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HPLC Method Development and Validation for Simultaneous Estimation of Dapagliflozin and Linagliptin in Bulk Drug and Pharmaceutical Formulation

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, often leading to severe complications such as cardiovascular disease, renal failure, and neuropathy. Linagliptin, a DPP-4 inhibitor, and dapagliflozin, an SGLT2 inhibitor, are frequently used together to treat type 2 diabetes mellitus in order to enhance glycemic control. The objective of this research is to develop and validate a novel new high-performance liquid chromatography (HPLC) technique for the simultaneous detection of linagliptin and dapagliflozin in pharmaceutical dosage forms and bulk.

The chromatographic separation was carried out using a Waters Reliant C18 column (150 × 4.6 mm) with a mobile phase consisting of acetonitrile and water (60:40 v/v) in isocratic mode. The flow rate was maintained at 1.0 mL/min, with UV detection at 244 nm. The retention times for Dapagliflozin and Linagliptin were found to be 1.607 and 3.637 minutes, respectively. The method was validated according to ICH guidelines for accuracy, precision, linearity, robustness, and ruggedness. Linearity was established in the concentration range of 60-210 ppm for Dapagliflozin and 1-10 ppm for Linagliptin, with correlation coefficients (r^2) above 0.998. Recovery studies confirmed accuracy within acceptable limits.

The developed method proved to be precise, accurate, and robust, making it suitable for routine quality control analysis of Dapagliflozin and Linagliptin in pharmaceutical formulations. This validated method ensures reliable quantification, facilitating effective therapeutic monitoring of these antidiabetic agents.

Keywords: HPLC, Dapagliflozin, Linagliptin, Diabetes Mellitus, Pharmaceutical Analysis, Method Validation

1. Introduction

Continuous hyperglycaemia is a hallmark of diabetes mellitus [DM], a chronic metabolic condition [1]. Hyperglycaemia is a common symptom of diabetes mellitus (DM), a chronic metabolic syndrome that is becoming more and more prevalent worldwide. Diabetic renal failure, limb amputation, blindness, and cardiovascular disease are among the serious adverse consequences of the condition [2]. According to reports, 415 million people between the ages of 20 and 79 have diabetes mellitus in 2015. The source of this data is the "International Diabetes Federation"[34]. Dapagliflozin [DAPA] (Fig. 1) is a pill that is part of a class of drugs known as sodium-glucose cotransporter 2 (SGLT2) inhibitors. These drugs support the kidneys' ability to eliminate glucose from the blood and excrete it as urine, which lowers blood glucose levels [5]. When it comes to chemistry, DAPA is known as (2S, 3R, 4R, 5S, 6R)-2-{4-chloro-3-[(4-ethoxyphenyl) [methyl]phenyl} -6-(hydroxymethyl) oxane-3,4,5triol. That is $C_{21}H_{25}CLO_6$ in molecular formula. 408.873 g/m is the molecular weight. While it supports weight loss, DAPA may increase the risk of UTI and vaginal thrush [6]. Approximately 100 times more selective for SGLT2 than SGLT, DAPA is a first-generation

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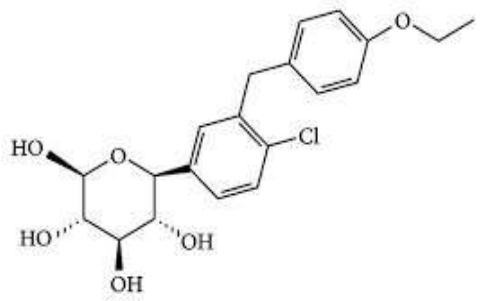
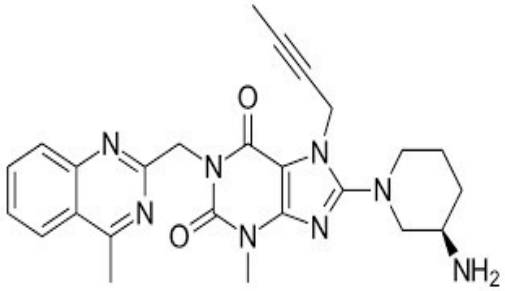
selective SGLT inhibitor that inhibits glucose transport [7].

Linagliptin (LINA) (Fig. 2), 8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4methylquinazolin-2-yl) methyl]-3,7-dihydro-1*H*-purine-2,6-dione] is a novel hypoglycaemic drug that belongs to dipeptidyl-peptidase-4 inhibitor class [89].Molecular weight 472.5 g/mol [10]. LNG was approved in 2011 by USA, Japan and Europe for the treatment of type 2 diabetes[11].Gliptins acts by inhibiting dipeptidyl peptidase-4 (DPP-4) and increase levels of active peptide hormones, such as glucagon like peptide-I (GLP-I) and glucose dependent insulinotropic peptide (GIP) and displays better glycaemic control in patients with type 2 diabetes [12-15]. GLP-1's proteolytic breakdown by DPP-4 results in a shorter plasma half-life; hence, to lengthen the plasma half-life of GLP-1, LINA inhibition of DPP-4 is required[16,17].Adults with type 2 diabetes mellitus should take this enzyme-inhibiting medication either on its own as an addition to diet and exercise or in conjunction with metformin or a thiazolidinedione to enhance glycaemic control [18-22].



Structure of Dapagliflozin



Structure of Linagliptin

Literature survey shows that numerous analytical methods are reported for the individual estimation of DAPA with other pharmaceutical preparations, by various methods such as UV spectrophotometry [23], HPLC[24-28], HPTLC [29-31], UPLC [32], LC MS [33-35]. Review of literature demonstrates that linagliptin can be calculated either individually or in combination with another active

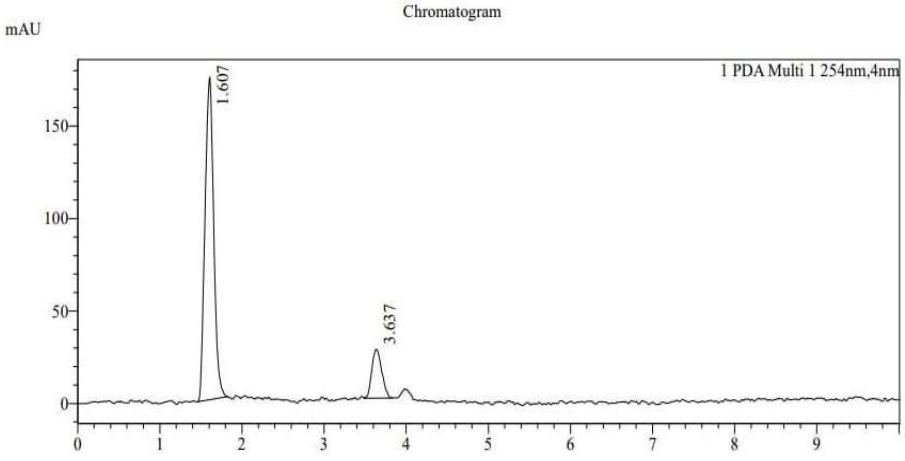


Fig. 1: Standard chromatogram of DAPA and LINA

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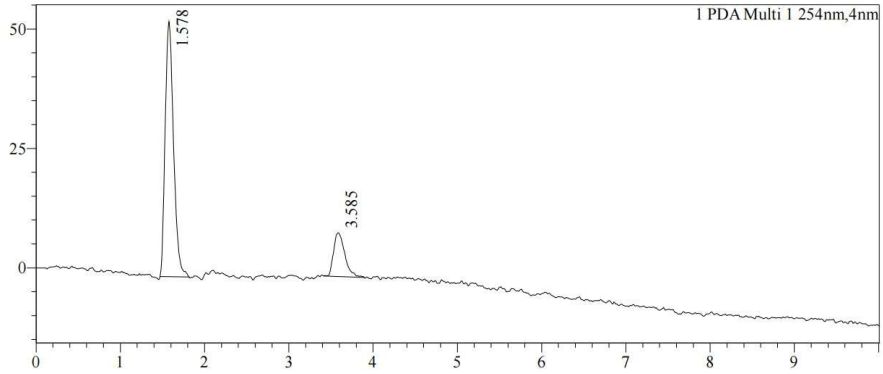

Fig. 2: Sample chromatogram of DAPA and LINA

pharmaceutical ingredient by many methods, which include by spectrophotometric [36-41], spectrofluorometric method [42,43], mass spectroscopic method [44-48], highperformance thin layer chromatography [49-51], electrochemical and amperometry methods [52] and the HPLC method [53-60]. However, there is some information available on the degradation behaviour of linagliptin and the isolation of impurities [61-62]. The developed method validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines [63].

Materials and Methods

Chemicals and Reagents

Acetonitrile purchased from QUALIGENS, HPLC Grade, Glacial acetic acid 100% From MERCK, Milli Q Water. Dapagliflozin and linagliptin was Purchased from ZENOMED HEALTH CARE. Fixed dose combination of tablet formulation OXAR (sun pharma) and ONDERO (lupin) containing 10mg/5mg of DAPA and LINA were procured form local market.

Instrumentation and Materials

Shimadzu HPLC was used for analysis. The separation was done on a UV detector and sampling was done by auto sampler. Data collection for chromatogram was done by LC solution. Acetonitrile and water (60:40) made up the mobile phase of the Waters reliant C_{18} µm column (150x4.6 mm) that was utilized. The mobile phase was filtered using a 0.45 µm membrane filter in an isocratic setting with a flow rate of 1.0 ml/min, an injection volume of 20 µL, and an elution monitor set for 10 minutes at 244 nm.

Preparation of Solution

Standard preparation

Standard stock solutions were set by dissolving 10 mg of DAPA and LINA in acetonitrile in 10ml volumetric flask to achieve concentration of 1000 µg/ml for DAPA and LINA. It was sonicated followed by filtration using 0.45 µm porosity filter paper. The stock solution was diluted by pipetted out 1 ml of above solution into 10 ml volumetric flask to produce reference standard solution containing DAPA and LINA (100 µg/ml), respectively.

Sample preparation

Ten tablets of Dapagliflozin and linagliptin combined dose 10mg/5mg were weighed separately and crushed. Weight equivalent to powder containing 10 mg of DAPA and 5 mg of LINA were dissolved in a 10 ml clean dry volumetric flask and acetonitrile was added. It was sonicated, followed by filtration using 0.45 µm porosity filter paper (stock solution). We further pipetted 1ml of Dapagliflozin and linagliptin from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent. The solutions were subject to analysis and results.

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Methods Development

The developed method was fully validated for the parameters as per ICH guidelines.

Accuracy

Accuracy of the method was determined by inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the amount found and amount added for Linagliptin and Dapagliflozin and calculate the individual recovery and mean recovery values.

Precision

To determine the precision, intra-day and inter-day analysis was performed. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. Solutions corresponding to each concentration level were injected in duplicate. The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample.

Linearity

Linearity is determined by a series of three to five injections of five or more standards. Plot a graph of peak area (heights) of the calibration standards are usually plotted in the Y-axis against the nominal standard concentration, and the linearity of the plotted curve is evaluated through the value of the co-relation coefficient (r^2). The methods were linear in the range of 60-210ppm for Dapagliflozin, 1-10ppm for linagliptin and inject each level into the chromatographic system and measure the peak area.

Robustness

Robustness of the method was performed in different conditions to find the variability of test results. The sample was analysed at 0.9 mL/min and 1.1 mL/min instead of 1mL/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded. Their effectson the retention time (R_t), tailing factor (T), theoretical plate numbers (N) and repeatability of peak areas (n = 6) were studied.

Ruggedness

Ruggedness of the proposed method was determined by analysing six assay sample solutions of linagliptin and dapagliflozin at nominal concentration by two analysts to check the reproducibility of the test results. The percentage recovery and percent relative standard deviation (% RSD) were calculated in both cases.

Limit of detection and Limit of quantification

The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. The determination of the limit of quantification is carried by minimum concentration at which the analyte can reliably be quantified is established.

A typical acceptable signal- to- noise ratio is 10:1. Other approaches depend on the determination of the slope of the calibration curve and the standard deviation of responses.

Results and Discussion

The goal of this study was to develop a HPLC method, several mobile phase compositions were tried for separation and quantification of Linagliptin and Dapagliflozin in bulk and pharmaceutical dosage forms. To develop an effective method for the analysis of the drugs preliminary tests were performed in orderto select adequate and optimum conditions (Table 1). Parameters such as detection wavelength, mobile phase composition and pH, mobile phase comprising of Acetonitrile: Water in 60: 40 v/v at a flow rate 1 mL/min to get a better reproducibility and repeatability.

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Quantification was achieved with UV detection at 240 nm and the retention time for Dapagliflozin and Linagliptin were found to be 1.607 and 3.637 mins respectively. A typical chromatogram of Linagliptin and Dapagliflozin is shown in (Figs. 1 and 2). The optimized method was validated as per ICH guidelines.

Table 1: Chromatographic conditions	
Column	Waters reliant C_{18} µm
Elution method	Isocratic
Mobile phase	Acetonitrile: water (60:40)
Flow rate	1 ml/min
Column temperature	40^{0}C
Volume of injection	20 µL
Detector	UV detector
Detection	wavelength 277
Run time	10 min

Accuracy

Three samples at three different concentration levels (50%, 100%, 150%) for two brands of Dapagliflozin and Linagliptinwere prepared by dissolving them in a acetonitrile and then diluting it in 10 mL mobile phase as in sample solution preparation. At every concentration level, it was injected in a triplicate and compared to the standard sample solution in the same way. The accuracy is presented as the percentage of the analyte recovery, and by measuring peak areas, the recovery was calculated (Tables 2 to 5).

Precision

Analysis was conducted to determine the accuracy within and between days. Every one of the six standard solution injections in HPLC had its area measured. The findings demonstrated that the six duplicate injections

Table 2: Accuracy studies
Data of accuracy and % recovery of Linagliptin brand 1 (OXARA- L5)

	RT	Peak Area	Mean	SD	RSD	%RSD	% Recovery
	1.621	183010		83.43	0.00455	0.455	99.85%
50%	1.612	183046	183128.3	57.98	0.00031	0.031	99.87%
	1.593	183329		142.12	0.00077	0.077	100.02%
	1.585	301183		1059.9	0.00353	0.353	99.04%
100%	1.591	301568	299684.3	1332.18	0.00444	0.444	99.17%
	1.589	296302		2391.43	0.00797	0.797	97.44%
	1.578	364920		2655.89	0.0072	0.72	96.58%
150%	1.583	371073	368676.3	1694.93	0.00459	0.459	98.21%
	1.578	370036		961.66	0.0026	0.26	100.88%

Table 3: Data of accuracy and % recovery of Dapagliflozin brand 1(OXARA L5)

	RT	Peak Area	Mean	SD	RSD	%RSD	%Recovery
	3.655	41063		65.05	0.00158	0.158	99.18%
50%	3.616	41390	41155.67	116.16	0.00403	0.403	99.97%
	3.606	41014		99.69	0.00242	0.242	99.06%
	3.618	56107		10.6	0.00018	0.018	98.66%
100%	3.607	56676	56092.33	412.95	0.00736	0.736	99.82%
	3.598	55494		422.82	0.00753	0.753	97.74%
	3.6	83033		106.77	0.00128	0.128	98.49%
150%	3.601	83289	83184.67	74.24	0.00089	0.089	98.80%
	3.586	83232		33.94	0.000408	0.04	99.09%

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Table 4: Data of accuracy and % recovery of Linagliptin brand 2(ONDERO-D5)							
	RT	Peak Area	Mean	SD	RSD	%RSD	%Recovery
	1.586	178742		1236.72	0.000685	0.685	97.52
50%	1.58	180254	180491.7	167.58	0.00092	0.092	98.35
	1.576	182479		1405.7	0.00778	0.778	99.56
	1.582	302185		1192.88	0.00396	0.396	99.37
100%	1.58	303230	300498.3	1931.81	0.00642	0.642	99.72
	1.583	296080		3123.99	0.01039	1.039	97.37
	1.605	374789		329.51	0.00088	0.088	99.19
150%	1.591	374616	374323.3	207.19	0.00055	0.055	99.15
	1.593	373565		535.98	0.00143	0.143	98.87

Table 5: Data of accuracy and % recovery of Dapagliflozin brand 2 (ONDERO-D5)							
	RT	Peak Area	Mean	SD	RSD	%RSD	%Recovery
	3.639	40209		255.97	0.0063	0.63	97.11
50%	3.645	40662	40571.33	63.35	0.00156	0.156	98.21
	3.638	40843		192.33	0.00474	0.474	98.64
	3.644	55517		63.92	0.000113	0.113	97.78
100%	3.626	55258	55428	120	0.00216	0.216	97.32
	3.627	55509		57.27	0.00103	0.103	97.76
	3.614	81771		312.54	0.00384	0.384	97
150%	3.635	80199	81329.67	799.03	0.00982	0.982	95.13
	3.643	82019		487.9	0.00598	0.598	97.29

Table 6: Precision data of standard Linagliptin						
Injections	RT	Peak Area	Mean	SD	RSD	%RSD
1	1.563	183294		499	0.0027	0.27
2	1.57	185889		403.83	0.00218	0.218
3	1.564	184366	184412	176.2	0.00095	0.095
4	1.564	184018		20.57	0.000111	0.001
5	1.57	183509		660.53	0.00358	0.358
6	1.58	185399		441.3	0.00239	0.239

% RSD fell within the specified range. For eachconcentration level, duplicate injections of the solutions were created. The results are discussed in (Tables 6 & 7).

System precision

Standard stock solution is injected for the 6 times and record the chromatogram. Calculate the relative standard deviation.

Acceptance criteria

After the successful 6 standard injection, peak area of the retention time is measured for %RSD which is NMT 1% Peak area of the standard linagliptin and dapagliflozin

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Table 7: Precision data of standard Dapagliflozin						
Injections	RT	Peak Area	Mean	SD	RSD	%RSD
1	3.557	59209		213.7	0.00358	0.358
2	3.567	60222		239.25	0.004	0.4
3	3.554	59071	59687.83	275.4	0.00461	0.461
4	3.44	60470		350.16	0.00586	0.586
5	3.555	60194		226.73	0.00379	0.379
6	3.58	58961		324.67	0.00543	0.543

Table 8: Inter-day precision of Linagliptin						
S. No	RT	Peak Area	Mean	SD	RSD	%RSD
1	1.563	183294		1110	0.006	0.6
2	1.58	185399	184864	378.3	0.00204	0.204
3	1.57	185889		731.85	0.0039	0.395

Table 9: Inter-day precision of Dapagliflozin						
S. No	RT	Peak Area	Mean	SD	RSD	%RSD
1	3.557	59209		173.24	0.00291	0.291
2	3.649	58961	59454.66	348.6	0.00586	0.586
3	3.555	60194		523.25	0.0088	0.88

Table 10: Intra-day precision of Linagliptin						
S. No	RT	Peak Area	Mean	SD	RSD	%RSD
1	1.566	181143		1385.22	0.00756	0.756
2	1.57	184449	183102.7	952.47	0.0052	0.52
3	1.57	183716		434.16	0.00237	0.237

Table 11: Inter-day precision of Dapagliflozin						
S. No	RT	Peak Area	Mean	SD	RSD	%RSD
1	3.581	58326		745.99	0.0125	1.25
2	3.578	59716	59441.33	194.45	0.00327	0.327
3	3.567	60222		552.24	0.00929	0.929

should have %RSD NMT 2%, after the 6 injections.

Method precision

Inter-day precision

Inter-day precision is performed at the same concentration of 3 replicates to determine whether the standard sample drug linagliptin and dapagliflozin has the same peak area at theirretention which isfurther calculated for the %RSD. The results are discussed in (Tables 8 & 9).

Intra-day precision

Intraday precision is carried out for the standard samples of dapagliflozin and linagliptin which is usually performed 3 replicates on three different days of each one replicate. % RSDis calculated with peak area of the retention time. The results presented in (Tables 10 & 11).

Method precision Inter-day precision (Tables 8 and 9)

Intra-day precision (Tables 10 and 11)

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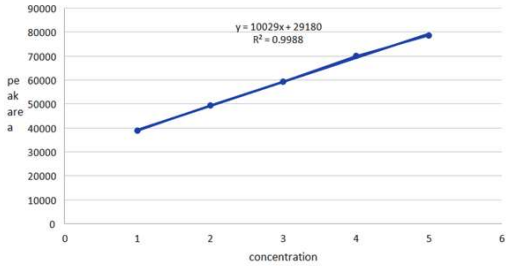

Fig. 3: Linearity curve of Dapagliflozin

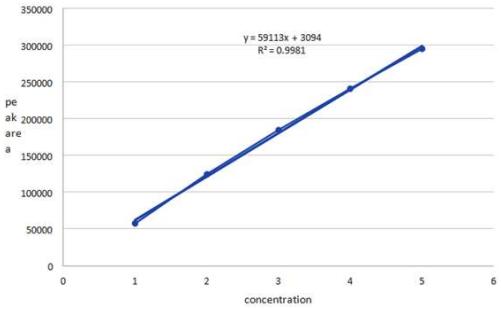

Fig. 4: Linearity curve of Linagliptin

Linearity

From the lower concentration to higher concentration minimum of five concentration is mandatory to obtain linearity graph. The results (Tables 12 & 13) and the linearity graph is presented in (Figs. 3 & 4).

Robustness

The robustness test on the developed HPLC method was investigated by applying small changes in mobile phase +2(or)-2, temperature +2(or)-2 and flow rate at 0.99 and 1.1ml/min and substituting in the obtained polynomial equations. No significant change was observed, indicating the robustness of the obtained method (Tables 14-19).

Ruggedness

Ruggedness is the method which is performed to determine nominal concentration of the standard drugs, which is compared with the test results.

Table 12: Linearity values of Linagliptin

Concentration	Peak
0	0
0.4 µg/ml	57422
0.8 µg/ml	124534
1.2 µg/ml	184580
1.6 µg/ml	240742
2.0 µg/ml	294881

Table 13: Linearity values of Dapagliflozin

Concentration	Peak
0	0
1.6 µg/ml	38814
2.0 µg/ml	49446
2.4 µg/ml	59363
2.8 µg/ml	70055
3.2 µg/ml	78653

Table 14: Change in temperature of Linagliptin

Temperature	RT	Peak Area	Peak Shape
+2	1.562	182553	Symetrical Shape
-2	1.567	192565	Symetrical Shape

Table 15: Change in temperature of Dapagliflozin

Temperature	RT	Peak Area	Peak Shape
+2	3.553	74087	Symetrical Shape
-2	3.567	75097	Symetrical Shape

- Change of mobile phase which is usually performed by the two analysts to determine the area of the standard sample. Data are presented in the (Tables 20 & 21).
- Change in the instrument is performed by the two analysts, the obtained area value is compared with the standard sample peak area. Data are presented in the (Tables 22 & 23).

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Table 16: Change in mobile phase of Linagliptin

Mobile Phase	RT	Peak Area	Peak Shape	RT Difference
+2	1.561	187842	Symetrical Shape	RT remains same
-2	1.576	199557	Symetrical Shape	RT remains same

Table 17: Change in mobile phase of Dapagliflozin

Mobile Phase	RT	Peak Area	Peak Shape	RT Difference
+2	3.559	74087	Symetrical Shape	RT remains same
-2	3.567	75097	Symetrical Shape	RT remains same

Table 18: Change in flow rate of Linagliptin

Flow Rate	RT	Peak Area	Peak Shape
1.1	1.445	178608	Symetrical Shape
0.99	1.592	231073	Symetrical Shape

Table 19: Change in flow rate of Dapagliflozin

Flow Rate	RT	Peak Area	Peak Shape
1.1	3.322	73170	Symetrical Shape
0.99	3.651	82061	Symetrical Shape

Conclusion

Developed a novel green HPLC simultaneous method for quantification of dapagliflozin and linagliptin in bulk drug and pharmaceutical. The proposed method could achieve the quantification with in10 min.

Table 20: Change inmobile phase of Linagliptin

	RT	Peak Area	Peak Shape
Analyst 1	1.566	181143	Symetrical Shape
Analyst 2	1.563	188748	Symetrical Shape

Table 21: Change in mobile phase of Dapagliflozin

	RT	Peak Area	Peak Shape
Analyst 1	3.581	58386	Symetrical Shape
Analyst 2	3.557	60244	Symetrical Shape

Table 22: Change in instrument for Linagliptin

	RT	Peak Area	Peak Shape
Person 1	1.570	184054	Symetrical Shape
Person 2	1.564	184366	Symetrical Shape

Table 23: Change in instrument for Dapagliflozin

	RT	Peak Area	Peak Shape
Person 1	3.567	63476	Symetrical Shape
Person 2	3.544	61772	Symetrical Shape

By using this method samples were separated with sufficient with accuracy, precision and linearity. Hence the proposed green method could be successfully employed for the simultaneous quantification of dapagliflozin and linagliptin in various pharmaceutical formulations.

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