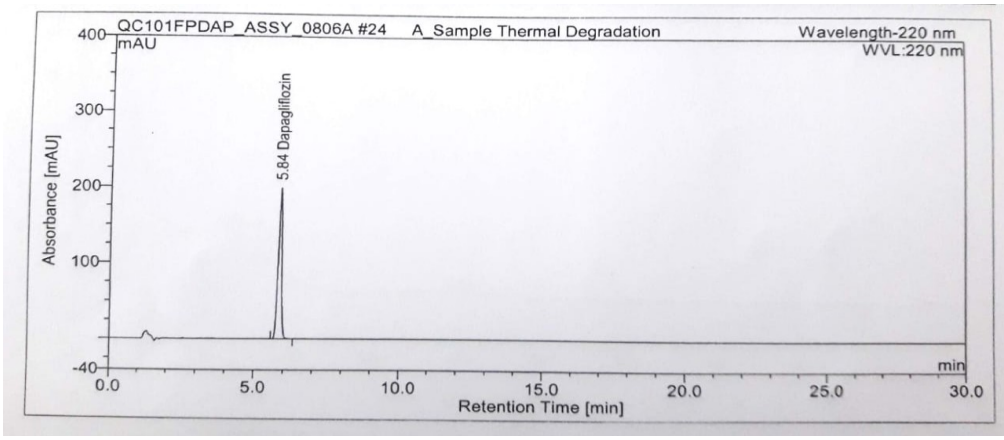


figure 6: thermal degradation of dapagliflozin

Photolytic Degradation:

Sample preparation

1 vial kept into UV chamber for 1.2 million lux hours (11.5 days), reconstitute vial with 19 mL water and transfer in 100 mL volumetric flask, make up the volume with diluent. Pipette out 5 mL of solution in 50 mL volumetric flask and make volume up to mark with diluent.

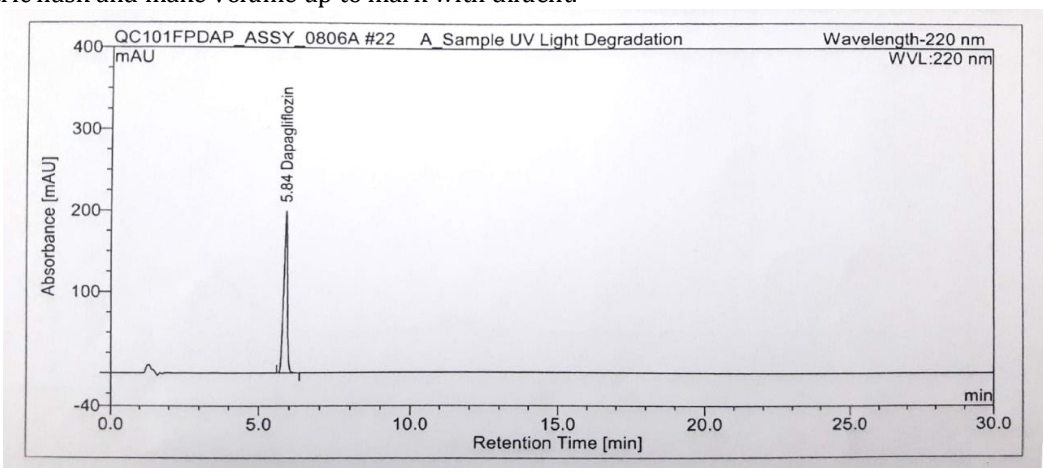


figure 7: photolytic degradation of dapagliflozin

Table 4.1: Degradation summary

Sr. No.	Stress Condition	Duration	Area after Degradation	% Degradation	% Mass Balance
1	Acid Hydrolysis (1N HCl)	110ºC for 4 hour	1567.132	6.0	98.1
2	Base Hydrolysis (2N NaOH)	100ºC for 3 hour	1610.135	0.0	95.8
3	Peroxide Degradation (10 % H_2O_2)	60ºC for 2 hour	1609.632	0.8	96.6
4	Thermal Degradation	60ºC in Oven for 5 days	1648.820	0.6	98.9
5	Photolytic degradation	UV chamber for 1.2 million lux hours	1659.567	0.1	98.8

Method Validation:

System Suitability/ System Precision

System suitability and precision was demonstrated by making five replicate injections as per the test method. The peak area of analyte for replicate injection was recorded. The theoretical plates and tailing factor were evaluated for the analyte peak.

Standard Sample Preparation (24 ppm)

Transfer an accurately weighed quantity of 96.0 mg of Dapagliflozin API to a 200mL volumetric flask, add about 100mL of diluent and sonicate to dissolve. Make up the volume up to the mark with diluent and mix dilute 5.0mL of above solution to 100mL with diluent and mix.

Table 4.2: System suitability/ system precision parameter

Injection No.	Peak area of Dapagliflozin
1	1714.840
2	1713.887
3	1714.049
4	1714.844
5	1717.253
Average	1714.975
% RSD	0.1

- Theoretical plates of Dapagliflozin peak : 11432
- Tailing factor of the Dapagliflozin peak : 1.1
- % RSD for five replicates standard injections is not more than 2 %.

Method Precision:

Method precision was demonstrated by preparing six samples as per the test method representing a single batch. The assay of these samples was determined and the precision and the precision of method was evaluated by computing the percentage relative standard deviation of assay results.

Table 4.3: Method precision parameter

Method Precision	% Assay
1	99.8
2	100.1
3	97.9
4	99.0
5	99.0
6	99.2
Average	99.2
% RSD	0.8

Acceptance criteria:

% RSD for assay of six preparations should not more than 2.0.

Sonication Time optimization:

Three sample dilution were made as per the proposed test methods, sonicate the above three preparations for different time period namely 30 minutes and 45 minutes and 60 minutes after adding a suitable quantity of diluent. After completion of sonication they were suitably diluted and the assay of these samples were determined.

Table 4.4: Sonication time optimization parameter

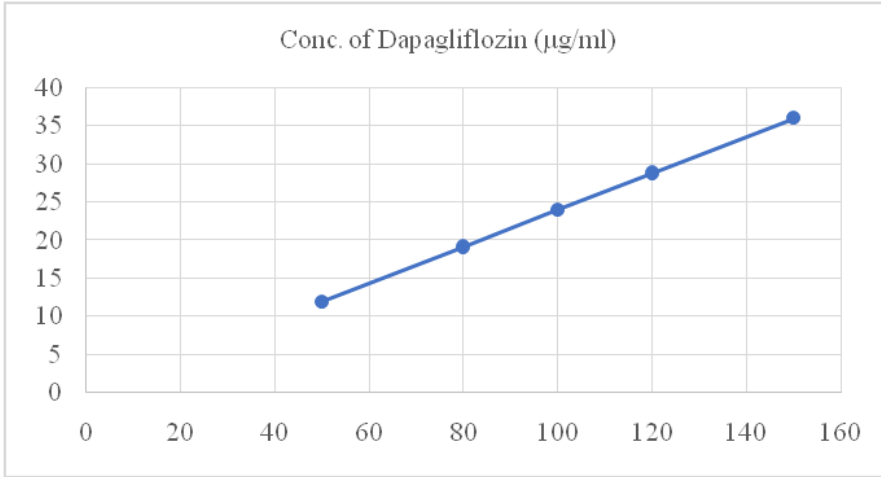
Sonication time	% Assay
30 min	97.6
45 min	98.2
60 min	98.3

Linearity:

The linearity of detector response for Dapagliflozin was demonstrated by preparing solutions of Dapagliflozin working standard over the range of 50- 150 % of standard concentrations. These solutions were injected into the HPLC system and the area of analyte peak was recorded. A graph of concentration vs. analyte peak response was plotted. The concentration co efficient between concentration & analyte peak response and Y- intercept of the correlation plot was evaluated.

Table 4.5: Linearity parameter

Linearity Level (%)	Conc. Of Dapagliflozin (µg/ml)	Peak area of analyte
50	12.003	887.438
80	19.206	1408.824
100	24.0075	1753.616
120	28.809	2098.941
150	36.011	2618.041



The plot was found to be linear with a correlation co efficient of for Dapagliflozin 0.9999 with respect to 100% linearity level response.

Accuracy:

The accuracy of the test method was demonstrated by preparing recovery samples (i.e. spiking of placebo with known quantities of API at the level of 50%, 100%, 150% of target concentration. The recovery samples were prepared in duplicate. The above samples were chromatograph and the percentage recovery for amount added was estimated. The precision of the recovery was determined by computing the relative standard deviation of duplicate recovery results.

Table 4.6: Accuracy parameter

Recovery at 50 % level:

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	48.47	48.23	99.5
2	48.54	48.27	99.4
Average			99.6
% RSD			0.3

Recovery at 100 % level:

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	49.88	49.71	99.7
2	49.88	49.69	99.6
Average			99.1
% RSD			0.1

Recovery at 150 % level:

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
1	146.17	143.18	98.0
2	146.13	143.36	98.1
Average			98.1
% RSD			0.1

Acceptance criteria:

The recovery is 98- 102 % and the RSD is NMT 2 %.

Filter compatibility & saturation:

The filter paper saturation was verified by preparing the assay samples with optimized samples preparation and analyzed the samples by discarding different volume of analyte. The assay of these samples was determined. Different filters are used. Here the filters are used are as follows, Millipore PVDF (0.45 µm), Millipore PVDF (0.22 µm), Millipore Nylon (0.2 µm).

Table 4.7: Filter compatibility and saturation parameter

Millipore PVDF (0.45 µm)

Discarded volume	% of Dapagliflozin	% Difference
Unfiltered	97.6	-
After 1 ml	97.7	0.1
After 3 ml	98.4	0.8
After 5 ml	98.1	0.5
After 7 ml	98.1	0.5
After 9 ml	97.8	0.2

Millipore PVDF (0.22 µm)

Discarded volume	% of Dapagliflozin	% Difference
Unfiltered	97.6	-
After 1 ml	97.9	0.3
After 3 ml	97.9	0.3
After 5 ml	97.8	0.2
After 7 ml	97.9	0.3
After 9 ml	97.7	0.1

Millipore Nylon (0.2 µm)

Discarded volume	% of Dapagliflozin	% Difference
Unfiltered	97.6	-
After 1 ml	97.7	0.1
After 3 ml	97.7	0.1
After 5 ml	97.8	0.2
After 7 ml	97.8	0.2
After 9 ml	97.7	0.1

Acceptance criteria:

The difference between the unfiltered and filtered samples is NMT 2.0 %.

Robustness:

According to Robustness, there is minor but deliberate change made in chromatographic parameter with reference of Floe rate and column temperature. To observe robustness, 100% level solution is used.

Table 8: Robustness parameter

Condition	% RSD	Theoretical plates	Tailing Factor
Normal	0.1	11538	1.0
Column temperature was changed by -5°C	0.1	10984	1.0
Column temperature was changed by +5°C	0.1	11972	1.1
Organic phase ratio of Mobile phase was changed by -2 % absolute [i.e., Buffer solution: Acetonitrile: Methanol (62:36:02) % v/v/v]	0.2	11972	1.0
Organic phase ratio of Mobile phase was changed by -2 % absolute [i.e., Buffer solution: Acetonitrile: Methanol (58:38:04) % v/v/v]	0.1	11045	1.1
Flow rate was changed by -10 % (i.e., 0.9mL/min)	0.1	12235	1.1
Flow rate was changed by -10 % (i.e., 1.1mL/min)	0.1	10904	1.0

CONCLUSION:

Stability Indicating RP-HPLC method has been developed and validated for the estimation of Dapagliflozin from tablet dosage form. The methods are found to be specific as there was no interference of any co-eluting impurities after degradation study. The degraded products are well resolved, indicating the method can also be useful for determination of degraded products.

All the parameters and results are found within the acceptance limit as given in the validation protocol. So, we can conclude that developed RP-HPLC method is found to be specific, linear, accurate and robust. Therefore, method is to be specific with good resolution. Thus, the proposed method can be used in pharmaceutical analysis for forced degradation study and routine quality control samples for Dapagliflozin form tablet dosage form.

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REFERENCES:

1. Marvin MD. 2005. "High Blood pressure and Diabetes-control them and live Longer" Le Jacq Ltd, 1,2.
2. Jeffery GH. 198., Bassett J., Mehdham J. and Denney RC., Text of quantitative Chemical Analysis; 5thEdn;,3-4.
3. Chatwal GR. 2002. and Anand SK., Instrumental Methods of Chemical Analysis; 5th Revised and Enlarged Edn; Himalaya Publication House, Mumbai, , 2.566
4. International Conference on harmonization of Technical Requirements for registration of Pharmaceuticals for human use, Impurities in New Drug Substance ICH Q3 A (R2).2006
5. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.
6. Willard HH., Merritt LL., Dean JA. And Frank AS.1986. Instrumental method of analysis; 7th edition; CBS publishers and Distributors, New Delhi, pp. 1-5.
7. Sanagapati MN., Dhanalakshmi K, Nagarjuna RG., Kavitha B.2014. "Method development and validation of Dapagliflozin API by UV spectroscopy", Int. J. Pharm. Sci. Rev. Res., Vol. 27(1), 270-272
8. Mohammad YN., Gowari SD. 2015. "A validated stability indicating high-performance liquid chromatographic method for simultaneous determination of Metformin HCl and Dapagliflozin in bulk drug and tablet dosage form", Asian Journal of Pharamaceutical and Clinical Research, volume-8, Issue 3, 320-326.
9. Kapupara PP., Shah KV., Jani BR. 2015. "Development and validation of UV-spectroscopic method for simultaneous estimation of Dapagliflozin and Metformin Hydrochloride in synthetic mixture", International Journal of Research and Development in Pharmacy and Life sciences, Vol. 4, No.3, 1569-1576.