GMR
The Original

ADVANCED METHODOLOGICAL ESTABLISHMENT OF STABILITY INDICATING ASSAY METHOD AND FORCED DEGRADATION STUDY OF DAPAGLIFLOZIN- DRUG HAVING ACTIVITY AGAINST METABOLIC DISORDERS BY USING RP-HPLC TECHNIQUE

Gabhale Nikhil K.[1*], Jadhav Shailaja B.[2]

[1]Progressive Education Society's Modern College of Pharmacy, affiliated to Savitribai Phule Pune University, Yamuna Nagar, Nigdi, 411044, Pune, India
[2]Progressive Education Society's Modern College of Pharmacy, affiliated to Savitribai Phule Pune University, Yamuna Nagar, Nigdi, 411044, Pune, India
*Corresponding Author: Gabhale Nikhil K, E-mail: nikhilgabhale77@gmail.com

ABSTRACT
It is a faster, precise and accurate method of analysis for Dapagliflozin using HPLC. This method includes Thermo scientific BDS C18 Hypersil (150cm × 4.6 mm, 5 µ) column, Mobile phase solution A: 0.1% OPA in Water (100%), Mobile phase solution B: ACN: Methanol (40:60 %V/V) and mixture of A: B in the proportion of (40:60 %v/v) at wavelength of 224 nm. The linearity, accuracy, precision, and robustness of this method were all confirmed. The range of linearity was discovered 20-60µg/mL & The correlation coefficient value was 0.999. The precision and robustness of this method was proven hence percent RSD was found less than 2%. The percentage recovery was in the range of 99.35-101.54%. LOD and LOQ were 11.75 S/N and 20.81 S/N. To evaluate the stability indicating properties degradation study by exposed with Acid (3 N HCL in a temperature range of 80°C) 3.97%, Base (3 N HCL in a temperature range of 80°C) 4.02%, A solution containing hydrogen peroxide (6% H_2O_2) 3.90%, Neutral for 1 hour 4.08%, UV light exposure at 294nm for 24 hours 3.02%, thermal degradation at 80^{O}C for 1hr 3.90%.

KEYWORDS: Development and validation of analytical method, HPLC, Dapagliflozin, Stability, Forced degradation.

1.0 INTRODUCTION

In Present study analytical method was developed for analysis of Dapagliflozin. Linearity, precision, accuracy, robustness, linearity range parameters of validation for developed analytical method done [1,2]. Stability of substance and specificity of the method were determined by degradation study. Acidic, basic, oxidation, photolytic, thermal degradation study done by performed by exposing of working standard solution of Dapagliflozin [7].

2.0 MATERIALS AND METHODS

1. **Drug:** Dapagliflozin

Dapagliflozin is a white crystalline powder.

2. Structure:

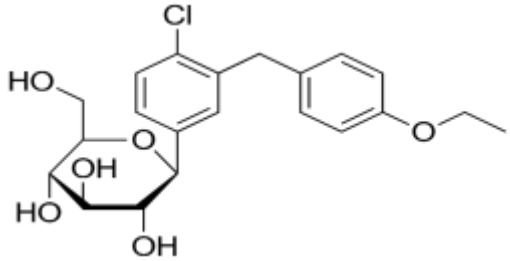


Figure 1: Structure of Dapagliflozin

3. Physicochemical properties of drug:

Molecular Formula : $C_{21}H_{25}ClO_6$
Molecular Weight : 408.9 g/mol
Solubility:
a.In Water: Poorly soluble

b. In Acetonitrile: Slightly soluble
c.In Methanol: Sparingly soluble
d. In Toluene: practically insoluble

4. MATERIALS AND METHOD

Dapagliflozin was procured as gift samples from Pharmaceutical Industry. The HPLC system employed was Mags Labs Pvt. Ltd. HPLC instrument Waters 2695 equipped with UV/ Visible detector (Pune, India), coming volumetric flasks and pipettes of borosilicate glass were used in the study. Methanol, Acetonitrile (HPLC Grade), Orthophosphoric acid, was procured from S.D. Fine Chemicals Ltd., Mumbai, India. Whatman filter paper No. 41 was also used in the study. The solution of 3N NaOH was prepared in double distilled water as per IP 1996 procedure. 3N HCl was prepared following the procedure outlined in BP 1999 and USP 24.

Preparation of standard stock solution and selection of detecting wavelength:

1] Standard stock solution:

Weighed accurately 25 mg of Dapagliflozin in 25.0 ml volumetric flask, dissolved in HPLC grade methanol and volume was made up to mark with same solvent (conc. 1000 ppm). Diluted 2 ml of stock solution in 50 ml volumetric flask with the same solvent to obtain the concentration of 40µg/ml. This solution was scanned in 1 cm cell using double beam UV- Visible Spectrophotometer over the range of 400-200 nm and the UV absorbance spectrum was recorded. From the spectrum, the detecting wavelength selected for estimation of the drug was 224.0 nm.

Selection of mobile phase

The pure drug of Dapagliflozin was injected into the HPLC system and run in different solvent systems. Each mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. Different mobile phases like methanol and water, acetonitrile, and water in various proportions were tried. Different individual solvent, as well as combinations of solvents, were tried to get a stable peak. Each mobile phase was filtered through 0.45µm nylon membrane filter and sonicated on the ultrasonic bath.

Table 1: Selection of mobile phase

Sr. No	Mobile Phase	Concentration	Comment
1.	Water	100%	System suitability parameters not satisfactory
2.	CAN	100%	System suitability parameters not satisfactory
3.	ACN: Water	50:50	System suitability parameters not satisfactory
4.	Methanol	100%	System suitability parameters not satisfactory
5.	Methanol: Water	80:20	System suitability parameters not satisfactory
6.	ACN: Methanol	80:20	System suitability parameters not satisfactory
7.	ACN: Water	40:60	System suitability parameters not satisfactory
8.	A: 0.1% OPA in Water B: ACN: Methanol	A: 100% B: 80:20	System suitability parameters not satisfactory
9.	A: 0.1% OPA in Water B: ACN: Methanol	A: 100% B: 40:60	System suitability parameters found satisfactory

After several trials Mobile phase - Mixture of Solution A (0.1% OPA in Water) : Solution B ACN: Methanol (40:60 v/v) and A: B (40:60 %v/v) was found to be the most satisfactory since it gave sharp, peak with symmetry within limits and significant reproducible retention time.

Selection of chromatographic parameters

The method was developed using Thermoscientific BDS C18 Hypersil (150× 4.6 mm), 5 µm column. The mobile phase used was A: 0.1% OPA in Water (100%), B: ACN: Methanol (40:60) and Mobile phase - Mixture of Solution A: Solution B (40:60 v/v). Flow rate adjusted was 0.8 mL/min. Detection was carried out at 224.0 nm. The mobile phase and samples were degassed by ultrasonic vibrations for 20 min. and filtered through 0.45µm nylon membrane filter. All determinations were performed at constant temperature.

Preparation of mobile phase and construction of calibration curves

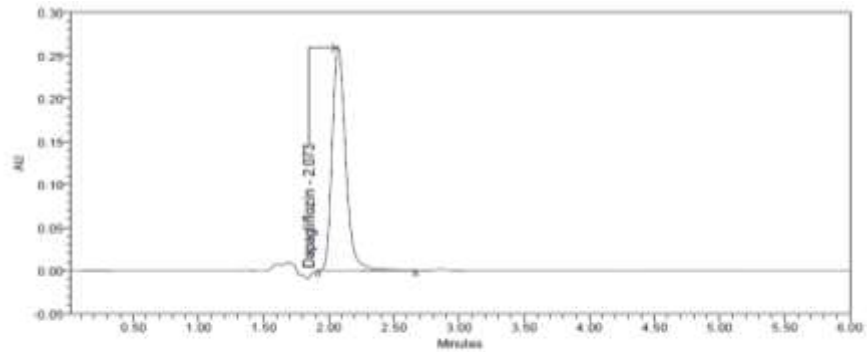
Mobile phase A was prepared by mixing 1ml OPA in Water 1000 ml water & B was prepared by mixing of 400 ml Acetonitrile in 600 ml Methanol. Both mobile phases were sonicated for 15min on ultrasonic bath and then it was filtered through 0.45µm nylon membrane filter.

The aliquot portion of the standard stock solution was diluted appropriately with same to obtain concentration of 40 µg/mL. The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. The appropriate aliquot of Dapagliflozin stock solution was transferred in series of 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to obtain the various concentration of 20-60 µg/ mL. These solutions were injected using a 20µL fixed loop system separately in triplicate and chromatographed under conditions described above and peak areas were recorded. The graph was plotted as concentration of drug Vs peak area.

Chromatographic conditions:
The following chromatographic conditions were optimized by trial and error for effective separation and densitometric evaluation of drugs:

Table 2: Chromatographic condition

Stationary phase	Thermoscientific BDS C18 Hypersil (150× 4.6 mm), 5 µm column
Mobile phase	Solution A: 0.1% OPA in Water (100%), Solution B: Mixture of ACN: Methanol (40:60). Mobile phase - Mixture of Solution A : Solution B (40:60)
Flow Rate	1.0 mL/min
Injection volume	20 µl
Sampler temperature	25ºC
Run Time	6 minutes
Oven Temperature	Ambient 25ºC
Wavelength	224 nm
Detector	UV
Software	Empower



	Peak Name	RT	Area	% Area	USP Tailing	USP Plate Count
1	Dapagliflozin	2.073	1876570	100.00	1.24	2048

Figure 2: Typical chromatogram for Dapagliflozin.

Analytical Method validation
To prove the reliability and reproducibility, the developed method was validated for following validation parameters.
Analysis of bulk drug
a. Preparation of Standard Solution:
Five solutions were prepared and analyzed in following manner:
An accurately weighed quantity of 25.25 mg DAPA was transferred to 25.0 ml volumetric flasks dissolved and diluted up to the mark with methanol. From this solution, 2.0 mL was transferred to 50.0 ml volumetric flask and diluted to the mark with methanol (Concentration 40 µg/ml). Five replicates of standard solutions injected for system suitability criteria.
1. Linearity and range
As per ICH Q2(R2) guidelines, for establishment of linearity of DAPA by proposed method, the calibration curve was obtained at five levels in the concentration range of 20-60µg/mL. For this the different levels of DAPA working standard (40 µg/ml) were injected. For evaluation of linearity, observed peak area and concentrations

were subjected to least square regression analysis to calculate calibration equation and correlation coefficient. The observed linearity confirming adherence of the system to Beer's law. The regression analysis equation was y=48398x+22783 with correlation coefficient (r) was 0.999.

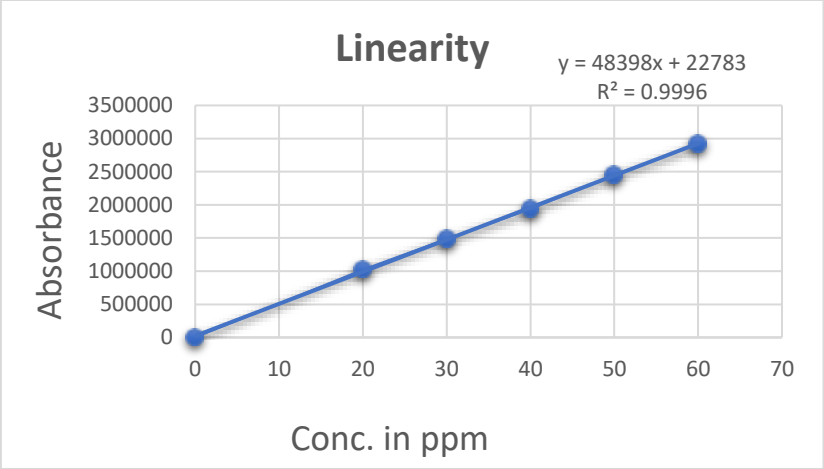


Figure 3: Linearity curve of Dapagliflozin.

Table 3: Preparation of different linearity levels of DAPA as per ICH Q2(R2) guidelines.

Linearity Level	Concentration (ppm)	Area
I	20	1022879
II	30	1488856
III	40	1936818
IV	50	2452275
V	60	2915515

Table 4: Statistical data of DAPA by HPLC

Sr. No.	Parameters	Results
1	Linearity range	20-60µg/mL
2	Regression equation	y=48398x+22783
3	Correlation coefficient	0.999

2. Method Precision:

Precision of the method was verified by repeatability and intermediate precision studies.

I) Repeatability:

In the repeatability studies, six replicates of one concentration of DAPA were prepared and injected on HPLC. From the obtained data, %RSD of Dapagliflozin were found to be less than 2%.

II) Intermediate Precision:

In the intermediate precision studies, three replicates of one concentration were prepared and injected on HPLC two consecutive days. From the obtained data, %RSD of DAPA were found to be less than 2%. The intermediate precision results of Dapagliflozin shown in Table.

Table 5: Method precision of DAPA (Intraday)

	Method Precision Dapagliflozin (Intraday)						AVG	%RSD
Intraday_Dapa	**STD**	**1876570**	**1886298**	**1888136**	**1887235**	**1899451**	**1887538**	**0.43**
	SPL	**1896937**	**1894083**	**1889627**	-	-	**1893549**	**0.19**

Table 6: Intermediate precision of DAPA (Interday)

	Method Precision Dapagliflozin (Interday)						AVG	%RSD
Interday_Dapa	**STD**	**1925203**	**1941054**	**1931709**	**--**	**--**	**1932655**	**0.41**

3. Accuracy

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method, as per ICH guidelines.

Preparation of Sample Solutions:

An accurately weighed quantity of pre-analyzed tablet powder equivalent to about 25 mg DAPA was transferred individually in nine different 25 ml volumetric flasks. To each of the flask following quantities of DAPA was added.

Table 7: Preparation of sample solution

Flask no.	Weight of Dapagliflozin (in mg)	Flask no.	Weight of Dapagliflozin (in mg)
1	21.22 mg	6	25.76 mg
2	21.34 mg	7	30.91 mg
3	21.12 mg	8	30.74 mg
4	25.95 mg	9	30.25 mg
5	25.55 mg	-	-

Then 5 ml methanol was added to each flask and contents of the flask were ultrasonicated for 20 minutes, volume was made up to the mark with methanol. The solution was individually mixed and filtered through 0.45µm PVDF syringe. From the filtrate, 2.0 ml solution was diluted to 50 ml with methanol. The result of accuracy study is given in table. The accuracy of the method was determined by calculating the recovery of Dapagliflozin by the standard addition method at three concentration levels (80%, 100% and 120%). The percentage recoveries of Dapagliflozin were found to be in the range of 101.64% to 103.98%. The Accuracy results of Dapagliflozin shown in table. The weight of the drug sample was taken is 25 mg.

Table 8: Accuracy results of DAPA by HPLC

Level of recovery (%)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery	% Recovery Mean	SD	%RSD
80	20	22.18	104.54	103.98	1.05	1.01
	20	21.92	102.76			
	20	22.10	104.64			
100	25	26.33	101.47	101.64	0.18	0.18
	25	25.96	101.62			
	25	26.23	101.84			
120	30	31.63	102.33	102.66	1.09	1.06
	30	31.94	103.89			
	30	30.79	101.78			

4. LOD & LOQ:

Determined by S/N ratio method in HPLC.

4.1 Limit of Detection (LOD)

Table 9: LOD (Determined by S/N ratio method in HPLC)

		Area	s/n	T.F.	R.T.
LOD	**Conc. 0.132 ppm**	60833	27.99	1.67	2.207

4.2 Limit of Quantitation (LOQ)

Table 10: LOQ (Determined by S/N ratio method in HPLC)

		Area	**s/n**	**T.F.**	**R.T.**
LOQ	**Std conc. 0.4 ppm**	79741	38.6	1.76	2.198
	Std conc. 1 ppm	111243	91.16	1.51	2.186

Table 11: LOD & LOQ (Determined by S/N ratio method in HPLC)

Parameters	**DAPA (S/N Ratio)**
Limit of Detection	27.99
Limit of Quantification	38.60

5. Robustness:

Robustness of method was evaluated by optimization of method parameters like change in flow rate, change in wavelength. Less than 2% RSD was observed.

Table 12: Change in Mobile phase composition (±1ml) of DAPA

Chromatographic Changes			
Factor	**Level**	**RT values**	**Tiling Factor**
Flow Rate			
0.8 ml/min	-0.2	2.67	1.38
1.0 ml/min	0.0	2.07	1.17
1.2 ml/min	+0.2	1.78	1.28
Wavelength			
Low Wavelength 222nm	-2	1.94	-
224 nm	0	2.07	-
High Wavelength 226nm	+2	1.94	-

Table 13: Summary of Method validation result by HPLC

Sr. No.	**Parameters**	**Results**
1	Linearity (n=6)	20-60 ng/band
2	Correlation coefficient (R^2)	0.999
3	Precision (%RSD)	
	Interday Pression	0.19 & 0.14
	Intraday precision	0.19 & 0.55
4	Accuracy (%Recovery)	101.64% to 103.98%
5	Limit of Detection (LOD)	27.99 S/N
6	Limit of Quantitation (LOQ)	38.60 S/N
7	Robustness (RT)	
	a) Change in Flow Rate (±2 ml/min)	
	+0.2min	1.78
	-0.2min	2.67
	b) Change in Wavelength	
	Low Wavelength 222nm	1.94
	High Wavelength 226nm	1.94

6. Forced Degradation Study

For Dapagliflozin forced degradation studies done as per ICH guidelines. In forced degradation, stress conditions like acid, base, hydrolytic, oxidative, dry heat (thermal) and light exposure applied on drug.

Forced degradation study of DAPA:

25 mg drug taken in 25 ml volumetric flask and dissolved completely using diluent. Further 2.0 ml of this stock solution taken in six 50 ml volumetric flasks. In flask no 1,2,3 2.0 ml of 3 N HCl, 3 N NaOH & 6% H_2O_2 added and refluxed at 80°C for 1 hr. In flask 4, 2.0 ml solution in HPLC grade water kept for 3 hrs. Flask 5 sample make up to the mark with diluent and kept at 80°C for 1 hr for heat/thermal degradation. The sample containing Flask No. 6 was make up to the mark with diluent and kept in UV Chamber at 294nm for 24 hrs. After completion of degradation all flasks were analyzed same as that of method.

The chromatogram obtained from acidic, alkaline, oxide, Neutral, heat and UV exposure, are as follows:

6.1 Acid degradation:

In Acid degradation, on exposure to 3N HCl 0.74% degradation was observed.

Table 14: Acid degradation result by HPLC

Area of Standard	1955109	% Degradation = 0.74%
Area after Acid Degradation	1940447	

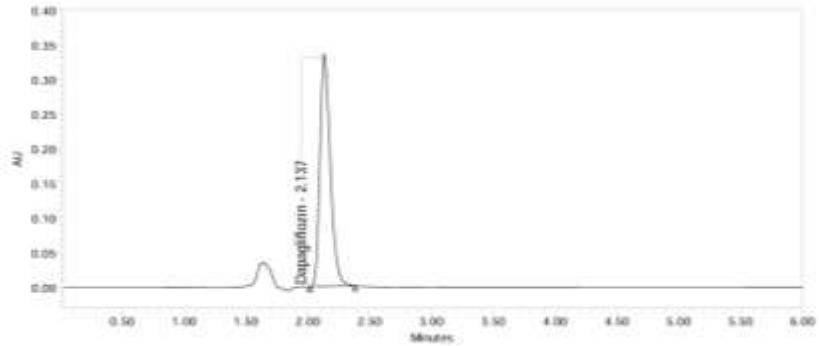


Figure 4: HPLC Chromatogram for acid degradation

6.2 Alkaline/ Base degradation:

In alkaline degradation, on exposure of 3N NaOH 2.23% degradation was observed.

Table 15: Alkaline/ Base degradation result by HPLC

Area of Standard	1955109	% Degradation = 2.23%
Area after Base Degradation	1911366	

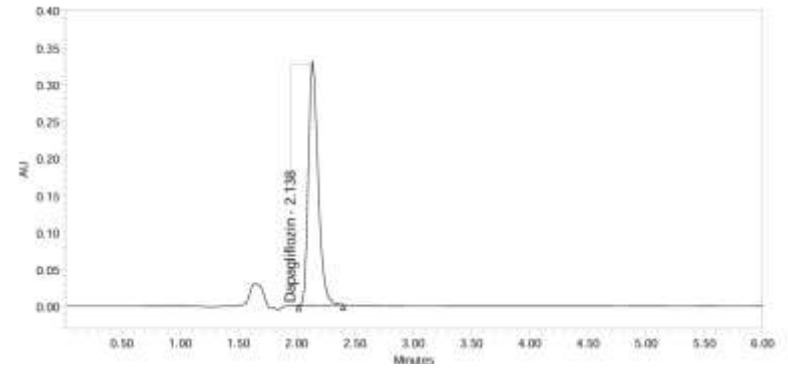


Figure 5: HPLC Chromatogram for alkaline degradation

6.3 Oxidation degradation:

In oxidation degradation, on exposure of 6% H_2O_2 9.20% degradation observed.

Table 16: Oxidation degradation result by HPLC

Area of Standard	1955109	% Degradation = 9.20%
Area after Oxidation Degradation	1775087	

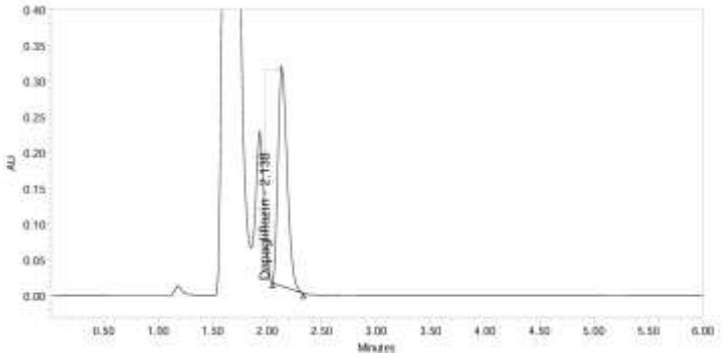


Figure 6: HPLC chromatogram for oxidation degradation.

6.4 Photolytic stress degradation:

In photolytic degradation, on exposure to UV light (294 nm) for 24 hrs 2.49% degradation observed.

Table 17: Photolytic stress degradation result by HPLC

Area of Standard	1955109	% Degradation = 2.49%
Area after Photolytic Degradation	1906407	

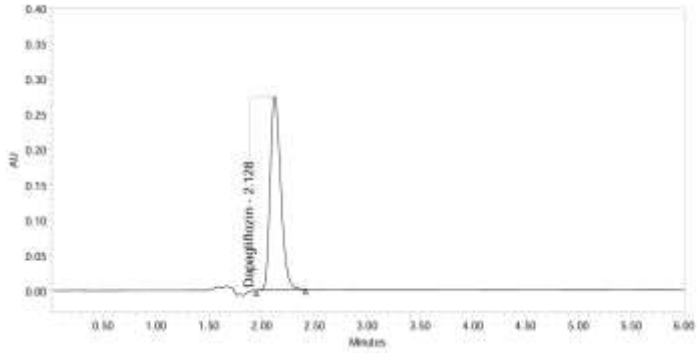


Figure 7: HPLC chromatogram for photolytic degradation.

6.5 Thermal degradation:

In thermal degradation, on exposed to 60°C for 45 min 1.69% degradation observed.

Table 18: Thermal degradation result by HPLC

Area of Standard	1955109	% Degradation = 1.69%
Area after Thermal Degradation	1922002	

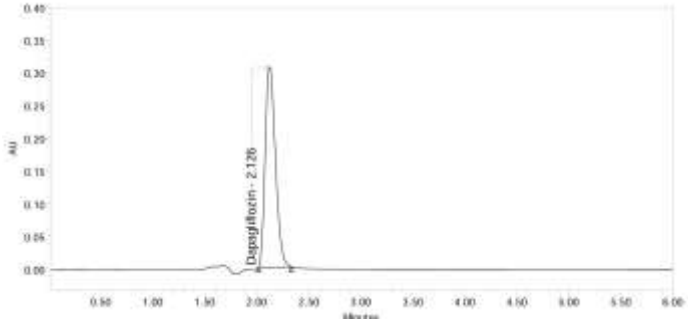


Figure 8: HPLC chromatogram for Thermal degradation.

6.6 Neutral degradation:

In Neutral stress degradation, exposed at room temperature for 45 min 0.24% degradation observed.

Table 19: Neutral degradation result by HPLC

Area of Standard	1955109	% Degradation = 0.24%
Area after Neutral Degradation	1950344	

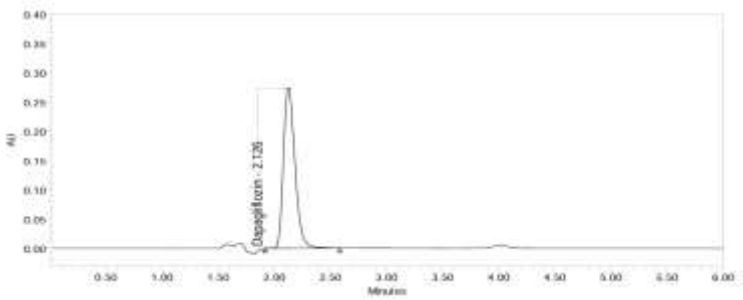


Figure 9: HPLC chromatogram for Neutral degradation.

Table 20: Degradation studies summary

Sr. No.	Degradation	Temp and time	Percent Degradation
1.	Acid (3 N HCl)	80°C temp for 1 hr	0.74%
2.	Base (3 N NaOH)	80°C temp for 1 hr	2.23%
3.	Oxide (6 % H_2O_2)	80°C temp for 1 hr	9.20%
4.	Photolytic Degradation	UV light (294 nm) for 24 hrs	2.49%
5.	Thermal	80°C temp for 1 hr	1.69%
6.	Neutral (H_2O)	Room temp. for 45 min	0.24%

- **Forced Degradation summary:**

In forced degradation studies Acid, base, photolytic, heat, neutral degradation shows minimum degradation as compared to oxidative degradation. Hence, we can conclude that Dapagliflozin degraded in oxidative degradation.

CONCLUSION

Present analytical method has been successfully validated according to the ICH guidelines. This method was the intended use of the determination of assay of a Dapagliflozin drug substance and product. Method validation was conducted in accordance with the regulatory guidelines, such as ICH guidelines and pharmacopeial standards. The method met the pre-defined acceptance criteria validation parameter such as Accuracy, Precision, Specificity, Linearity and range, Limits of detection (LOD) and quantitation (LOQ), Robustness etc. Hence this method was considered fit for its intended purpose for routine quality control and stability testing.

Acknowledgments: I would like to thank Dr. Shailaja Jadhav Madam for their valuable guidance, Dr. P. D. Chaudhari Sir for inspiration. I am also thankful to all my friends, family for supporting me.

Conflict of Interest: No Conflict of Interest.

REFERENCES

1. M. D. Game, Naglaxmi Bopudi, Development and Validation of Stability Indicating HPLC Method for Estimation of Dapagliflozin in Marketed Formulation, IJPER, June 2018 Vol.:12, Issue:3.
2. Shakir Basha , Sravanthi P, Development and validation of dapagliflozin by reversed-phase high-performance liquid chromatography method and it's forced degradation studies, Asian J Pharm Clin Res, Vol 10, Issue 11, 2017, 101-105.
3. Phani RSCH, Prasad KRS, Useni Reddy Mallu, A Study of New Method Development, Validation and Forced Degradation for Simultaneous Analysis of Dapagliflozin and Saxagliptin in Pharmaceutical Dosage Form by HPLC Method, Der Pharma Chemica, 2017, 9(20):96-103.
4. Manasa Sanagapati, Dhanalakshmi K, Nagarjuna Reddy G, Sreenivasa SDevelopment and Validation of stability-Indicating RP-HPLC method for determination of Dapagliflozin, Journal of Advanced Pharmacy Education & Research,Jul-Sep 2014, Vol 4 Issue 3.
5. Vaghela Yavantika V., Dr. Pragnesh Patani2, Digesh Patel, Development and validation of stability indicating estimation method of dapagliflozin in its tablet dosage form, IJRAR, volume 6, issue 2, april – june 2019.
6. Md. Rageeb Md. Usman, Sufiyan Ahmad, Chandrakant Deore, Tanvir Shaikh, Bharat Jain, Md. Imran, Stability Indicating RP-HPLC Method for Determination of Saxagliptin and Dapagliflozin in Bulk and Tablet Dosage Forms, J. Pharm. Sci. & Res. Vol. 12(4), 2020, 499-506
7. Mr. Tarang Patel, Ronak Parikh, Method Development, Validation and Forced Degradation Studies of Dapagliflozin and Pioglitazone Hydrochlorides in Synthetic Mixtures by RP-HPLC, IJTSRD, Volume – 6, Issue – 6, September-October 2022, Page 1858