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Research Article

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Development of a RP-HPLC Method for Simultaneous Estimation of Empagliflozin and Linagliptin

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Abstract This work outlines the creation of a novel Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative detection of Empagliflozin and Linagliptin in bulk and tablet forms. The approach is quick, accurate, sensitive, and sensitive. The approach may be utilised for regular monitoring of Empagliflozin and Linagliptin in industry in the assay of bulk medication and dosage form due to the low percent relative standard deviation (% RSD) results in the precision research.

Keywords Empagliflozin, Linagliptin, RP-HPLC, Dosage form, Method Development

Introduction

Empagliflozin {(2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-Chloro-3-[[4-[(3*S*)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymeth-yl) oxane-3,4,5-triol} is a is an antidiabetic medication (sodium glucose cotransporter-2 (SGLT-2) inhibitor) and used to improve glucose control in people with type 2 diabetes [1-2].

Linagliptin {8-[(3*R*)-3-Aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione} is used to treat type 2 diabetes (but not type 1) in conjunction with exercise and diet. Linagliptin is a dipeptidyl peptidase-4 inhibitor that works by increasing the production of insulin and decreasing the production of glucagon by the pancreas [3-4].

Literature review revealed that few methods were described for the determination of Empagliflozin and Linagliptin alone or in combination with other drugs from pharmaceutical dosage forms and in human plasma including spectrophotometry, ultra-performance liquid chromatography (LC), LC–mass spectroscopy, and high-performance LC (HPLC) techniques [5-25].

The aim of present study to develop RP-HPLC method for simultaneous estimation of Empagliflozin and Linagliptin. Various literature assessments showed that there are numerous analytical method has been reported for the estimation of Empagliflozin and Linagliptin. There is a continued need for developing more efficient, sensitive, accurate and precise methods for the analysis of Empagliflozin and Linagliptin alone and in combination in dosage forms. So, the present work aims to develop assay method that is accurate, simple, rapid, economic, precise and reliable for the estimation of Empagliflozin and Linagliptin alone and in combination in dosage form.

Experimental Work

The reference standards of empagliflozin and linagliptin were procured from Clearsynth, Hyderabad, India. Boehringer Ingelheim International branded tablet formulation Glyxambi® (empagliflozin 10 mg and linagliptin 5 mg) was purchased from local market.

Acetonitrile (HPLC-grade) was obtained from Merck Fine Chemicals, Mumbai, India. Analytical grade Sodium Hydroxide, Hydrochloric Acid and Hydrogen Peroxide were from SD Fine Chemicals, Hyderabad, India. Water was purified through Milli-Q® Integral water purification system. 0.45 µm nylon syringe filters were obtained from Chromatography World, Mumbai, India.

Instrumentation: Instruments used during the development and validation is given in the following table. Detailed instrumentation of the HPLC and PDA detector is given in the introduction.

Preparation of Solutions

Buffer (pH 2.16): 1.0 mL of ortho phosphoric acid, 85% solution was transferred into a 1000 mL of volumetric flask. The volume was made up with water and mixed well.

Mobile Phase: The above buffer solution (pH 2.16) was mixed with HPLC grade acetonitrile in a ratio of 45:55 V/V and degassed. This mixture was used as the mobile phase.

Diluent: Acetonitrile and water were mixed in 45:55 V/V ratio and mixed well.

Standard drug solution (Stock and working): 10 mg of empagliflozin and 5 mg of linagliptin reference standards were weighed into a 10 mL volumetric flask. Aboutthree fourths volume of the diluent was added it and sonicated for 10 min. Thevolume was made up with the diluent and mixed well. This solution was used as thestock standard solution (1 mg/mL of empagliflozin and 0.5 mg/mL of linagliptin).

2.0 mL of the above stock solution was transferred into a 20 mL volumetric flask and made up the volume with the diluent and mixed well. This solution wasused as a working standard solution (100 µg/mL of empagliflozin and 50µ mg/mL of linagliptin).

Formulation Sample Solution: 20 tablets were weighed, and the average weight of the tablet was calculated. Tablets were pulverized using a motor.

A quantity of powder equivalent to one tablet was weighed and transferred into a 10 mL volumetric flask. Around 7 mL of the diluent was added to the volumetric flask and sonicated for around 10 min. The volume was made up with the diluent and mixed well. This solution was used as the stock sample solution (1.0 mg/mL of empagliflozin and 0.5 mg/mL of linagliptin).

An aliquot of the sample stock solution was filtered through a 0.45 µm nylon syringe filter. 2.0 mL of the filtrate was transferred into a 20 mL volumetric flask.The volume was made up with the diluent and mixed well. The final theoretical concentrations of empagliflozin and linagliptin were 100 µg/mL and 50 µg/ml of respectively.

Preparation of sample solutions for forced degradation studies: The final degradation solution concentrations were 100 µg/mL and 50 µg/mL of empagliflozin and linagliptin respectively. (Refer to page number 29 in the introduction (chapter-1) for preparation of the samples for forced degradation studies).

Optimized chromatographic conditions

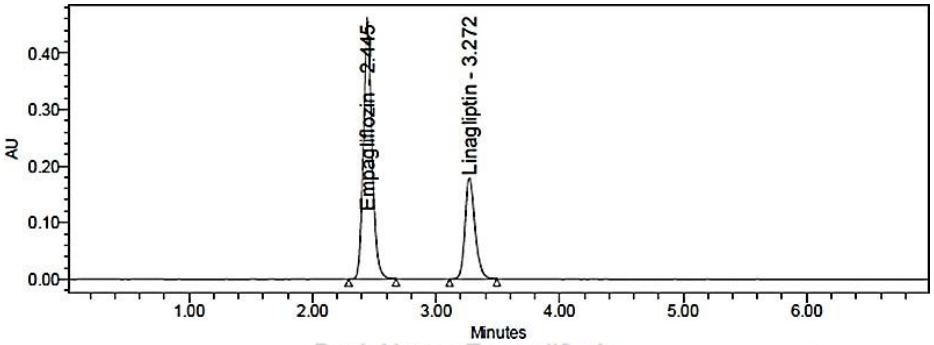
Table 1: Optimized Chromatographic conditions

Column	Thermo Scientific™ Hypersil™ ODS C18 (150 mm x 4.6 mm; 5µ)
Mobile phase	0.1% Ortho phosphoric acid in water - Acetonitrile in45:55 V/V
Volume of injection	10 µL
Flow rate	1.0 mL/min
Sample temperature	25°C
Column temperature	30°C
Wavelength	257 nm
Run time	6 min
Retention time	Empagliflozin: 2.44 min Linagliptin: 3.27 min

Validation of the Developed Method

The objective of validation of an analytical method is to verify the characteristics of the proposed method suitability for its intended purpose. After developing a suitable method, it was validated for accuracy (recovery), linearity, specificity, ruggedness, precision, robustness, limit of quantification and limit of quantification.

System suitability: The standard solution was prepared as per the proposed method and injected into the HPLC system. The results of the system suitability assessment for initial evaluation study parameters were given in figure 1. Percent relative standard deviation (% RSD) of 0.7 % indicates good system precision. System suitability was established before running the sample for all the validation parameters.



Peak Name: Empagliflozin

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Empagliflozin	2.437	218929	6127	1.15
2	Empagliflozin	2.437	220361	6034	1.14
3	Empagliflozin	2.437	219437	5759	1.14
4	Empagliflozin	2.437	222380	5611	1.18
5	Empagliflozin	2.439	219792	5653	1.14
6	Empagliflozin	2.445	217997	6099	1.14
Mean			219816		
Std. Dev.			1490.7		
% RSD			0.7		

Peak Name: Linagliptin

	Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	3.261	100068	8133	1.23	5.8
2	Linagliptin	3.262	101418	7983	1.23	5.9
3	Linagliptin	3.263	100648	8028	1.22	5.9
4	Linagliptin	3.264	100913	8191	1.24	5.9
5	Linagliptin	3.265	100104	8006	1.23	5.8
6	Linagliptin	3.272	100054	8188	1.22	5.9
Mean			100534			
Std. Dev.			560.45			
% RSD			0.6			

Figure 1: Representative chromatogram and relevant system suitability data

Method precision: The precision of the proposed method was evaluated by performing six independent assays of the test sample preparation and calculating the % RSD. The intermediate precision of the proposed method was checked by performing the same procedure on a different day under the same experimentalconditions. The % RSD

was found to be below 2.0 % which indicates the proposed method was precise. Data obtain from precision experiments are given in Table 2.

Table 2: Precision results of empagliflozin and linagliptin

Sample number	% Assay			
	Empagliflozin		Linagliptin	
	Day 1	Day 2	Day 1	Day 2
1	100.058	100.456	99.218	100.359
2	99.674	100.100	98.175	100.103
3	99.045	100.080	98.664	99.889
4	100.141	100.202	99.348	100.142
5	99.666	100.131	99.468	100.805
6	99.007	100.074	98.880	99.860
Average	99.598	100.174	98.959	100.193
SD	0.484	0.146	0.487	0.351
%RSD	0.49	0.15	0.49	0.35

Specificity: Interference with the diluent was evaluated by injecting the diluent into HPLC as per the proposed method. No peaks were found except for the solvent front peaks.

Specificity was also evaluated by forced degradation studies. Glyxambi® tablets were stressed and solutions were prepared with respective stressed samples and each stressed sample was injected into HPLC as per the proposed method. Peak purity was established by using Empower 2 Software form 200 – 400 nm.

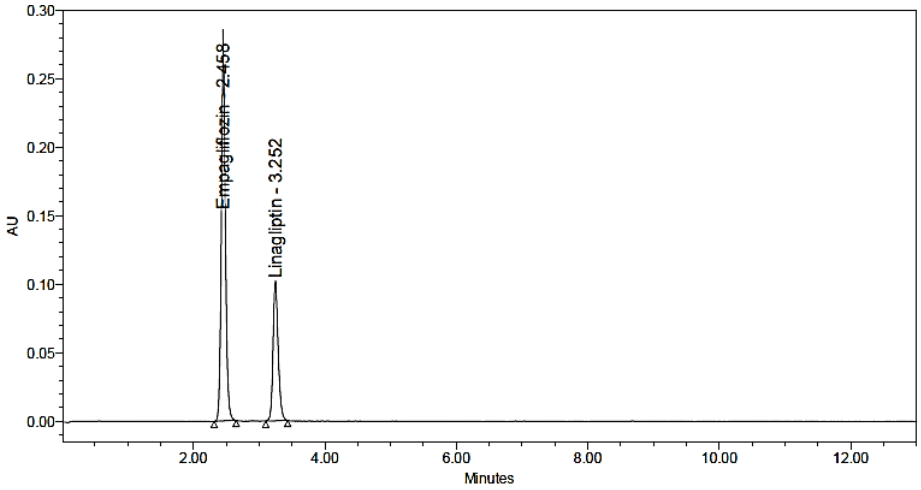
Data obtained from each degradation condition along with the relevant degradation percent and the peak purities are given in the Tables 3 and 4. The results of the study show that the stressed conditions did not affect empagliflozin andlinagliptin.

Table 3: Forced degradation results of empagliflozin

Nature of degradation	Empagliflozin		Percent Assay
	Purity Angle	Purity Threshold	
Acid	0.185	0.285	99.3
Base/Alkali	0.135	0.354	98.9
Peroxide	0.179	0.296	98.6
Dry heat	0.129	0.365	99.4
Photolytic	0.147	0.289	99.3
Neutral (control)	0.159	0.287	99.6

Table 4: Forced degradation results of linagliptin

Nature of degradation	Linagliptin		Percent Assay
	Purity Angle	Purity Threshold	
Acid	0.233	0.432	100.7
Base/Alkali	0.365	0.756	100.6
Peroxide	0.235	0.436	99.5
Dry heat	0.380	0.768	100.5
Photolytic	0.134	0.342	100.8
Neutral (control)	0.139	0.335	100.8



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Empagliflozin	2.458	218155	68.51	0.185	0.285	8078	1.1
2	Linagliptin	3.252	99879	31.49	0.233	0.432	8310	1.2

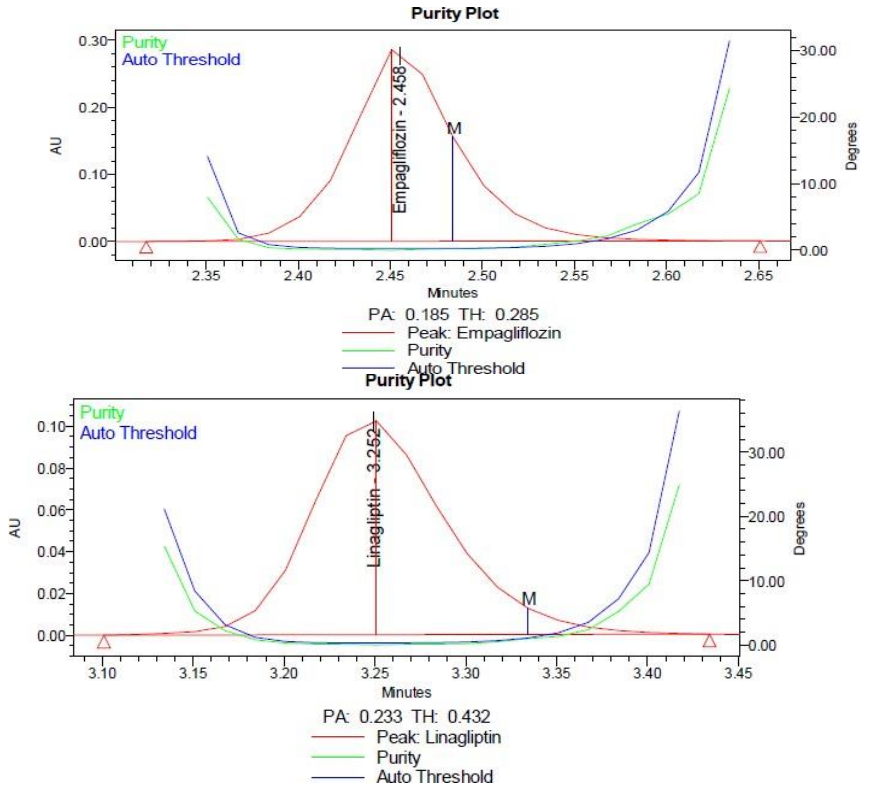
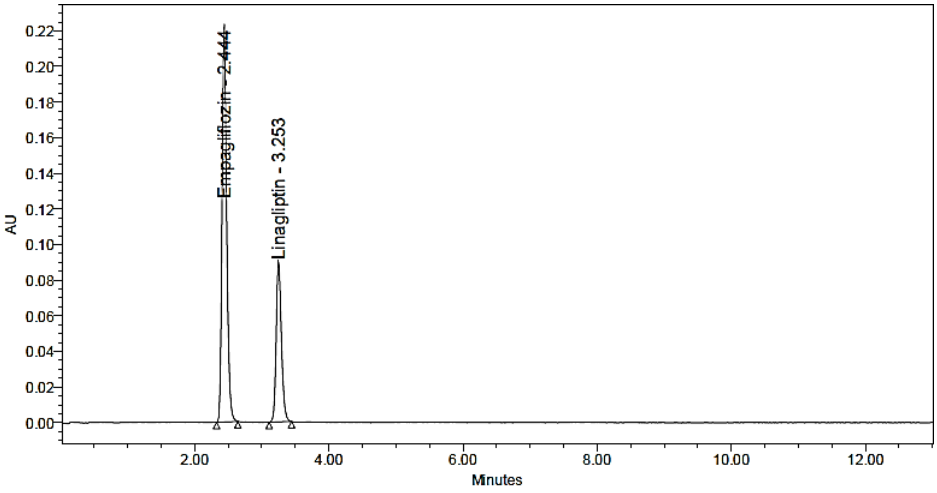


Figure 2: Acid degradation chromatogram and purity plot of empagliflozin andlinagliptin



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Empagliflozin	2.444	217233	68.56	0.135	0.354	5938	1.2
2	Linagliptin	3.253	99783	31.44	0.365	0.756	8866	1.2

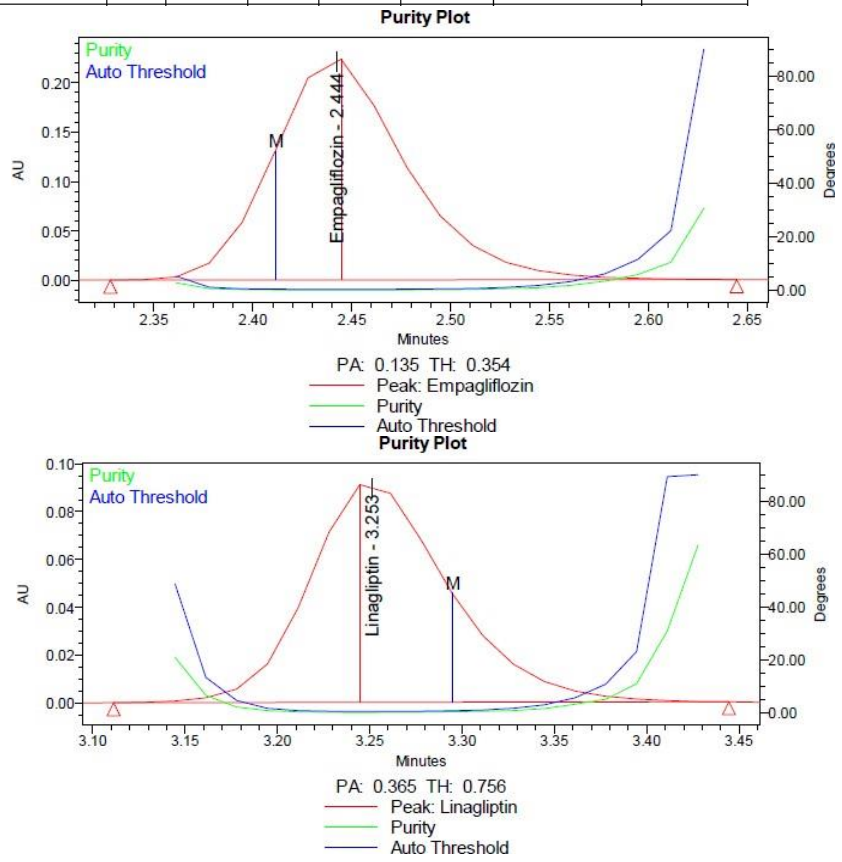
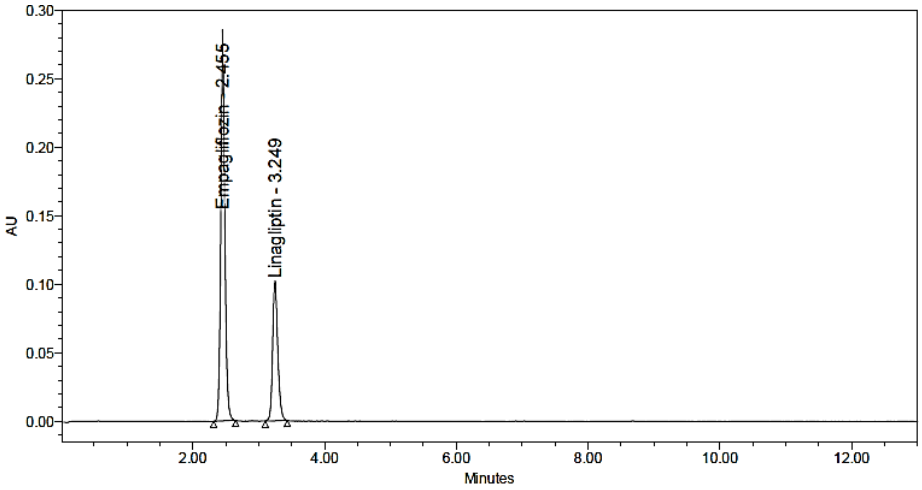


Figure 3: Alkali degradation chromatogram and purity plot of empagliflozin and linagliptin



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Empagliflozin	2.455	216597	68.51	0.179	0.296	8051	1.1
2	Linagliptin	3.249	98713	31.49	0.235	0.436	8358	1.2

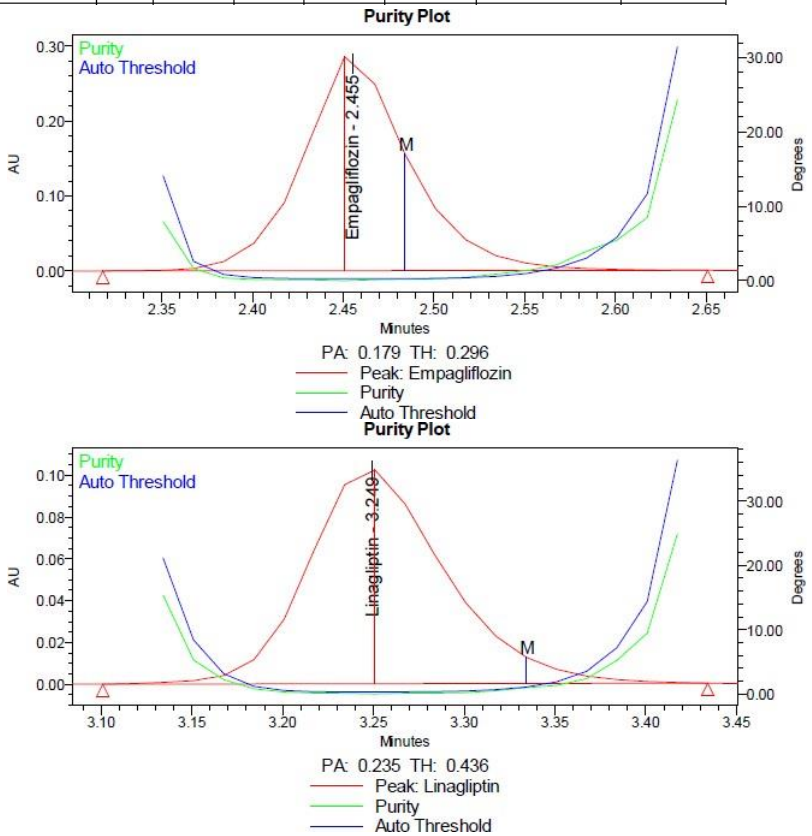
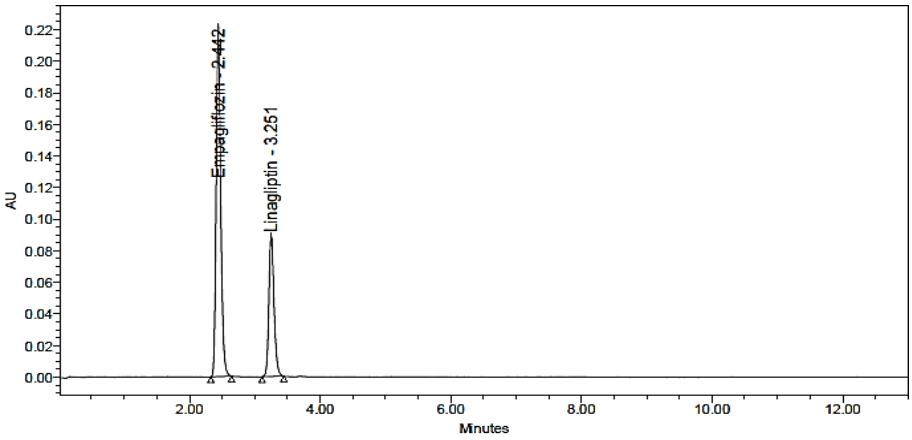


Figure 4: Peroxide degradation chromatogram and purity plots of empagliflozin and linagliptin



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Empagliflozin	2.442	218332	68.56	0.129	0.365	5982	1.2
2	Linagliptin	3.251	99769	31.44	0.380	0.768	8845	1.2

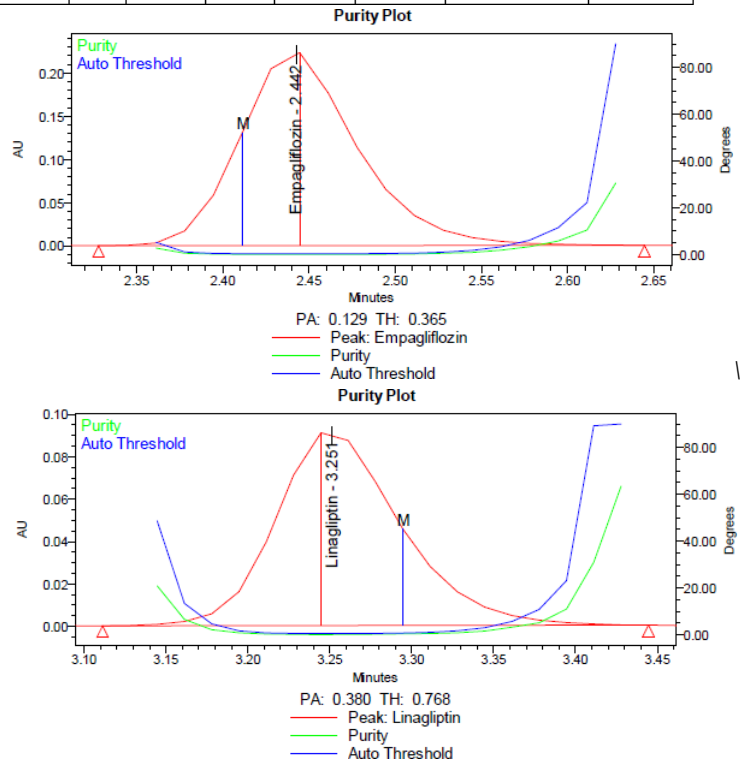
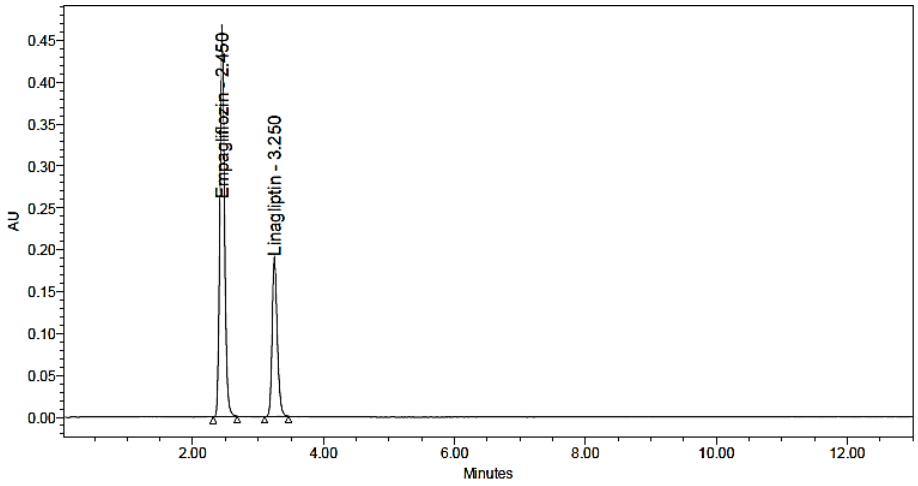


Figure 5: Thermal degradation chromatogram and purity plot of empagliflozin and linagliptin



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Empagliflozin	2.450	218247	68.62	0.147	0.289	5808	1.1
2	Linagliptin	3.250	100035	31.38	0.134	0.342	8430	1.2

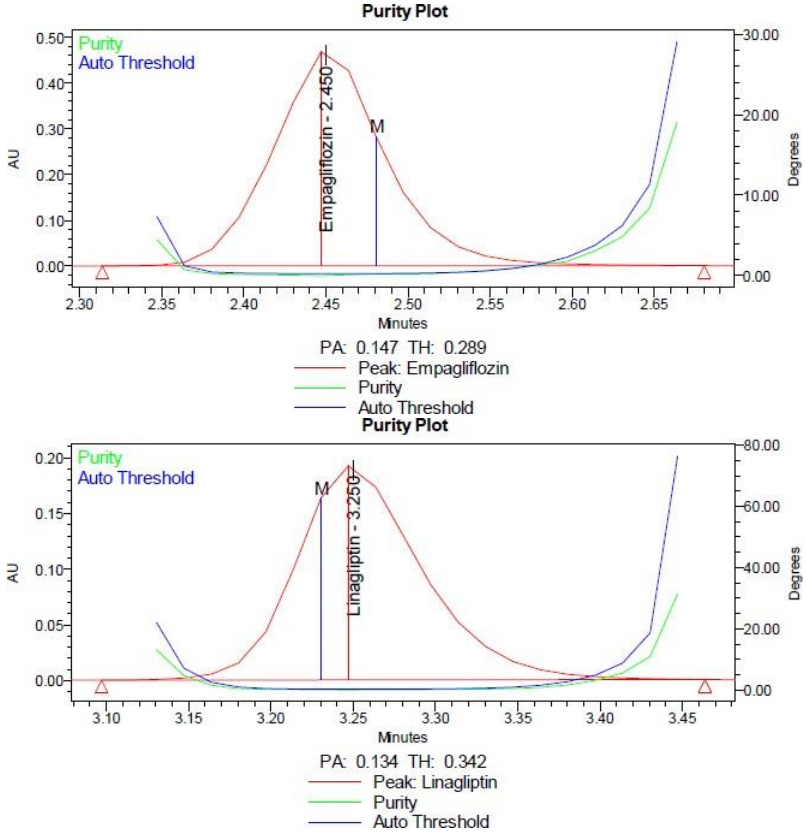
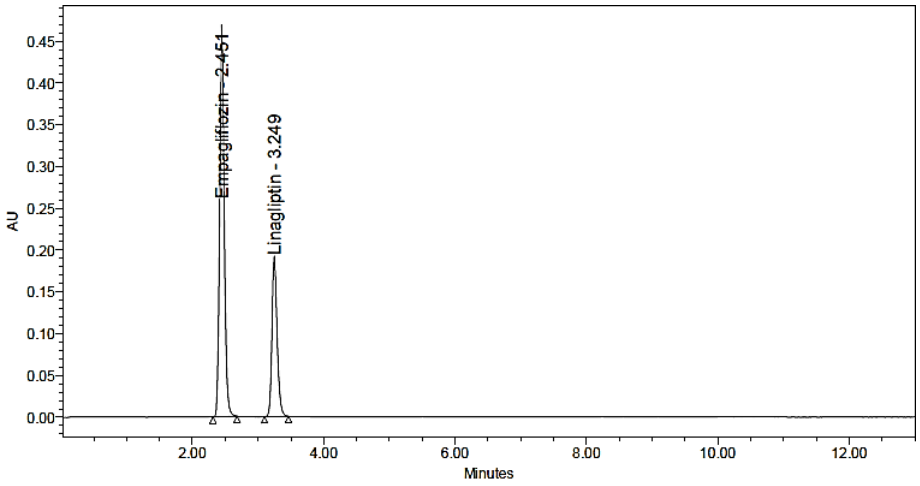


Figure 6: Photolytic degradation chromatogram and purity plot of empagliflozin and linagliptin



	Peak Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing
1	Empagliflozin	2.451	218721	68.57	0.159	0.287	5825	1.1
2	Linagliptin	3.249	100018	31.43	0.139	0.335	8245	1.2

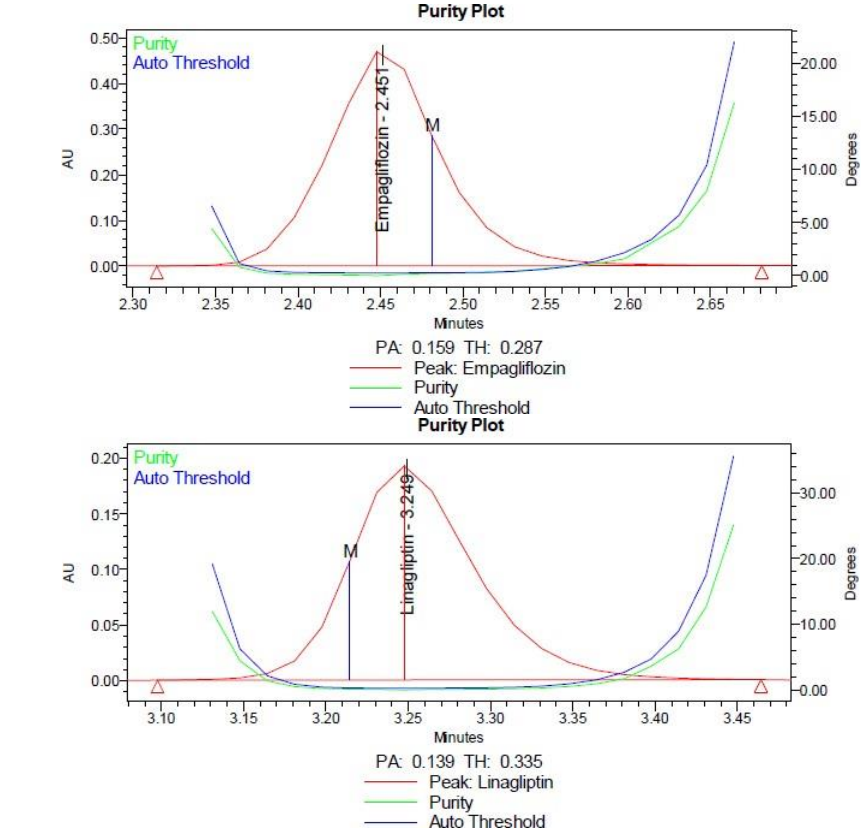


Figure 7: Control chromatogram and purity plot of empagliflozin and linagliptin

Accuracy: Accuracy was performed using the commercial drug product and active pharmaceutical ingredients, so it is not possible to obtain the exact placebo components to perform accuracy. So, accuracy was performed by adding known quantities of the analyte (standard solution) to a prequalified commercial tablet composite.

Weighed and transferred weight equivalent to one tablet of prequalified commercial tablet composite into a 100 mL volumetric flask. The amount present in each sample was calculated from the weight of the powder transferred into

the individual volumetric flask. Standard with known concentration was prepared and spiked into the prequalified commercial tablet dosage form. Three sets of empagliflozin and linagliptin accuracy samples were prepared at approximately50%, 100% and 150% levels (n=9, three at each level). The recovery data was presented in Tables 5 and 6.

Table 5: Percent Recovery results of empagliflozin

Accuracy	Standard added (µg/ mL)	Prequalified sample (µg/mL)	Total Amount (µg/mL)	Area of accuracy solution	Amount Recovered (µg/mL)	Percent Recovered	Mean % Recovery	SD	%RSD
	47.956	96.016	143.972	317427	144.38646	100.288			
50%	47.956	97.064	145.020	316941	144.16540	99.411	99.532	0.703	0.71
	47.956	97.004	144.960	315173	143.36120	98.897			
	95.912	97.167	193.079	420692	191.35811	99.109			
100%	95.912	97.131	193.043	420068	191.07427	98.980	99.136	0.171	0.17
	95.912	97.362	193.274	422011	191.95807	99.319			
	143.868	99.166	243.033	530774	241.43057	99.341			
150%	143.868	97.177	241.045	523922	238.31383	98.867	99.391	0.550	0.55
	143.868	97.239	241.106	529870	241.01937	99.964			
			Overall Mean			99.353			
			Overall Standard deviation			0.487			
			Over all % RSD			0.49			

Table 7: Percent Recovery results of linagliptin

Accuracy	Standard added (µg/ mL)	Prequalified sample (µg/mL)	Total Amount (µg/mL)	Area of accuracy solution	Amount Recovered (µg/mL)	Percent Recovered	Mean % Recovery	SD	%RSD
	26.341	52.014	78.355	159526	79.37012	101.296			
50%	26.341	51.829	78.170	159836	79.52436	101.733	101.551	0.227	0.22
	26.341	51.859	78.199	159725	79.46913	101.624			
	52.681	51.750	104.431	212551	105.75203	101.265			
100%	52.681	51.973	104.655	210999	104.97986	100.310	100.632	0.548	0.54
	52.681	52.156	104.838	211391	105.17489	100.322			
	79.022	51.970	130.993	263759	131.22992	100.181			
150%	79.022	52.169	131.191	264668	131.68218	100.374	100.398	0.230	0.23
	79.022	51.776	130.798	264572	131.63442	100.640			
			Overall Mean			100.860			
			Overall Standard deviation			0.616			
			Over all % RSD			0.61			

Linearity: Linearity was demonstrated by preparing different concentrations of drug substance and analyzed as per the proposed method. A linear relationship was evaluated by calculation of a regression line by the method of least squares. A plot of the area of the peak as a function of analyte concentration was prepared. The correlation coefficient, y-intercept and slope of the regression line were calculated.

Table 8: Linearity results of empagliflozin

Concentration(µg/mL)	Repetition 1Area	Repetition 2Area	Repetition 3Area	Average
25.149	58395	58856	58846	58699
50.298	101840	102681	102009	102177
75.446	154378	154634	154999	154670
100.595	214406	213569	214368	214114
125.744	263995	264807	263821	264208
150.893	316490	319167	318123	317927
201.191	420692	420068	422011	420924
251.488	530774	523922	529870	528189
slope	2103.075	2079.819	2101.811	2094.901
y intercept	41.260	2481.381503	580.711	1282
correlationcoefficient	0.9998	0.9998	0.9998	0.9998

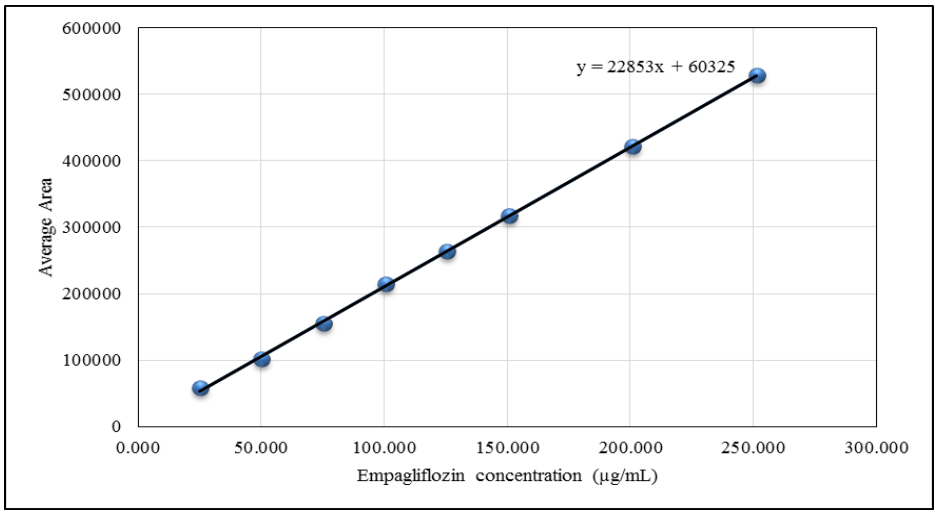


Figure 8: Linearity plot of empagliflozin

Table 9: Linearity results of linagliptin

Concentration(µg/mL)	Repetition 1Area	Repetition 2 Area	Repetition 3 Area	Average
12.624	30652	30426	30589	30556
25.248	55984	56159	56034	56059
37.872	82467	82396	82399	82421
50.496	105582	105980	105555	105706
63.120	135182	134529	135128	134946
75.744	158589	159397	158535	158840
100.992	212551	210999	211391	211647
126.240	263759	264668	264572	264333
slope	2056.889	2057.858	2057.775	2057.507
y intercept	4010.561	3924.434	3885.659	63.929
Correlationcoefficient	0.9999	0.9999	0.9999	0.9999

Limit of Detection and Limit of Quantification (LOD and LOQ): The limit of detection and limit of quantification were determined based on the calibration curve using the values of average slope, the standard deviation of y-intercepts of regression lines that have been obtained from the linearity study.

Table 10: Theoretical LOD and LOQ of empagliflozin and linagliptin

Analyte	SD	Slope	LOD (µg/ mL)	LOQ (µg/ mL)
Empagliflozin	1281.779	2095	2.019	6.119
Linagliptin	63.929	2058	0.103	0.309

Robustness: Effect of flow rate, column temperature and mobile phase composition were studied. This test was carried out by injecting the working standard six times. The chromatograms showed that all the system suitability parameters such as peak area, theoretical plates, tailing factor and retention time of the analytes found satisfactory. Hence the developed stability indicating method was robust.

Table 11: Robustness results of empagliflozin

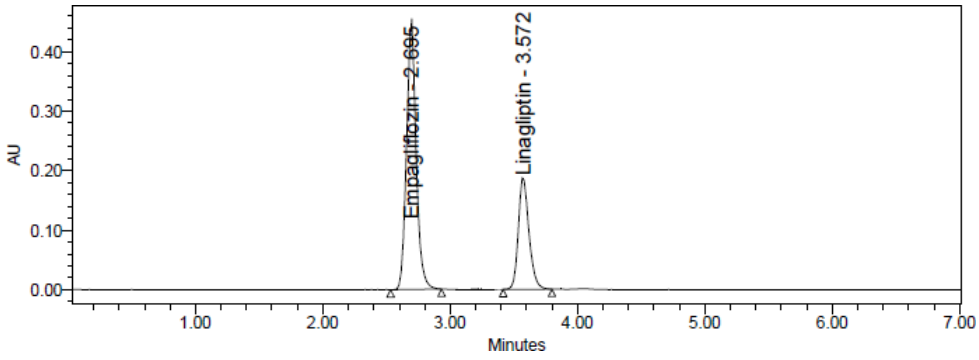
Parameters	Empagliflozin		
	Retention time(min)	USP Plate Count	USP Tailing
Flow rate 0.8 mL	2.695	6101	1.12
Flow rate 1.2 mL	2.256	5778	1.15
Temperature 25ºC	2.465	6009	1.10
Temperature 35ºC	2.465	5769	1.10
Mobile phase *	2.463	5420	1.07
Mobile phase **	2.466	6137	1.16
Optimized condition	2.437	6127	1.15

Table 12: Robustness results of linagliptin

Parameters	Linagliptin			
	Retention time (min)	USP Plate Count	USP Tailing	USP Resolution
Flow rate 0.8 mL	3.574	9103	1.20	5.9
Flow rate 1.2 mL	2.987	8346	1.20	5.7
Temperature 25 ºC	3.263	8164	1.21	5.6
Temperature 35 ºC	3.262	8148	1.20	5.7
Mobile phase *	3.219	8422	1.20	5.4
Mobile phase **	3.306	8200	1.20	5.9
Optimized condition	3.272	8133	1.23	5.8

* Buffer solution and acetonitrile in 40: 60 V/V

** Buffer solution and acetonitrile in 50: 50 V/V



Peak Name: Empagliflozin

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Empagliflozin	2.464	223666	5833	1.09
2	Empagliflozin	2.465	223859	6141	1.10
3	Empagliflozin	2.466	224221	5927	1.10
4	Empagliflozin	2.465	223622	6033	1.10
5	Empagliflozin	2.465	223564	6141	1.10
6	Empagliflozin	2.466	224678	5980	1.10
Mean		2.465	223935	6009	1.10
Std. Dev.		0.001	435.337	121.626	0.004
% RSD		0.03	0.19	2.02	0.37

Peak Name: Linagliptin

	Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	3.261	103096	8486	1.22	5.7
2	Linagliptin	3.263	101872	8100	1.20	5.6
3	Linagliptin	3.265	103929	7970	1.22	5.6
4	Linagliptin	3.261	103096	8321	1.22	5.7
5	Linagliptin	3.263	101872	7977	1.20	5.6
6	Linagliptin	3.265	103929	8130	1.22	5.6
Mean		3.263	102966	8164	1.21	5.6
Std. Dev.		0.002	925.441	203.071	0.010	0.052
% RSD		0.05	0.90	2.49	0.85	0.92

Figure 9: Robustness chromatograms of empagliflozin and linagliptin at flowrate 0.8 mL/min.

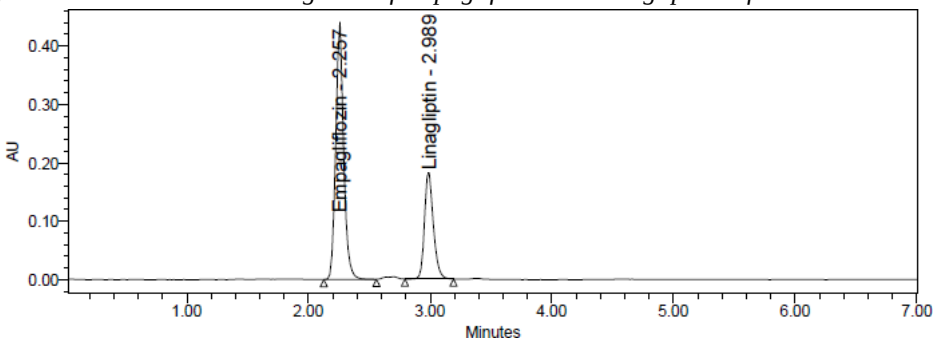


Figure 10: Robustness chromatograms of empagliflozin and linagliptin at flowrate 1.2 mL/min

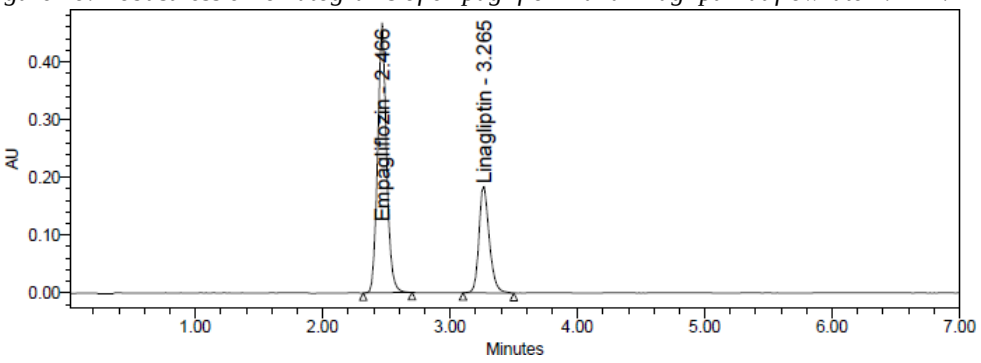
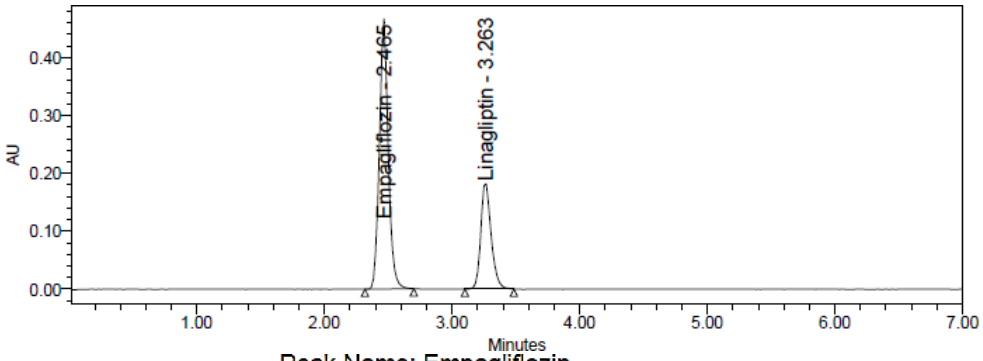


Figure 11: Robustness chromatograms of empagliflozin and linagliptin attemperature 25 ºC.



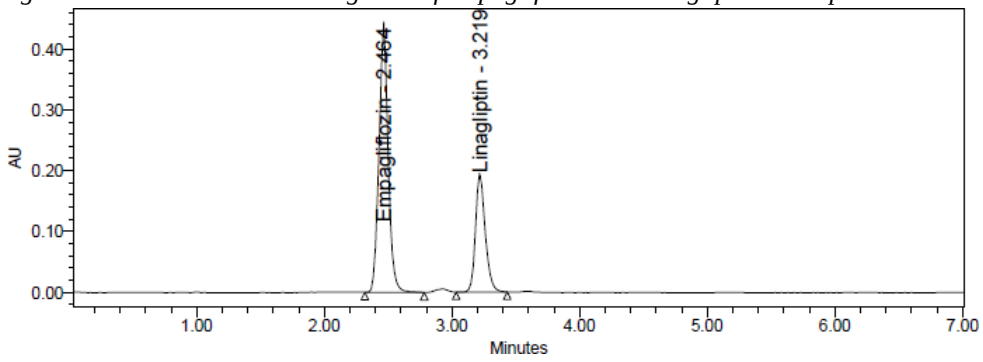
Peak Name: Empagliflozin

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Empagliflozin	2.464	224668	5671	1.10
2	Empagliflozin	2.465	224360	5776	1.08
3	Empagliflozin	2.465	225032	5859	1.11
4	Empagliflozin	2.465	224145	5677	1.10
5	Empagliflozin	2.465	224110	5754	1.10
6	Empagliflozin	2.465	224377	5878	1.11
Mean		2.465	224449	5769	1.10
Std. Dev.		0.000	348.795	87.548	0.011
% RSD		0.02	0.16	1.52	1.00

Peak Name: Linagliptin

	Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	3.259	102832	8303	1.19	5.7
2	Linagliptin	3.263	102803	8145	1.19	5.7
3	Linagliptin	3.263	103017	8022	1.21	5.6
4	Linagliptin	3.259	102784	8165	1.19	5.7
5	Linagliptin	3.263	102877	8171	1.19	5.7
6	Linagliptin	3.263	103411	8079	1.20	5.7
Mean		3.262	102954	8148	1.20	5.7
Std. Dev.		0.002	238.886	95.381	0.008	0.041
% RSD		0.06	0.23	1.17	0.70	0.72

Figure 12: Robustness chromatograms of empagliflozin and linagliptin at temperature 35 ºC



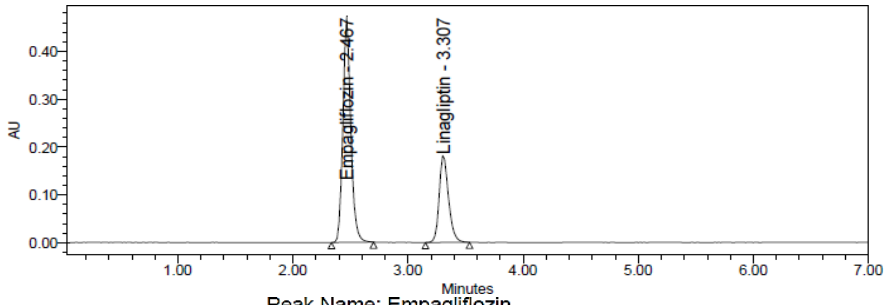
Peak Name: Empagliflozin

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Empagliflozin	2.461	217774	5315	1.05
2	Empagliflozin	2.462	219022	5485	1.08
3	Empagliflozin	2.464	220719	5476	1.08
4	Empagliflozin	2.464	217625	5355	1.07
5	Empagliflozin	2.464	219479	5419	1.08
6	Empagliflozin	2.463	220471	5472	1.07
Mean		2.463	219182	5420	1.07
Std. Dev.		0.001	1307.242	71.155	0.012
% RSD		0.05	0.60	1.31	1.09

Peak Name: Linagliptin

	Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	3.218	101283	8242	1.19	5.4
2	Linagliptin	3.219	101618	8525	1.21	5.3
3	Linagliptin	3.219	102875	8471	1.20	5.3
4	Linagliptin	3.218	101294	8442	1.19	5.4
5	Linagliptin	3.218	102576	8427	1.20	5.4
6	Linagliptin	3.219	102695	8423	1.20	5.3
Mean		3.219	102057	8422	1.20	5.4
Std. Dev.		0.001	737.495	95.745	0.008	0.055
% RSD		0.02	0.72	1.14	0.63	1.02

Figure 13: Robustness chromatograms of empagliflozin and linagliptin at mobile phase - Buffer and Acetonitrile in the ratio 40:60 V/V



Peak Name: Empagliflozin

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Empagliflozin	2.465	223195	5886	1.19
2	Empagliflozin	2.467	223461	6258	1.14
3	Empagliflozin	2.467	223466	6153	1.14
4	Empagliflozin	2.465	223535	6187	1.18
5	Empagliflozin	2.467	223287	6175	1.15
6	Empagliflozin	2.467	223777	6164	1.15
Mean		2.466	223454	6137	1.16
Std. Dev.		0.001	203.003	12.507	0.021
% RSD		0.04	0.09	2.09	1.84

Peak Name: Linagliptin

	Peak Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	3.306	102373	8124	1.19	6.0
2	Linagliptin	3.306	102202	8242	1.20	5.8
3	Linagliptin	3.307	102355	8284	1.21	5.8
4	Linagliptin	3.306	102287	8120	1.20	5.9
5	Linagliptin	3.306	102465	8229	1.20	5.8
6	Linagliptin	3.306	102153	8199	1.20	5.8
Mean		3.306	102306	8200	1.20	5.9
Std. Dev.		0.000	115.548	66.087	0.006	0.084
% RSD		0.01	0.11	0.81	0.53	1.43

Figure 14: Robustness chromatograms of empagliflozin and linagliptin at mobile phase Buffer and Acetonitrile in the ratio 50:50 V/V

Stability of the Sample Solution: A study was conducted to establish the stability of the sample solution. Sample solutions were stored in tightly stoppered Pyrex glassware on the bench top. The sample solution was analyzed as per methodology at initially and at different time intervals. Calculated the percent assay at different time points.

Table 13: Stability of sample solution

Time	Empagliflozin		Linagliptin	
	% Assay	Difference	% Assay	Difference
Initial	99.6	+ 0.2	98.9	+ 0.2
48 hours	99.8		99.1	

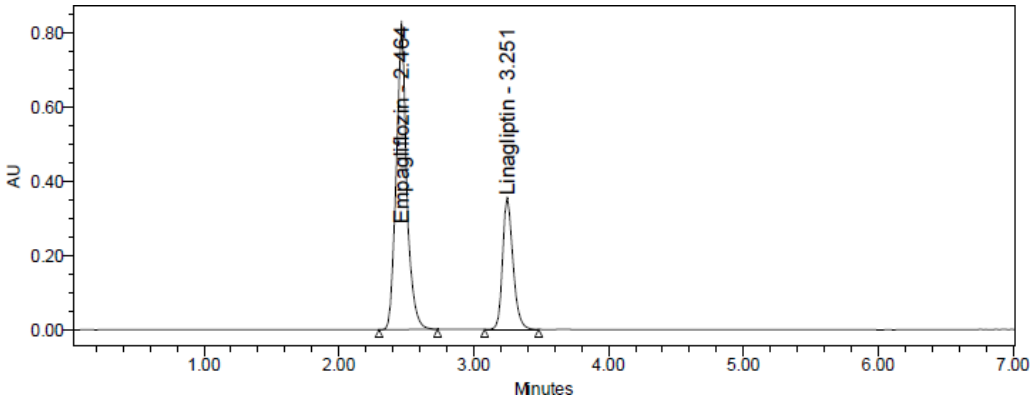


Figure 15: Chromatogram of the commercial formulation

Discussion

A novel stability indicating RP-HPLC method was developed and validated for simultaneous estimation of empagliflozin and linagliptin in the tablet dosage form. The proposed method can give faster elution of the analytes with good resolution.

Table 14: Summary of the validation

Parameter	Result	
System suitability	**USP Plate Count**	
	6127	8133
	USP tailing	
	1.15	1.23
	% RSD	
	0.7	0.6
Method precision	**% RSD**	
	0.49 and 0.15	0.49 and 0.35
Specificity	Forced degradation (acid, base/alkali, peroxide, photolytic, neutral (control)	
Accuracy	**Percent Recovery**	
Linearity	**Correlation coefficient**	
LOD and LOQ(µg/mL)	2.019 and 6.119	0.103 and 0.309
Robustness	With respect to temperature, mobile phase composition and flow rate.	
Stability of the Sample Solution	Performed sample stability up to 48 hours	

Forced degradation studies were conducted to know the stability of the analytes under specified conditions. During the forced degradation study, the analytes were stable in all the conditions and no interferences were found, both

drug peaks have purity angles lesser than their purity threshold indicating peak purity. The percent recovery and precision studies showed that the method is accurate and precise.

Conclusion

Thus, the present stability indicating RP-HPLC method was shown to be simple, specific, accurate, precise and robust and this method is suitable for simultaneous determination of empagliflozin and linagliptin in tablet formulations and for monitoring the degradation pattern of these drugs.

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