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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL AND EPLERENONE IN SYNTHETIC MIXTURE

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ABSTRACT

A simple, precise, accurate, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of Dapagliflozin Propanediol and Eplerenone in a synthetic mixture. Chromatographic separation was achieved using a Cosmosil C18 column (250 mm ×4.6 mm, 5µm) with a Isocratic mobile phase of buffer and methanol (30:70%v/v), at a flow rate of 1.0 mL/min and detection at 228 nm. The developed RP-HPLC method showed excellent performance with good chromatographic separation of Dapagliflozin Propanediol and Eplerenone. The method exhibited strong linearity in the ranges of 10-30 ppm for Dapagliflozin and 25-75 ppm for eplerenone, with correlation coefficients exceeding 0.999. Validation studies, conducted in accordance with the International Council for Harmonization (ICH) Q2(R2) guidelines, confirmed that the method was precise, accurate, robust, specific, and sensitive. The stability-indicating capability was verified through forced degradation experiments under acidic, alkaline, oxidative, and thermal conditions, where degradation products were adequately resolved from the parent compounds using the developed method. The validated method was successfully applied to the analysis of a synthetic mixture, yielding assay results of 100.33% for dapagliflozin and 99.65% for eplerenone. Therefore, the proposed method is suitable for routine quality control analysis and stability studies.

INTRODUCTION

Eplerenone is a selective mineralocorticoid (aldosterone) receptor antagonist, primarily used in cardiovascular settings to mitigate the harmful effects of aldosterone excess (e.g. in heart failure or post-myocardial infarction). Dapagliflozin is an inhibitor of sodium–glucose cotransporter 2 (SGLT2), a newer class of medicines originally developed to enhance urinary glucose excretion and thus improve glycemic control in type 2 diabetes mellitus, but which has also shown substantial benefits on heart failure and renal outcomes. The hypothesis of combining eplerenone with dapagliflozin is to target complementary mechanisms of disease and thereby achieve additive or even synergistic benefits, while balancing safety considerations.[1-2]

Dapagliflozin (DAPA) is a sodium-glucose cotransporter 2 inhibitor used to treat type 2 diabetes mellitus. Dapagliflozin, when used alongside diet and exercise in adults, enhances glycemic management by blocking glucose reabsorption in the proximal tubule of the nephron and inducing glycosuria.[3]

Eplerenone (EPLE) is a mineralocorticoid receptor antagonist. It binds to the receptor and locks the binding of aldosterone, a component of the renin-angiotensin-aldosterone-system (RAAS). Aldosterone binds to mineralocorticoid receptors in tissues and increases blood pressure through induction of sodium reabsorption and possibly other mechanisms. Eplerenone works by blocking the actions of aldosterone and resulting in decreasing blood pressure.[4-5]

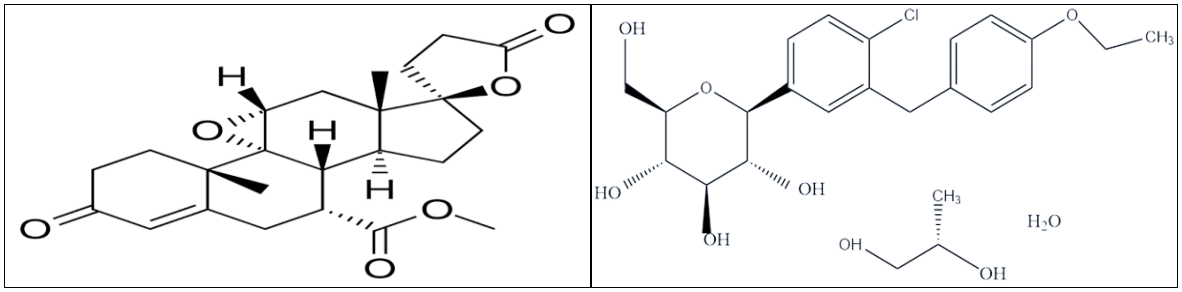

Fig. 1: Structure of Dapagliflozin propanediol monohydrate and Eplerenone.

The literature review reveals that few analytical methods were reported like RP-HPLC methods[6-7], Spectrophotometric method[8], LC/MS[9] and Stability study[10-14] in single or in Combination with other drug in bulk and pharmaceutical dosage form. But no method is reported. Hence present study aimed to develop a new Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Dapagliflozin propanediol and Eplerenone in Synthetic Mixture suitable for routine quality control analysis.

MATERIALS AND METHOD

Chemicals and Reagent

Sunij Pharma Pvt. Ltd. (Gujarat, India) provided dapagliflozin, while MSN laboratories (Gujarat, India) provided a gift sample of eplerenone. Other common reagents such as methanol, orthophosphoric acid, hydrochloric acid and sodium hydroxide provided as a Anachem and acetonitrile and HPLC grade water provided in a Astron chemicals.

Preparation of Solutions

Dapagliflozin stock solution (200 µg/ml)

20 mg of Dapagliflozin propanediol monohydrate was transferred into 100 ml flask. 70 ml Methanol added and dissolved by sonication. Volume made with Methanol.

Eplerenone stock solution (500 µg/ml)

50 mg of Eplerenone was transferred into 100 ml flask. 70 ml methanol added and dissolved by sonication. Volume made with methanol.

Mixed standard solution (20 µg/ml dapagliflozin + 50 µg/ml eplerenone)

1 ml Dapagliflozin stock solution + 1 ml Eplerenone stock solution into 10 ml volumetric flask. Volume made with diluent.

Preparation of synthetic mixture

The mixture contained dapagliflozin and eplerenone along with commonly used pharmaceutical excipients, including mannitol, microcrystalline cellulose, hydroxypropyl methylcellulose and magnesium stearate.

Forced Degradation Studies

To evaluate the stability of the drugs stress studies were performed on a synthetic mixture confirm the separation of dapagliflozin and eplerenone from potential degradation products. Stress testing involved exposing the mixture to acidic and alkaline conditions using 0.1 N HCl and 1 N NaOH at room temperature for 1 hour, followed by neutralization. Oxidative stress was induced by treating the mixture with 3% hydrogen peroxide for 3 hours at room temperature, while thermal degradation was assessed by subjecting the sample to a conical flask and was kept in preheated oven at 60 °C for 3 hours. In all stress conditions, the degraded samples were neutralized or diluted with the mobile phase.

Method Validation[15]

Adhering to the ICH Q2(R2) framework, the study assessed vital performance metrics, specifically robustness, sensitivity, accuracy, precision, Working range, specificity, and system suitability.

Specificity

The specificity of the developed HPLC method was critically evaluated through the analysis of individual standard solutions and their combined mixture for Dapagliflozin and Eplerenone. The chromatographic profiles demonstrated that both analytes produced sharp, symmetrical, and well-resolved peaks at distinct retention times.

Range and Linearity

The linearity of the proposed HPLC method was established by analyzing a series of standard solutions of Dapagliflozin and Eplerenone at different concentration levels. Standard stock solutions of both analytes were appropriately diluted to obtain five concentration levels, namely 10+25 µg/mL, 15+37.5 µg/mL, 20+50 µg/mL, 25+62.5 µg/mL, and 30+75 µg/mL for Dapagliflozin and Eplerenone, respectively.

Precision

Precision was evaluated at three levels: intermediate precision (intraday precision), reproducibility (interday precision), and repeatability. The solution containing 20µg/ml of Dapagliflozin and 50µg/ml of Eplerenone was injected six times for repeatability study. Intermediate precision and reproducibility study was performed by injecting 10, 20, 30 µg/ml of Dapagliflozin and 25, 50, 75 µg/ml of Eplerenone solutions three times for each aliquot. The %RSD for precision was calculated.

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

These parameters are useful for understanding the sensitivity of the developed analytical method. Hence, LOD and LOQ were evaluated for Dapagliflozin and Eplerenone. The LOD & LOQ were calculated on basis of formula

$$LOD = 3.3 \times \sigma / S$$
$$LOQ = 10 \times \sigma / S$$

Accuracy

Accuracy represents the closeness of agreement between the experimentally obtained values and the true value of the analytes. Accuracy of the method was evaluated at three concentration levels corresponding to 50%, 100%, and 150%, i.e., 10 µg/mL and 25 µg/mL, 20 µg/mL and 50 µg/mL, and 30 µg/mL and 75 µg/mL for Dapagliflozin and Eplerenone, respectively.

Robustness

Robustness evaluates the reliability of an analytical method under small and deliberate variations in method parameters. The robustness of the developed RP-HPLC method was assessed by introducing slight variations in mobile phase composition and flow rate.

Assay of synthetic mixture

The synthetic mixture was prepared in a fixed ratio of Dapagliflozin to Eplerenone of 2:5. Accordingly, the mixture contained 20 mg of Dapagliflozin and 50 mg of Eplerenone along with common pharmaceutical excipients. Synthetic mixture powder eq. to Dapagliflozin 20 mg, Eplerenone 50 mg was taken into 100 mL volumetric flask. 70 mL methanol was added. Kept in sonicator for 20 minutes. Then volume was made up with methanol. Solution was filtered through Whatman 0.45 PVDF syringe filter. Transfer 10 mL of the above stock solution into a 100 mL volumetric flask and dilute to volume with methanol. This results in an intermediate solution having concentrations of 20 µg/mL of Dapagliflozin and 50 µg/mL of Eplerenone. This solution being subjected to HPLC analysis under optimized conditions.

RESULT AND DISCUSSION

Analytical wavelength detection

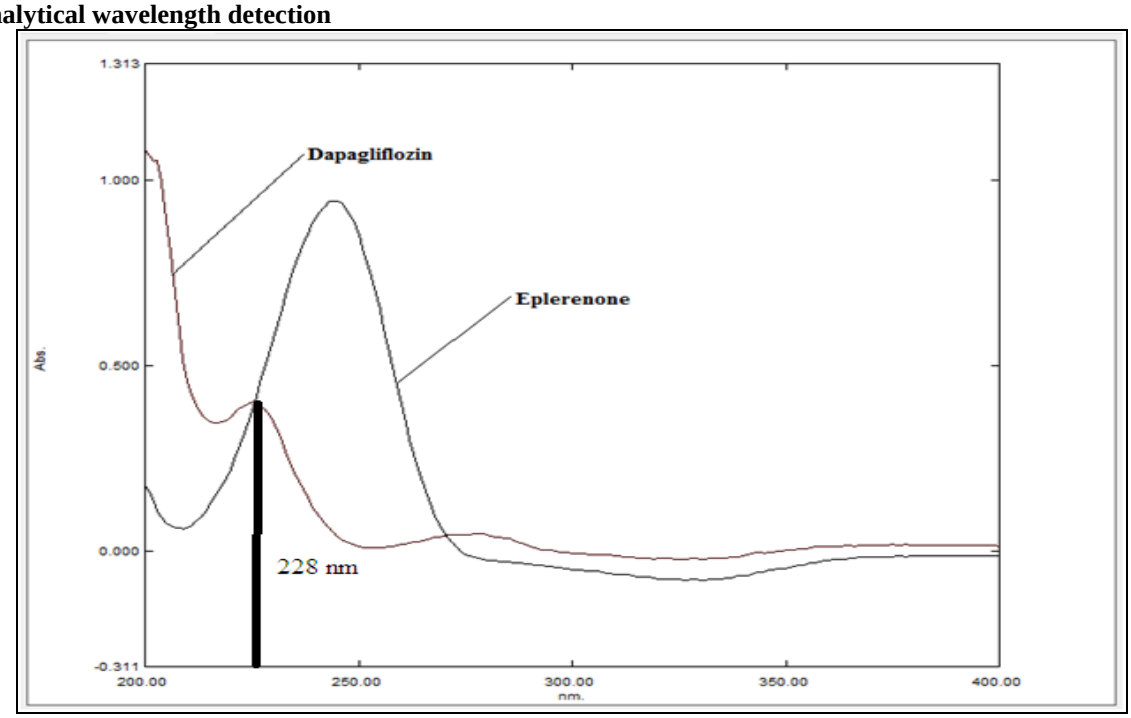

Figure 2: Overlay spectra of Dapagliflozin and Eplerenone.

UV preparation: 10 ppm of dapagliflozin and 25 ppm of eplerenone solutions were prepared in methanol and scanned in the range of 200 to 400 nm. Results: Isosbestic point 228 nm.

Optimized Chromatographic Conditions

Shimadzu HPLC system was used for method development, degradation studies and validation. Data acquisition was performed on HPLC. The separations were achieved on Cosmosil C18 (250 mm×4.6 mm, 5µm), column. The column was maintained at 25 °C temperature and the eluent was monitored at 228 nm using detector. The mobile phase of Buffer: Methanol (30:70 % v/v) mixture at a flow rate of 1 ml/min was used as a mobile phase. The injection volume was 20µl.

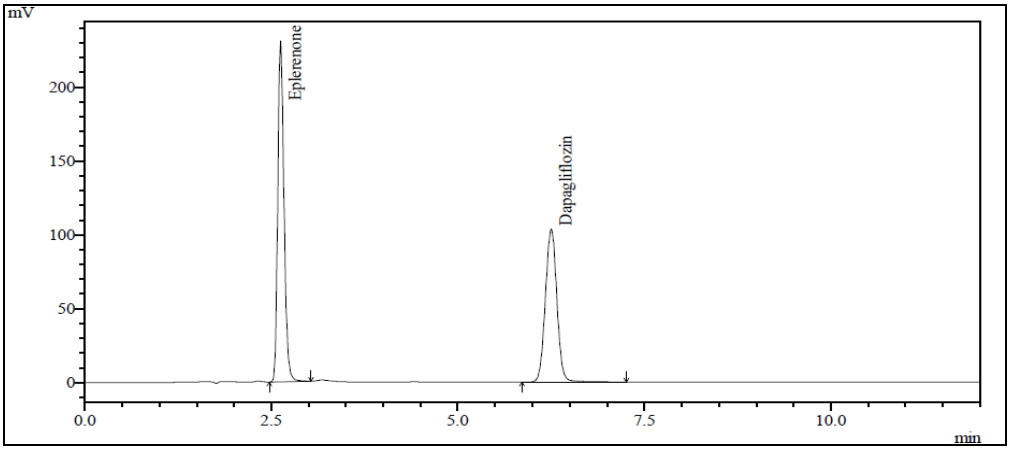

Figure 3 Optimized Chromatogram 20 µg/ml dapagliflozin + 50 µg/ml eplerenone, M.P- Phosphate buffer : Methanol (30:70 %v/v).

Table 1: Optimized chromatographic conditions.

Stationary Phase	**Cosmosil C18 (250 mm×4.6 mm, 5µm)**
Mobile Phase (v/v)	Phosphate Buffer: Methanol (30:70%v/v)
Diluent	Methanol
Mode of elution	Isocratic
Flow Rate (mL/min)	1.0 ml/min
Detection Wavelength (nm)	228 nm
Column Temperature	25 °C
Injection Volume (µL)	20 µL
Run Time (minutes)	12 min
Retention time (minutes)	DAPA- 6.2 min, EPLE- 2.6 min

System suitability parameters

The DAPA + EPLE solution at concentrations of 20 and 50 µg/mL was injected five times to evaluate system suitability test (SST) parameters, including retention time (Rt), number of theoretical plates, peak area, resolution and tailing factor (n = 5).

Table 2: System suitability parameters for DAPA (n = 5).

	DAPA		**EPLE**	
Parameters	**Mean ± SD**	**% RSD**	**Mean ± SD**	**% RSD**
Retention time	6.19 ± 0.04	0.72	2.66 ± 0.03	0.97
Tailing factor	1.03 ± 0.02	1.47	1.28 ± 0.01	0.78
Theoretical plates	7502.60 ±16.62	0.22	4045.80 ± 9.15	0.23
Area	111676.80 ± 15.07	0.01	134361.80 ± 19.24	0.01
Resolution	16.30 ± 0.24	1.46	-	-

Forced Degradation studies

1. Acid hydrolysis

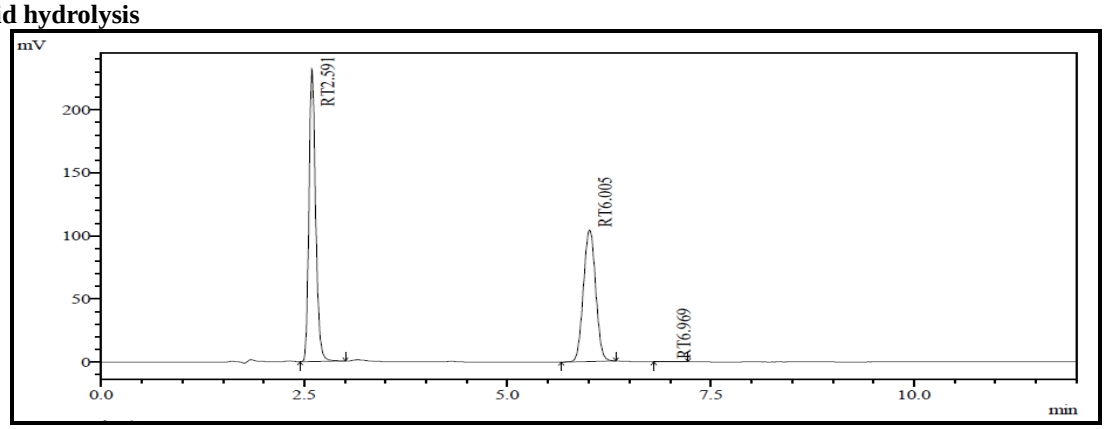

Fig. 4: Chromatogram of Acid Degradation of Standard API.

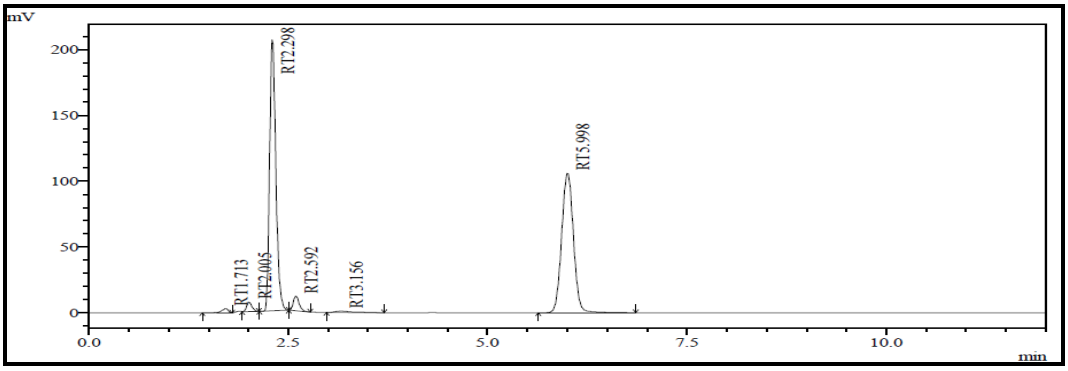

Fig. 5: Chromatogram of Acid Degradation of Sample.

2. Base hydrolysis

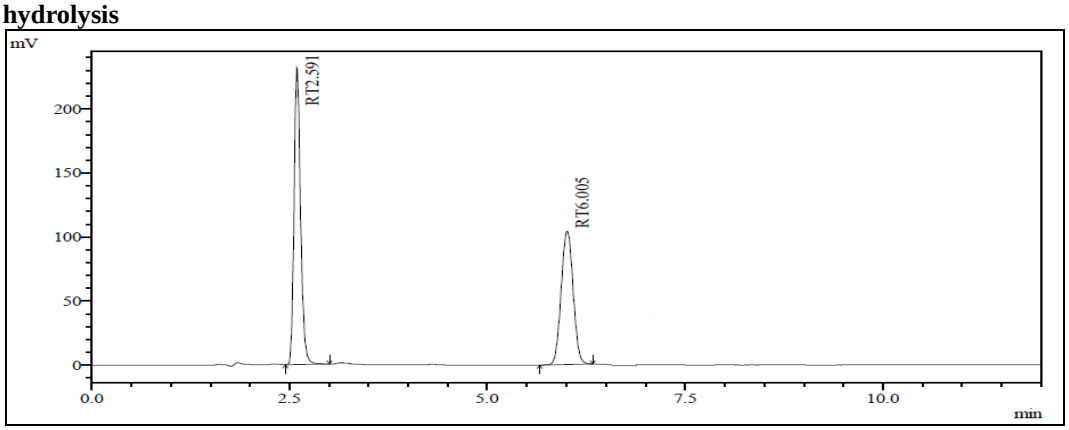

Fig. 6: Chromatogram of Base Degradation Standard API.

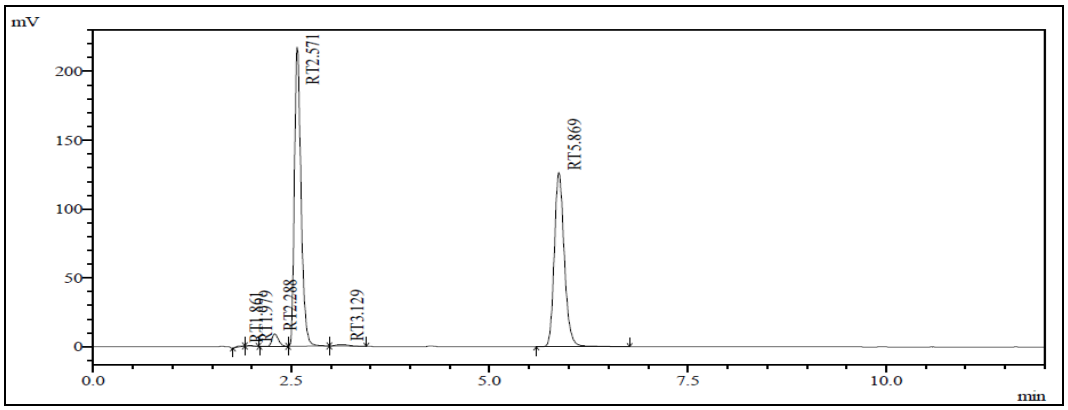

Fig. 7: Chromatogram of Base Degradation Sample.

3. Peroxide hydrolysis

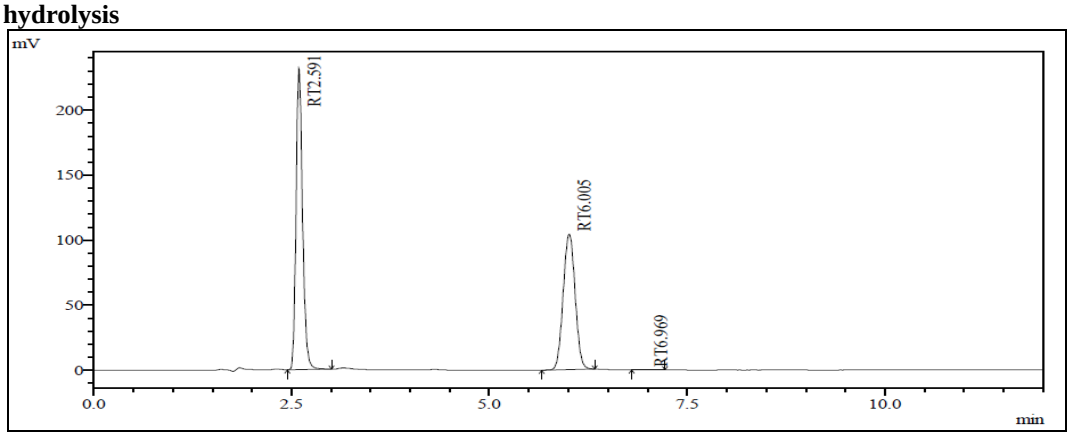

Fig. 8: Chromatogram of Oxidative Degradation of Standard API.

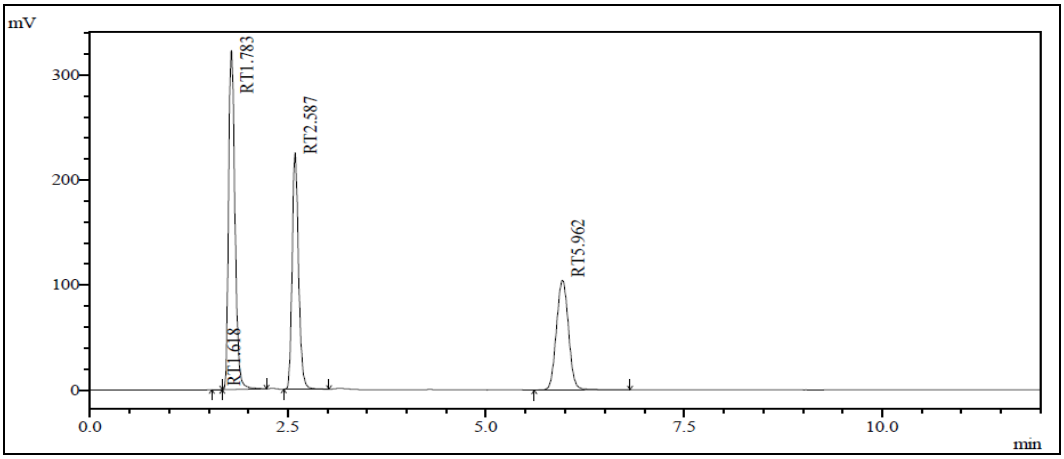

Fig. 9: Chromatogram of Oxidative Degradation of Sample.

4. Thermal Degradation

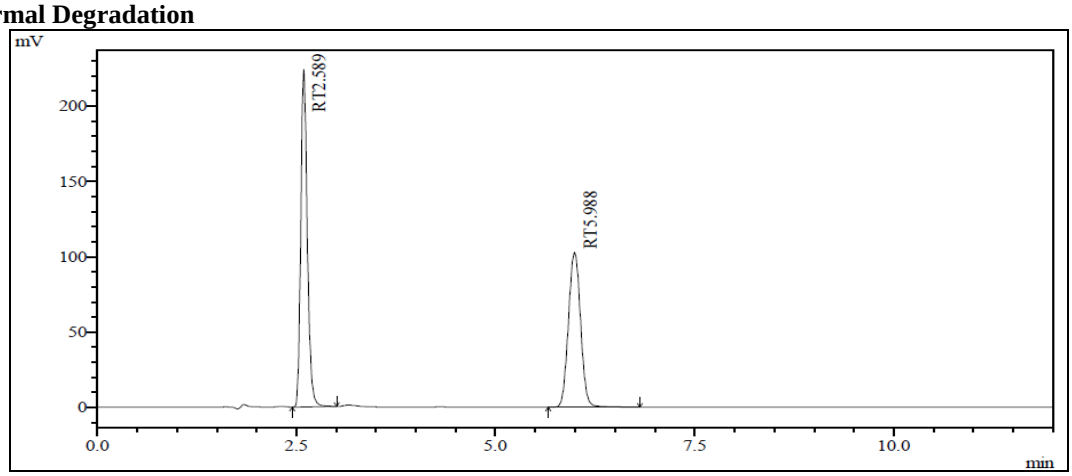

Fig. 10: Chromatogram of Thermal Degradation of Standard API.

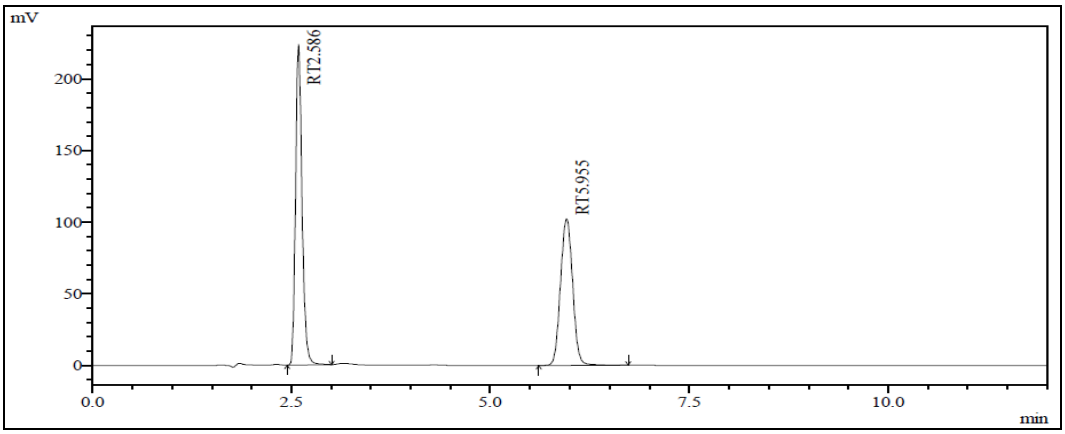

Figure 11: Chromatogram of Thermal Degradation of Sample.

Table 3: Summary of Force Degradation.

Stress Condition	Compound	Std. Area	Obs. Area	% Degradation
Acid	DAPA	111682	105151	5.848
	EPLE	134350	111923	16.693
Base	DAPA	111478	100512	9.837
	EPLE	134478	126429	5.985
Oxidative	DAPA	111528	101051	9.394
	EPLE	134587	127655	5.151
Thermal	DAPA	111748	108828	2.613

	EPLE	134357	128053	4.692

Validation of RP-HPLC method

Specificity

No extraneous or co-eluting peaks were detected at or near the retention times of the analytes, indicating the absence of interference from formulation excipients or other potential impurities.

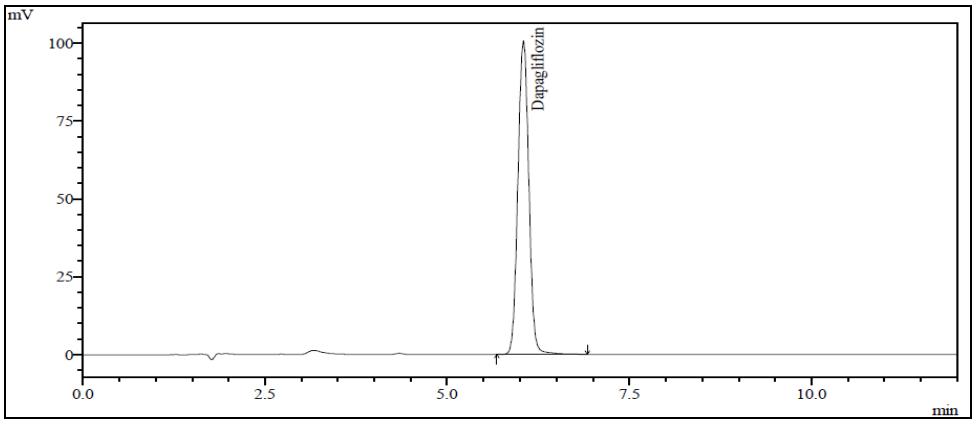

Fig. 12: Peak identification for Dapagliflozin.

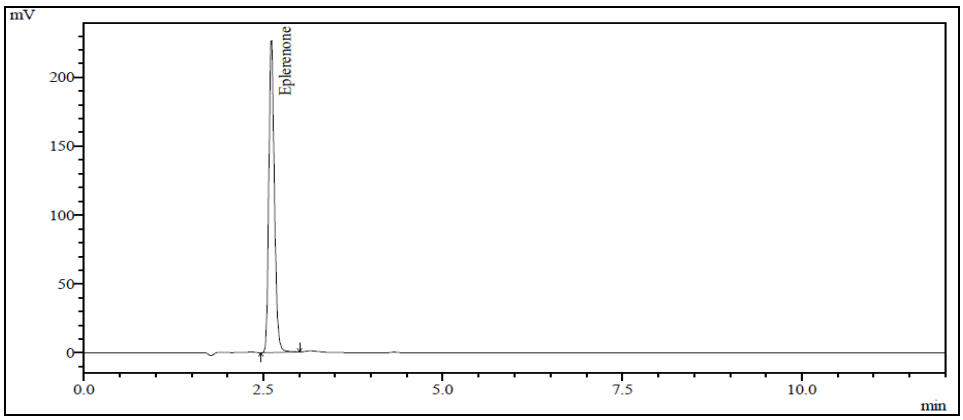

Fig. 13: Peak identification for Eplerenone.

Linearity

Linearity was assessed by preparing five standard solutions of DAPA and EPLE. The method demonstrated linearity over the concentration range of 10-30 µg/ml for DAPA with a correlation coefficient (R^2 = 0.9996) and 25-75 µg/ml for EPLE with a correlation coefficient (R^2 = 0.9998). The results of the linearity study for DAPA and EPLE are presented in Table 1.

Table 4: Linearity of DAPA.

Sr. No.	Concentration (µg/mL)	Peak Area (Mean ± SD)	% RSD
1.	10	55587.00 ± 53.11	0.10
2.	15	82281.33 ± 63.57	0.08
3.	20	111883.33 ± 113.78	0.10
4.	25	137372.67 ± 56.08	0.04
5.	30	166461.67 ± 134.40	0.08
Linear Regression equation			y=5535.5x – 5.7333
Linear Regression Coefficient			R^2 =0.9996

Table 5: Linearity data for EPLE.

Sr. No.	Concentration (µg/mL)	Peak Area (Mean ± SD)	% RSD
1.	25	68450.33 ± 96	0.14
2.	37.5	100577.00 ± 87.43	0.09
3.	50	137406.33 ± 278.99	0.20
4.	62.5	169586.00 ± 314.50	0.19
5.	75	199353.67 ± 223.51	0.11

Linear Regression equation	y=2646.5x +2748.4
Linear Regression Coefficient	R^2 =0.9988

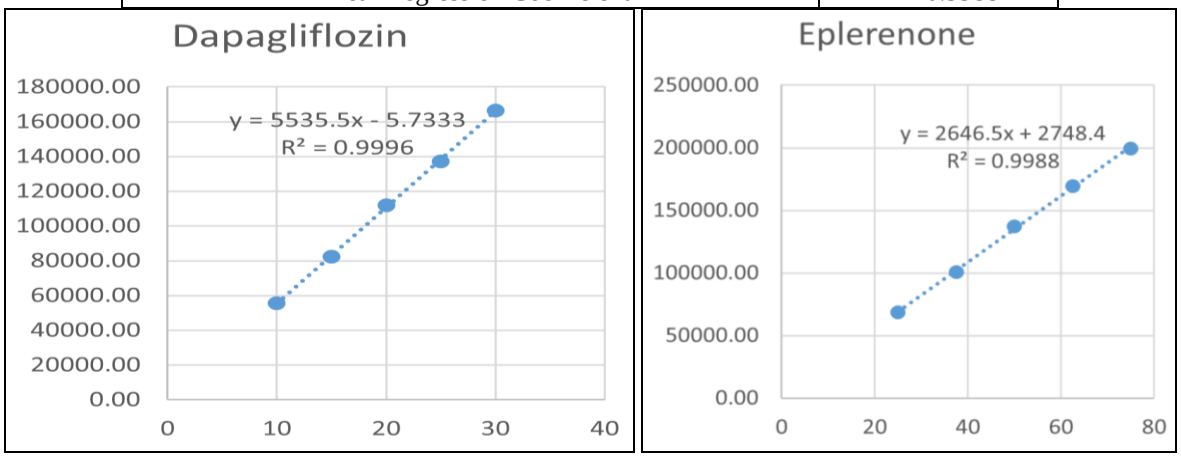

Fig. 14: Calibration Curve of DAPA and EPLE.

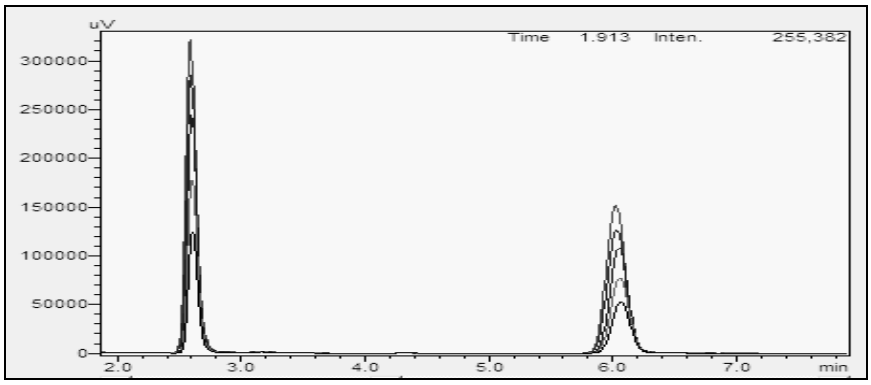

Fig. 15: Overlay Chromatogram of Linearity.

Precision

Table 6: Repeatability data of DAPA and EPLE.

Area	Concentration of DAPA (20 µg/mL)	Area	Concentration of EPLE (50 µg/mL)
1.	115928	**1.**	140467
2.	115268	**2.**	139650
3.	115654	**3.**	140102
4.	115123	**4.**	140987
5.	115789	**5.**	139784
6.	115874	**6.**	140620
Mean	115606.00	**Mean**	140268.33
SD	334.32	**SD**	514.63
% RSD	0.29	**% RSD**	0.37

Table 7: Data of Intraday precision of DAPA and EPLE.

Conc. DAPA (µg/mL)	Intraday (Mean Area ± SD)	% RSD	Conc. EPLE (µg/mL)	Intraday (Mean Area ± SD)	% RSD
10	55471.67 ± 86.41	0.16	25	68451.33 ± 94.50	0.14
20	115485.33 ± 546.87	0.47	50	140245.67 ± 521.50	0.37
30	166048.33 ± 819.48	0.49	75	199645.67 ± 300.91	0.15

Table 8: Data of Inter-day precision of DAPA and EPLE.

Conc. DAPA (µg/mL)	Inter-day (Mean Area ± SD)	% RSD	Conc. EPLE (µg/ mL)	Inter-day (Mean Area ± SD)	% RSD
10	55531.67 ± 370.24	0.67	25	68489.00 ± 414.34	0.60
20	115855.33 ± 851.05	0.73	50	140291.00 ± 623.37	0.44

30	166649.00 ± 697.36	0.42	75	199073.33 ± 767.51	0.39

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

Using slope and Y-intercept, the determined values of LOD and LOQ were evaluated. LOD and LOQ value for DAPA was found to be 0.034 µg/ml and 0.104 µg/ml respectively. LOD and LOQ value for EPLE was found to be 0.080 µg/ml and 0.243 µg/ml respectively.

Accuracy

Accuracy of the method was confirmed by recovery study at three levels (50%, 100% and 150%) of placebo addition.

Table 9: Accuracy data of DAPA and EPLE.

Accuracy Data for DAPA						
Level of Spiking	**Quantity of Placebo (mg)**	**Amount of drug Added (µg/mL)**	**Amount of drug Recovered (µg/mL)**	**Std area**	**Test area (Mean)**	**% Mean recovery ± SD**
Un-spiked	86	-	-	-	-	-
50%	86	10	9.999	55568	55565.00	99.995 ± 0.009
100%	86	20	19.999	111928	111924.67	99.997 ± 0.004
150%	86	30	30.000	166610	166609.33	100.000 ± 0.002
Accuracy data for EPLE						
Un-spiked	86	-	-	-	-	-
50%	86	25	24.999	68546	68489.00	99.996 ± 0.007
100%	86	50	49.995	137467	140291.00	99.990 ± 0.008
150%	86	75	74.998	199545	199073.33	99.997 ± 0.008

Robustness

Table 10: Robustness data for DAPA and EPLE.

DAPA				
PARAMETER	**LEVEL**	**AREA**	**MEAN**	**%RSD**
Mobile phase composition	28:72	116021	116437.67	0.35
		116841		
		116451		
	32:68	114512	114453.33	0.32
		114789		
		114059		
Flow Rate(ml/min)	0.8	115928	115418.67	0.39
		115241		
		115087		
	1.2	117841	117325.33	0.39
		116981		
		117154		
EPLE				
Mobile phase composition	28:72	158963	158553.00	0.26
		158149		
		158547		
	32:68	130214	130523.67	0.22
		130789		
		130568		
Flow Rate(ml/min)	0.8	140467	140393.00	0.17
		140587		
		140125		
	1.2	125412	125617.00	0.15
		125789		
		125650		

Assay of synthetic mixture

Table 11: Determination of DAPA and EPLE from synthetic mixture.

Drug	Amount taken (µg/mL)	Amount found (µg/mL) (Mean ± SD)	% Assay (Mean ± SD)
DAPA	20	20.07 ± 0.04	100.33 ± 0.03
EPLE	50	49.82 ± 0.54	99.65 ± 0.81

The obtained assay values for both analytes were within the acceptable limits, indicating that the method is accurate and suitable for quantitative estimation. The low standard deviation values further confirm the consistency and reliability of the method for routine analysis of Dapagliflozin and Eplerenone.

CONCLUSION

In the present investigation, a reliable and efficient RP-HPLC method was successfully developed for the simultaneous estimation of Dapagliflozin and Eplerenone in synthetic mixture. The method was systematically optimized to achieve satisfactory chromatographic separation with well-defined and resolved peaks for both analytes. In conclusion, the developed RP-HPLC method is simple, precise, accurate, robust, and stability-indicating. It can be confidently applied for routine quality control and stability studies of Dapagliflozin and Eplerenone in pharmaceutical formulations.

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