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SIMULTANEOUS QUANTIFICATION OF DAPAGLIFLOZIN AND LINAGLIPTIN IN PHARMACEUTICAL FORMULATION: A STABILITY-INDICATING RP-HPLC APPROACH

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ABSTRACT

Background: Type II diabetes is treated with linagliptin and dapagliflozin. Dapagliflozin works by specifically blocking the sodium-glucose co-transporter-2 (SGLT-2) protein, while linagliptin belongs to the dipeptidyl peptidase-4 (DPP-4) inhibitor class, a relatively new and developing class of medications.

Materials and Methods:

The aim of this study was to develop and validate a straightforward, accurate, and stable reversed-phase liquid chromatographic (RP-HPLC) method for determining the combination pharmaceutical dosage form of dapagliflozin and linagliptin, along with a stability-indicating assay. Forced degradation studies involving acid and base hydrolysis, oxidation, heat, and photodegradation were conducted on linagliptin and dapagliflozin. Separation and forced degradation were achieved using a reversed-phase Nova (C18, 250 mm x 4.6 mm, 5 µm) column and isocratic elution. The eluent consisted of methanol and phosphate buffer (65:35 % v/v), with the pH adjusted to 4.0 using ortho phosphoric acid, and a flow rate of 1 mL/min. Detection was performed at 226 nm with a photodiode array detector.

Findings:

The method effectively separated linagliptin, dapagliflozin, and their breakdown products. It demonstrated suitable accuracy, linearity, and precision for concentration ranges of 4–40 µg/mL for linagliptin and 8–80 µg/mL for dapagliflozin.

Conclusion:

The proposed method is innovative, straightforward, accurate, specific, sensitive, quick, and economically feasible, as it does not require any prior separation operations. It can be used for the simultaneous determination of dapagliflozin and linagliptin in tablet formulations.

Keywords: Dapagliflozin, Linagliptin, Chromatography, Force degradation, Diabetes

INTRODUCTION

Dapagliflozin and Linagliptin are prescribed for managing type 2 diabetes mellitus (T2DM) due to their complementary mechanisms and different metabolic pathways, which enhance therapeutic effects and reduce adverse interactions. This combination involves dapagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, and linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, both of which contribute to better glycemic control, weight reduction, and lower systolic blood pressure **[1-3]**.

Dapagliflozin works by selectively and irreversibly inhibiting SGLT2 in the kidneys, thereby reducing glucose reabsorption and increasing glucose excretion through urine, helping to regulate blood sugar levels. Its chemical structure is (1S)-1,5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl]-D-glucitol, (Figure 1) and it is classified under the Biopharmaceutical Classification System (BCS) as a class III compound, indicating high solubility but low permeability **[4]**.

Linagliptin, on the other hand, is an oral hypoglycemic agent within the DPP-4 inhibitor category **[5]**. Its chemical name is 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl) methyl]-4,5-dihydropurine-2,6-dione (Figure 2). Linagliptin can be used alone with lifestyle changes or in combination with other medications like metformin or thiazolidinedione to improve blood sugar control in T2DM patients **[6-10]**.

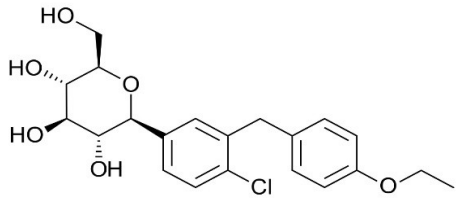

Figure 1: Structure of Dapagliflozin

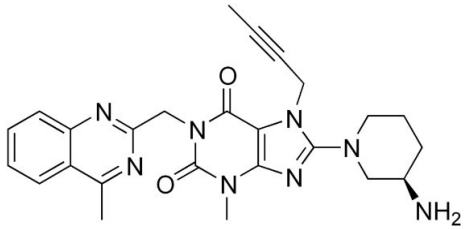

Figure 2: Structure of Linagliptin

Linagliptin functions by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4), which leads to higher levels of active incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This mechanism helps decrease glucagon release while enhancing insulin secretion **[11-12]**.

A comprehensive review of existing research reveals that numerous analytical

methods have been developed to analyze Dapagliflozin (DAPA) and Linagliptin (LINA), both individually and in combination with other medications. Nonetheless, there are relatively few reverse-phase high-performance liquid chromatography (RP-HPLC) methods for the simultaneous analysis of DAPA and LINA **[13-16]**. Most available RP-HPLC methods employ acetonitrile-based mobile phases. In contrast, this study utilizes a methanol-based mobile phase, which presents several benefits over acetonitrile for the simultaneous quantification of DAPA and LINA, as detailed in **Table 1 [17-19]**.

Table 1: Comparison of Methanol and Acetonitrile as Mobile Phases in RP-HPLC

Factor	Methanol	Acetonitrile
Polarity	Higher polarity, better for polar analytes like DAPA and LINA	Less polarity
Cost	Less expensive	More expensive
Safety	Less toxic, safer handling	More toxic
Volatility	Less volatile, consistent mobile phase composition	More volatile
Compatibility	Better for sharper peaks for some analytes	Often faster elution times
Environmental Factor	More environmentally friendly	Less environmentally friendly

MATERIALS AND METHODS

Instrumentation and Chemicals

Chromatogram analysis was performed using a Shimadzu SCL-10A VP model from Japan. The chromatographic setup included a photodiode array (PDA) detector and used Lab Solution software for acquiring and processing data. The system facilitated the efficient chromatographic separation of the compounds. Chromatographic separation was achieved on an ODS NOVA C-18 column [250 mm x 4.6 mm, 5 µm].

Reference standards of Dapagliflozin and Linagliptin were obtained from a reputable pharmaceutical company. Dapavel-L tablets were sourced from the local market. The method employed HPLC grade solvents and analytical grade chemicals. Additionally, all glassware was calibrated prior to use.

Preparation of standard stock solution

To prepare the standard stock solutions, 100 mg each of dapagliflozin and linagliptin were added to a 100 mL volumetric flask and dissolved in methanol. Methanol was then used to bring the solutions to the final volume, resulting in stock concentrations of 1000 µg/ mL for both DAPA and LINA. These stock solutions were further diluted to achieve concentrations of 100 µg/ mL each for DAPA and LINA. To create various concentrations ranging from 8 to 80 µg/mL for DAPA and 4 to 40 µg/mL for LINA, the standard solutions were further diluted accordingly.

Preparation of mobile phase

This was an isocratic elution method with methanol: buffer (potassium dihydrogen orthophosphate) (65:35 %v/v) pH adjusted

to 4.0 using o-phosphoric acid. The mobile phase was filtered through a 0.22 µm nylon membrane filter and degassed before use.

Chromatographic Separation and condition

In the column, standard and sample solutions were injected. Using a degassed mobile phase containing a combination of methanol and potassium dihydrogen orthophosphate (65:35 % v/v) and a PDA detector set at 226 nm, the chromatogram was run for an appropriate length of time. After total separation was attained, the chromatogram was ended. Lab Solution software was utilized to record various data relating to the peaks, including area, height, retention duration, and resolution. **Table 2** presents the optimized chromatography conditions used in the study.

Table 2: Optimized chromatographic conditions

Parameters	Condition
Method	RP-HPLC
Stationary Phase	ODS NOVA C-18 column [250 mm x 4.6 mm, 5 µm].
Mobile Phase	Methanol : Buffer (Potassium dihydrogen orthophosphate) (65:35 %v/v) and pH adjusted to 4.0 with o-phosphoric acid
Flow rate	1 mL min^{-1}
Total run time	15 Min
Injection volume	20 µL
Detection Wavelength	226 nm

Method validatıon

The assay, accuracy, precision, and limits of detection and quantification were all verified for the method. The International Council for Harmonization of Technical Requirements of Pharmaceuticals for Human Use's (ICH) suggested guidelines were followed during the method validation process **[20-22]**.

System suitability studies

System suitability was assessed using five replicates of the DAPA and LINA mixture. Column efficiency, resolution, and peak asymmetry were calculated for the standard solutions.

Linearity

The stated method's linearity was evaluated by measuring concentrations at five equidistant levels (n=6), ranging from 8 - 80 µg/mL for DAPA and 4 - 40 µg/mL for LINA. A graph of drug concentration vs peak area was generated, and the regression equation was determined.

Precision

Intraday precision was assessed by testing three concentrations levels—8, 40, and 80 µg/mL for DAPA, and 4, 20, and 40 µg/mL for LINA on the same day, each in triplicate. Interday precision involved evaluating these same concentration levels three times on three different days, also in triplicate. All solutions were prepared from separate stock solutions made on different days. The standard deviation and percentage relative standard deviation (RSD) were calculated to assess precision.

Accuracy (Recovery study)

By estimating the recovery of DAPA and LINA using the standard addition approach, accuracy was determined. This involved conducting % recovery study at 50%, 100%, and 150% by spiking the API into the sample.

Robustness

Modest modifications to each technique condition were used to assess the approach's robustness. This included variations in the HPLC pump flow rate, pH and mobile phase composition, to assess the method's reliability and performance under different conditions. The % RSD was calculated for all the parameters.

Assay

Accurately weighed 40 mg of powdered Dapavel-L tablet and added it to a 100 mL volumetric flask. 15 minutes were spent sonicating it after dissolving it in 25 mL of methanol. diluted with 100 mL of methanol and vigorously shaken. Using Whatman filter paper No. 42, filter the mixture. The filtered solution was pipetted into a 10 mL volumetric flask, and 20 µg /mL LINA and 40 µg m/mL DAPA were the final sample concentrations after the solution was diluted with methanol to the appropriate level.

Limit of Detection and Limit of Quantification

The drug's limit of detection (LOD) and limit level quantification (LOQ) were determined by utilizing the following ICH-designated formulas to compute the signal-to-noise ratio (i.e., 3.3 for LOD and 10 for LOQ):

The LOQ may be expressed as:

LOQ = 10*SD/Slope

Where, SD = the standard deviation of the response

Slope = the slope of the calibration curve

The LOD may be expressed as:

LOD = 3.3*SD/Slope

Where, SD = the standard deviation of the response

Slope = the slope of the calibration curve

Force degradation studies

Forced degradation studies were conducted to demonstrate whether the analytical method could unequivocally assess the analyte in the presence of impurities and degradation products. These studies were performed under stress conditions such as acidic, basic, oxidative, photolytic, and thermal degradation **[23]**.

Acid degradation

After pipetting 1 mL of the sample solution into a 10 mL volumetric flask, 5 N hydrochloric acid was added, and the mixture refluxed for 1 hour. Then, the solution was neutralized with the required volume of 5 N NaOH, and it was allowed to stand for a few minutes. The stability of the solution was assessed by diluting it with the

diluent to 10 mL, injecting it into the HPLC system, and recording the chromatogram.

Base degradation

1 mL of the sample solution was pipetted into a 10 mL volumetric flask, followed by 5 N NaOH. The mixture was refluxed for an hour. After an hour, the needed volume of 5 N hydrochloric acid was added to neutralize the solution, which was allowed to stand for a few minutes. To test the stability, the solution in the flask was diluted to 10 mL with diluent, injected into the HPLC system, and the chromatogram was recorded.

Oxidative degradation

1 mL of the sample solution was pipetted into a 10 mL volumetric flask, followed by the addition of 30 % H_2O_2 for 1 hour. To determine the stability, the solution in the flask was diluted to 10 mL with the diluent, then injected into the HPLC system, and the chromatogram was recorded.

Thermal degradation

A volumetric flask containing 1 ml of the drug solution was placed in an oven at 150°C for 24 hours. The solution was then diluted to 10 ml with the diluent before being injected into the HPLC system to determine stability.

Photolytic degradation

A volumetric flask containing 1 mL of the drug solution was exposed to UV light (254 nm) for 24 hours. The solution was then diluted to 10 mL with the diluent before being injected into the HPLC system to determine stability.

RESULTS AND DISCUSSION

Mobile phase selection

Distinct mobile phases **(Table 3)** were used, each with a different solvent percentage and pH level. Potassium dihydrogen orthophosphate: methanol (65:35%v/v) provided the optimal polarity for DAPA and LINA signal migration, separation, and resolution. The eluted peaks under the given circumstances were tail-free, sharply defined, and resolved. Chromatograms for the several mobile phases employed in the trials are displayed.

System Suitability data

Measurements were made for the column efficiency, resolution, and peak asymmetry of the standard solutions listed in **Table 4**.

Linearity

Calibration curve for Dapagliflozin and linagliptin were present in **Figure 3 and 4**. The HPLC overlain chromatogram depicted in **Figure 5** show 8 - 80 µg/ mL and 4 – 40 µg/ mL concentrations with retention time 8.757 min and 3.158 min for DAPA and LINA respectively. The method demonstrated linear with regression equation y = 22453x + 19014 for DAPA and y = 72643x + 13714 LINA. Table 5 and 6 represent the linearity and regression analysis of the drugs.

Precision

The results for intraday precision, and interday precision of the HPLC method are presented in **Tables 7**. The % RSD value for the precision study was found to be less than 2, indicating that the developed method is precise.

Accuracy (Recovery study)

This study included % recovery analysis at 50%, 100%, and 150% by spiking the API into the sample. The results of the recovery study are summarized in **Table 8**. The % recovery, ranging from 98 % to 102 %, justify the accuracy of the developed method. n: The value of % recovery shows that there is no interference from the excipients.

Robustness

The results for robustness is presented in **Table 9**. The % RSD value for all robustness parameters were found to be less than 2% indicated the developed HPLC method were robust.

Assay

The assay of DAPA and LINA was analyzed in the Dapavel-L tablet combined formulation. The % assay results, summarized in **Table 10**, fall within the acceptance criteria of 98-102%. This shows that excipients are not interfering, proving that the suggested method may be successfully used to analyze commercial formulations that contain DAPA and LINA.

LOD and LOQ

The result data for LOD and LOQ given in **Table 11**.

Force degradation study

These studies were conducted to evaluate the stability of the drug under defined stress conditions, including acid, base, peroxide hydrolysis, photo, and thermal degradation. The formulation was used in degradation experiments, and the degraded materials were injected. All assay results of the injected materials are within the acceptable degradation limits. The findings from the stress degradation studies are presented in **Table 12**, and the corresponding chromatograms are provided in **Figure** 5.

Table 3: Optimization of mobile phase

Sr. No.	Solvent System	Ratio (% v/v)	Remark
1	Water : Methanol	50:50	one peak observed. No separation
2	Buffer (Phosphate): Methanol	50:50	Peaks separation but shape is not proper
3	Buffer (Phosphate pH- 4 adjusted with OPA): Methanol	60:40	One peak observed. No separation
4	Buffer (Phosphate pH- 4 adjusted with OPA): Methanol	70:30	One peak observed. No separation
5	Buffer (Phosphate pH- 4 adjusted with OPA): Methanol	30:70	Separation observed and shape of peaks also good
6	Buffer (Phosphate pH- 4 adjusted with OPA) : Methanol	35:65	Separation observed and shape of peaks also good, less tailing

Table 4: Observed values for system suitability test *(n=6)

Parameters	Observed values		Specifications
	DAPA	LINA	
Retention Time (min)	8.757	3.158	-
Theoretical Plates	5410	2413	< 2000
Tailing Factor	1.11	1.39	< 2
Resolution	14.96		> 2

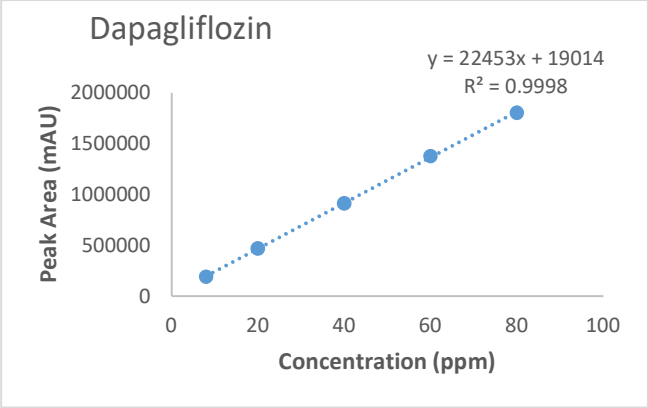

Figure 3: Calibration curve of Dapagliflozin

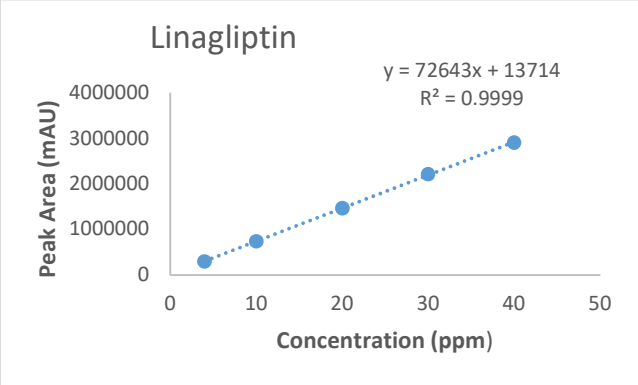

Figure 4: Calibration curve of Linagliptin

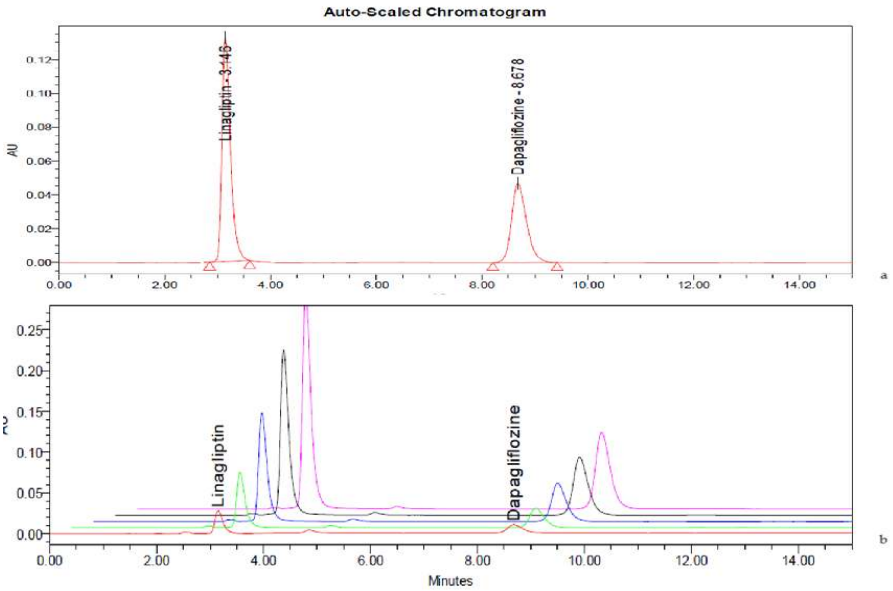

Figure 5: Chromatogram of Dapagliflozin and Linagliptin and Overlain chromatogram of Dapagliflozin and Linagliptin

Table 5: Linearity Data for DAPA and LINA. *(n=6)

Concentration (µg m/L)		Peak area ± SD*	
DAPA	LINA	DAPA	LINA
8	4	193724±1675	300110±6415
20	10	471192±4170	741083±1730
40	20	914362±3614	1464216±24927
60	30	1380170±12381	2209887±21841
80	40	1805931±7375	2908100±11802

Table 6: Regression analysis data for DAPA and LINA.

PARAMETERS	DAPAGLIFLOZIN	LINAGLIPTIN
Wavelength (nm)	226 nm	226 nm
Linearity (µg /mL)	8-80 (µg/mL)	4-40 (µg/mL)
Regression Equation (y= mx + c)	y = 22453x + 19014	y = 72643x + 13714
Regression Coefficient (R^2)	0.999	0.999
Slope (m)	22453	72643
Intercept (c)	19014	13714

Table 7: Precision data for estimation of DAPA and LINA

Drug	Conc. (µg/ mL)	Intraday (n=3)		Inter day (n=3)	
		Peak area ± SD	% RSD	Peak area ± SD	% RSD
DAPA	8	198696.7 ± 1722.72	0.96	194902 ± 1614.47	0.83
	40	913691± 4788.78	0.52	907760.33 ± 3047.63	0.34
	80	1830436 ± 5776.96	0.32	1838458 ± 7692.70	0.42
LINA	4	292299.67 ± 3448.63	1.18	303592.00 ± 1640.77	0.54
	20	1470002.33 ± 13167.86	0.90	1480866.33 ± 7151.41	0.48
	40	2910332.67 ± 9027.76	0.31	2905867.67 ± 15866.71	0.55

Table 8: Recovery study for DAPA and LINA

Concentration from Formulation	Amount of standard added (µg/mL)	Total Amount found Mean ± SD (n=3)	% Recovery ± SD (n=3)
40 µg/mL (DAPA)	20	60.36 ± 0.48	100.61 ± 0.81
	40	80.43 ± 0.57	100.54 ±0.72
	60	101.2 ± 0.74	101.19 ± 0.74
20 µg/mL (LINA)	10	30.2 ± 0.29	100.67 ± 0.95
	20	40.42± 0.10	101.04 ± 0.25
	30	50.30 ± 0.25	100.60 ± 0.50

Table 9: Robustness data for DAPA and LINA *(n=3)

Factor (For DAPA)	Level	*Peak area ± SD	% RSD	*Rt ± SD	% RSD
Mobile phase - Phosphate Buffer (pH-4 with OPA) : Methanol (35:65) Standard Rt: 8.678					
Change in flow rate ± 2	1.2	897428 ± 4500.36	0.53	7.90 ± 0.02	0.19
	0.8	938807 ± 9432.4	0.86	9.55 ± 0.09	0.89
Change in solvent Ratio ± 5	40:60	953403.3 ± 713.28	0.075	13.73 ± 0.14	1.02
	30:70	952324.7 ± 140.15	0.015	6.08 ± 0.04	0.72

Factor (For LINA)	Level	*Peak area ± SD	% RSD	*Rt ± SD	% RSD
Mobile phase - Phosphate Buffer (pH-4 with OPA) : Methanol (35:65) Standard Rt: 3.146					
Change in flow rate ± 2	1.2	1391561 ± 13911.02	1.0	2.86 ± 0.04	1.23
	0.8	1735204 ± 14973.29	0.86	3.50 ± 0.02	0.44
Change in Solvent Ratio ± 5	40:60	1431266 ± 16536.72	1.08	3.75 ± 0.05	1.35
	30:70	1484264 ± 4325.60	0.29	2.89 ± 0.03	1.04

Table 10: Assay data for DAPA and LINA Tablet *(n=3)

Sr. No	Amount taken		Amount found		% label claim	
	DAPA (µg/mL)	LINA (µg/mL)	DAPA (µg/mL)	LINA (µg/mL)	DAPA	LINA
1	40	20	40.23	20	100.575	100
2	40	20	39.49	20.29	98.725	101.45
3	40	20	39.51	20.13	98.775	100.65
4	40	20	39.73	20.14	99.325	100.7
5	40	20	39.63	20.31	99.075	101.55
6	40	20	40.21	19.97	100.525	99.85
Average			39.80	20.14	99.5	100.7
SD			0.34	0.14	0.84	0.71
% RSD			0.85	0.70	0.85	0.70

Table 11: LOD and LOQ data for DAPA and LINA *(n=3)

	DAPA	LINA
LOD	1.53 µg/mL	0.55 µg/mL
LOQ	4.63 µg/mL	1.66 µg/mL

Table 12: % degradation of LINA and DAPA

Sr. No	Stress conditions	% Degradation	
		DAPA	LINA
1	Acid Degradation (5N , 1 Hr)	6.73 %	8.28 %
2	Alkali Degradation (5N , 30 min.)	11.11 %	13.97 %
3	Peroxide Degradation (30%, 12 Hr)	14.87 %	16.42 %
4	Thermal Degradation (70 ºC, 24 Hr)	11.4 %	15.33 %
5	Photolytic Degradation (UV light, 7 days)	6.29 %	7.59 %

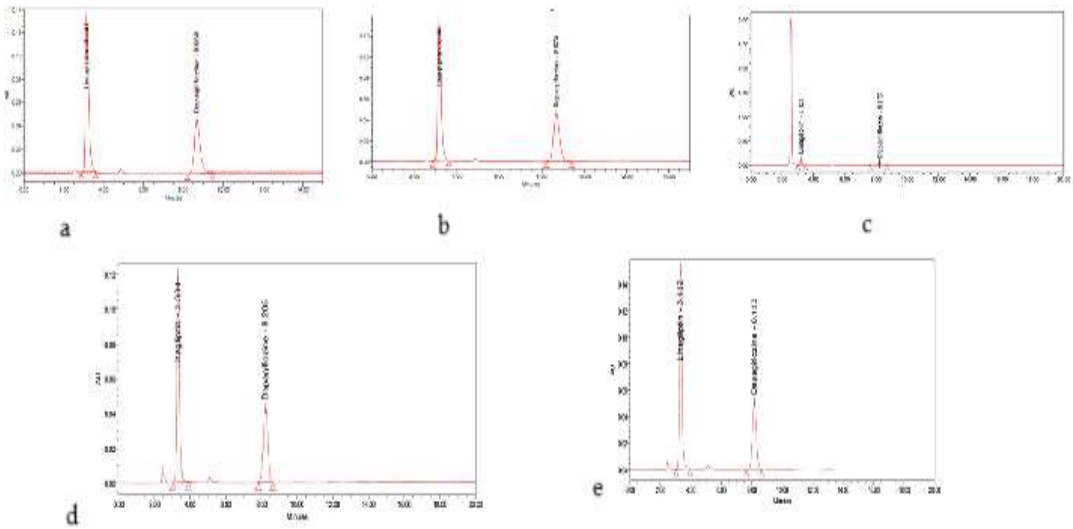

Figure 6: Chromatogram of degradation study in a. Acid degradation b. Alkali degradation c. Peroxide degradation d. Thermal degradation e. Photolytic degradation

CONCLUSIONS

The effective, reliable, and dependable RP-HPLC method has been established for the simultaneous measurement of Linagliptin (LINA) and Dapagliflozin (DAPA) in pharmaceutical formulations. This approach, which uses a mobile phase based on methanol, has a number of benefits, including improved safety, cost-effectiveness, and environmental friendliness. Excellent linearity, accuracy, and precision are provided, and the validated

parameters adhere to the requirements set forth by the International Conference on Harmonization (ICH). The capacity of the method to indicate stability in the presence of contaminants and degradation products was validated by stability tests, which also helped to evaluate the analytes. For regular quality control and dosage determination in pharmaceutical research and industry, this approach is appropriate.

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