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Stability Indicating Method Development and Validation for Simultaneous Estimation of Linagliptin and Empagliflozin in Tablets by HPLC

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Original Research Article

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Abstract: A very simple, accurate, precise, robust, rugged and stability indicating method with gradient elution was developed for simultaneous estimation of Linagliptin and Empagliflozin in tablets. The developed method was rapid with a run time of 25 minutes eluting the peaks at 5.388 min (Linagliptin) and 8.390 min (Empagliflozin) and economic. The Chromatographic separation was achieved gradiently on a Hypersil ODS 3V, 250 x 4.6 mm.5.0µ. Column by using Potassium di-hydrogen phosphate (adjusted to pH 2.20 with ortho phosphoric acid) as mobile phase –A. Water: Acetonitrile (5:95) is used as mobile phase-B.. Flow rate of 1mL/min with UV detection at 225nm was used. The retention times of Linagliptin & Empagliflozin are 5.388 min and 8.390 min respectively. The developed method was specific and well separated from the impurities of both Linagliptin & Empagliflozin. The method is linear in a range of 40% to 160 % against the standard concentration for both Linagliptin & Empagliflozin. The correlation coefficient was found to be R2= 0.995 & 0.996 for Linagliptin & Empagliflozin respectively. Both standard and test solutions proved to be stable for up to 48 Hrs. Forced degradation study showed that the method is stability indicating. The developed method can be used for routine analysis of Linagliptin & Empagliflozin fixed dose combination.
Keywords: RP-HPLC, Linagliptin & Empagliflozin, Gradient Elution, Stability Indicating, Validation.

INTRODUCTION

LINAGLIPTIN

8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-2,3,6,7 tetrahydro-1H-purine-2,6-dione is a White to pale yellow solid with a pKa of 9.86. This is soluble in methanol, soluble in ethanol and Dimethyl sulphoxide and practically insoluble in water. Its molecular formula is C25H28N8O2 & Molecular weight is 472.5422 g/Mol. Belongs to Class-III in BCS classification [1, 2].

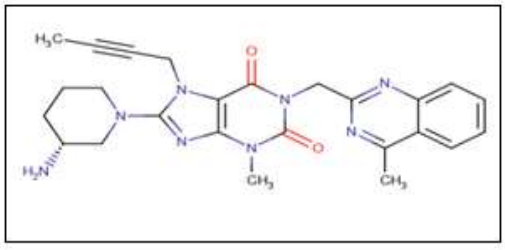

Fig-1: Linagliptin

EMPAGLIFLOZIN

((2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy]phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol is a white to yellowish powder with a pKa of 12.57. Empagliflozin is freely soluble in Dimethyl sulphoxide, very slightly soluble in acetonitrile and practically insoluble in water. Molecular formula = $C_{23}H_{27}ClO_7$ & Molecular Weight = 450.91. BCS classification of the molecule is Class-III [3, 2].

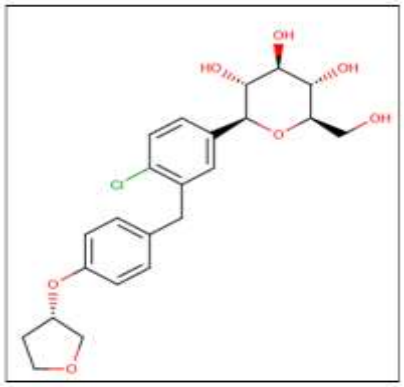

Fig-2: Empagliflozin

MATERIALS AND METHODS

Reagents and chemicals:

- Linagliptin - Active pharmaceutical Ingredient (API).
- Empagliflozin- Active pharmaceutical Ingredient (API).
- HPLC grade Acetonitrile, Potassium dihydrogen Orthophosphate-AR Grade, Orthophosphoric Acid-HPLC Grade, 0.45µ PVDF membrane filter, HPLC Grade water was used. Other chemicals and reagents like ammonium acetate, HCl, NaOH, H_2O_2 of AR grade were used.

Instruments used

Analysis was performed by using analytical balance precisa XB220A, HPLC used is of Waters make with PDA detector. Column used in Hypersil ODS 3V, 250 x 4.6 mm.5.0µ. Other equipments like sonicator, water bath, hot air oven of thermo make were used.

Preparation of Mobile Phase buffer

Weigh and transfer about 1.36 grams of Potassium di-hydrogen phosphate (KH2PO4) into 1000 mL of Milli-Q-Water and adjust the pH to 2.20 with diluted Ortho-phosphoric acid, filter and degas.

Mobile Phase Preparation:

Mobile phase-A: Buffer 100%

Mobile phase-B: Water: Acetonitrile (5:95)

Preparation of Diluent-1: Mix Acetonitrile and water in the ratio of 70:30.

Preparation of Diluent-2: Mix Acetonitrile and water in the ratio of 50:50.

Table-1: Optimized Chromatographic Conditions

Column	Hypersil ODS 3V, 250 x 4.6 mm.5.0µ.
Detector wavelength	225 nm
Flow rate	1.0 mL/min
Injection volume	10.0 µL
Column oven Temp.	30˚C
Sample tray	15°C
Run time	25.0 minutes
Elution	Gradient

Empagliflozin standard stock solution

Weigh and transfer 25.00 mg of Empagliflozin into 25 mL volumetric flask, add about 15 mL of diluent-1 and sonicate for 10 min, dilute to volume with diluen-1 and mix well.

Linagliptin standard stock solution

Weigh and transfer 25.00 mg of Linagliptin into a 100 mL volumetric flask, add about 60mL of diluent-1 and sonicate for 10 min, dilute to volume with diluent-1 and mix well.

Standard solution

Transfer 5mL of Empagliflozin and 4mL of Linagliptin standard stock solutions into a 20mL volumetric flask and dilute with diluent-2 and mix well. (Concentration of Empagliflozin and Linagliptin is about 250 µg/mL and 50 µg/mL)

Preparation of Placebo:

Weigh placebo equivalent to 250 mg of Empagliflozin and 25 mg of Linagliptin, add about 180mL of diluent-1 and sonicate for 30minutes with intermittent shaking, cool to room temperature, dilute to volume with diluent-1 and mix well. Centrifuge the sample at 5000 rpm for 10 minutes. Dilute 5mL of this solution into a 20mL volumetric flask with diluent-2 and mix well.

Preparation of Test solution:

Weigh and transfer 10 tablets into 250mL volumetric flask, add about 180mL of diluent-1 and sonicate for 30minutes with intermittent shaking, cool to room temperature, dilute to volume with diluent-1 and mix well. Centrifuge the sample at 5000 rpm for 10 minutes. Dilute 5mL of this solution into a 20mL volumetric flask with diluent-2 and mix well. (For 25/5 mg concentration of Empagliflozin and Linagliptin is about 250 µg/ml and 50 µg/ml).

METHOD VALIDATION [4]

SYSTEM SUITABILITY

Prepared the standard and injected in to the chromatograph.

Acceptance Criteria

% RSD should be NMT 2.0 for Empagliflozin & Linagliptin from the five replicate injections.

Table-2: System Suitability

SYSTEM SUITABILITY						
Analytes	**Empagliflozin**			**Linagliptin**		
Injections	**Area**	**Tailing**	**USP Plate Count**	**Area**	**Tailing**	**USP Plate Count**
1	850498	1.07	12232	678345	1.22	9874
2	849768	1.02	12934	687564	1.20	9823
3	852894	1.05	12933	678420	1.25	9846
4	849982	1.09	12345	668592	1.21	9789
5	876893	1.02	12738	687532	1.23	9823
Average	856007	1.05	12636	680091	1.22	9831
STDEV	11741.92	0.03	329.89	7894.33	0.02	31.49
% RSD	1.37	2.94	2.61	1.16	1.57	0.32

SPECIFICITY

Injected blank solution, placebo, standard, individual impurities and test solutions in to the chromatograph after system suitability.

No Interference was observed at the RT's of Empagliflozin and Linagliptin from blank, impurities and placebo solutions.

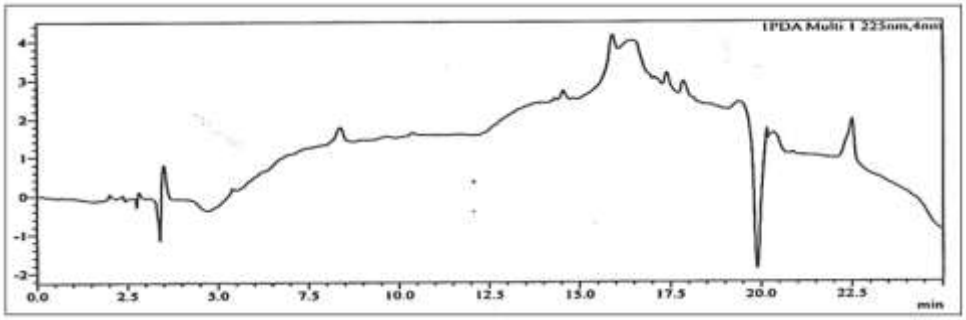

Fig-3: Blank Solution

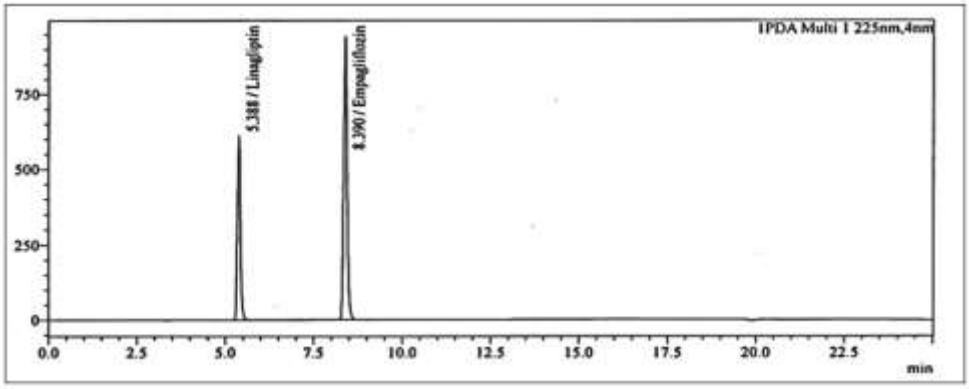

Fig-4: Standard Solution

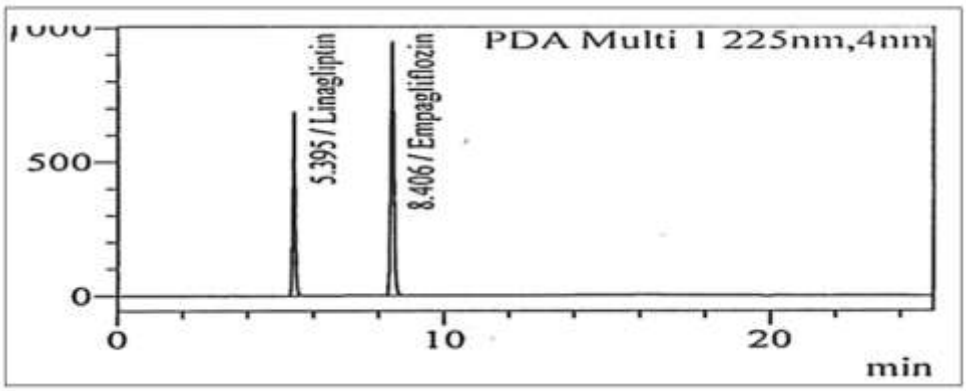

Fig-5: Test Solution

Table-3: Specificity

S. No.	Sample Name	Retention Time
1.	Blank Solution	No peaks were observed at the RT's of Empagliflozin & Linagliptin.
2.	Placebo Solution	No peaks were observed at the RT's of Empagliflozin & Linagliptin.
3.	Standard Solution	Empagliflozin : 5.435min Linagliptin : 8.345min
4.	Test Solution	Empagliflozin : 5.498 min Linagliptin : 8.398 min
Acceptance Criteria: No peak should be present at the RT's of Empagliflozin & Linagliptin.		

PRECISION

Determined the precision of the test method by preparing and injecting six samples of Linagliptin & Empagliflozin test solutions of 5-25 mg strength. Injected the solutions into HPLC and recorded the results. Intermediate precision was performed on a different day, on a different system by using the same lot of samples.

Calculation

Calculate the amount of Empagliflozin and Linagliptin for 25/5mg using following formula,

$$\text{mg/tablet of Empagliflozin} = \frac{A_{TE}}{A_{SE}} \times \frac{W_{SE}}{25} \times \frac{5}{20} \times \frac{250}{10} \times \frac{20}{5} \times \frac{P}{100}$$

$$\text{mg/tablet of Linagliptin} = \frac{A_{TL}}{A_{SL}} \times \frac{W_{SL}}{100} \times \frac{4}{20} \times \frac{250}{10} \times \frac{20}{5} \times \frac{P}{100}$$

Where,

A_{TE} = Area of Empagliflozin in test preparation
A_{SE} = Area of Empagliflozin in standard preparation
A_{TL} = Area of Linagliptin in test preparation
A_{SL} = Area of Linagliptin in standard preparation
W_{SE} = Weight of Empagliflozin Standard taken in mg
W_{SL} = Weight of Linagliptin Standard taken in mg
P = Potency of Standard on as is basis
LC = Label Claim

$$\% \text{ Assay} = \frac{\text{Assay (in mg/tablet)} \times 100}{\text{Label claim in mg}}$$

Table-4: Method Precision Linagliptin

METHOD VALIDATION_ASSAY_METHOD PRECISION_LINAGLIPTIN								
Sample Name		Weight of tablets						
Precision test Solution-1		2412.34		M/F	1	Ave. Wt.(mg)		241.5
Precision test Solution-2		2399.13		Volume(mL)	250			
Precision test Solution-3		2405.12		Dil. Rate	4			
Precision test Solution-4		2432.12						
Precision test Solution-5		2416.87		**Strength (mg)**	**5**			
Precision test Solution-6		2420.87						
Std. Wt.	**25.00**	**mg**	**Volume**	**100**	**Dil. Rate**	**5**	**Purity**	**99.8**
Sample Name	**STD Area**		**Sample Area**		**Assay (%)**	**Ave (%)**	**SD (%)**	**RSD (%)**
Test Solution-1	**680091**		**693475**		**101.89**	**100.74**	**0.96**	**0.95**
Test Solution-2	**680092**		**689328**		**101.84**			
Test Solution-3	**680093**		**680125**		**100.23**			
Test Solution-4	**680094**		**685602**		**99.92**			
Test Solution-5	**680095**		**679823**		**99.70**			
Test Solution-6	**680096**		**688737**		**100.84**			

Table-5: Method Precision Empagliflozin

METHOD VALIDATION_ASSAY_METHOD PRECISION_EMPAGLIFLOZIN								
Sample Name		Weight of tablets						
Precision test Solution-1		2412.34		M/F	1	Ave. Wt.(mg)		241.5
Precision test Solution-2		2399.13		Volume(mL)	250			
Precision test Solution-3		2405.12		Dil. Rate	4			
Precision test Solution-4		2432.12						
Precision test Solution-5		2416.87		**Strength (mg):**	**25**			
Precision test Solution-6		2420.87						
Std. Wt.	25.00	mg	Volume(mL)	25	Dil. Rate	4	**Purity**	99.98
Sample Name	**STD Area**		**Sample Area**		**Assay (%)**	**Ave (%)**	**SD (%)**	**RSD (%)**
Test Solution-1	856007		846734		99.02	99.42	1.05	1.06
Test Solution-2	856007		852345		100.23			
Test Solution-3	856007		860921		100.98			
Test Solution-4	856007		845293		98.05			
Test Solution-5	856007		852334		99.49			
Test Solution-6	856007		847652		98.78			

Table-6: Intermediate Precision Linagliptin

METHOD VALIDATION_ASSAY_INTERMEDIATE PRECISION_LINAGLIPTIN								
Sample Name		Weight of tablets						
Precision test Solution-1		2398.14		M/F	1	Ave. Wt.(mg)		240.5
Precision test Solution-2		2401.56		Volume (mL)	250			
Precision test Solution-3		2399.23		Dil. Rate	4			
Precision test Solution-4		2403.32						
Precision test Solution-5		2414.02		Strength (mg)	5			
Precision test Solution-6		2410.78						
Std. Wt.	25.00	mg	Volume (mL)	100	Dil. Rate	5	Purity	99.8
Sample Name	STD Area		Sample Area		Assay	Ave (%)	SD (%)	RSD (%)
Precision test Solution-1	680023		689766		101.50	100.82	0.63	0.63
Precision test Solution-2	680023		687234		100.98			
Precision test Solution-3	680023		690234		101.52			
Precision test Solution-4	680023		683459		100.35			
Precision test Solution-5	680023		683784		99.96			
Precision test Solution-6	680023		687204		100.59			

Table-7: Intermediate Precision Empagliflozin

METHOD VALIDATION_ASSAY_INTERMEDIATE PRECISION_EMPAGLIFLOZIN								
Sample Name		Weight of tablets						
Precision test Solution-1		2398.14		M/F	1	Ave. Wt.(mg)		240.5
Precision test Solution-2		2401.56		Volume(mL)	250			
Precision test Solution-3		2399.23		Dil. Rate	4			
Precision test Solution-4		2403.32						
Precision test Solution-5		2414.02		Strength (mg)	25			
Precision test Solution-6		2410.78						
Std. Wt.	25.00	mg	Volume(mL)	25	Dil. Rate	4	Purity	99.98
Sample Name	STD Area		Sample Area		Assay	Ave	SD	RSD
Precision test Solution-1	853247		842358		98.97	100.50	1.16	1.16
Precision test Solution-2	853247		845902		99.24			
Precision test Solution-3	853247		865342		101.62			
Precision test Solution-4	853247		864672		101.37			
Precision test Solution-5	853247		868734		101.39			
Precision test Solution-6	853247		859082		100.40			

Table-8: Method & Intermediate Precision Combined

METHOD & INTERMEDIATE PRECISION COMBINEDLY									
Method Precision		Intermediate Precision		Empagliflozin			Linagliptin		
Empagliflozin	Linagliptin	Empagliflozin	Linagliptin	Overall Avg.	STDEV	% RSD	Overall Avg.	STDEV	% RSD
99.02	101.89	98.97	101.50	100.44	1.20	1.19	100.78	0.77	0.77
100.23	101.84	99.24	100.98						
100.98	100.23	101.62	101.52						
98.05	99.92	101.37	100.35						
99.49	99.70	101.39	99.96						
98.78	100.84	100.40	100.59						

LINEARITY

Determined the Linearity by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis, from 40 % to the 160 % against standard concentrations for both the analytes.

Table-9: Linearity Of Empagliflozin

LINEARITY OF EMPAGLIFLOZIN				
Wt. taken		Conc.(ppm)		
250.23	mg			
250	mL	1000.92		
Vol. of Stock Soln. taken (mL)	Diluted to (mL)	Conc. (ppm)	% Against Std. Conc.	Area
0	0	0	0	0
2	20	100.09	40	404387
4	20	200.18	80	698765
5	20	250.23	100	856733
6	20	300.28	120	1019822
8	20	400.37	160	1329834

Table-10: Linearity of Linagliptin

LINEARITY OF LINAGLIPTIN				
Wt. taken		Conc.(ppm)		
50.34				
250		200		
Vol. of Stock Soln. taken (mL)	Diluted to (mL)	Conc. (ppm)	% Against Std. Conc.	Area
0	0	0	0	0
2	20	20.14	40	356432
4	20	40.27	80	698765
5	20	50.34	100	856733
6	20	60.41	120	1019822
8	20	80.54	160	1286740

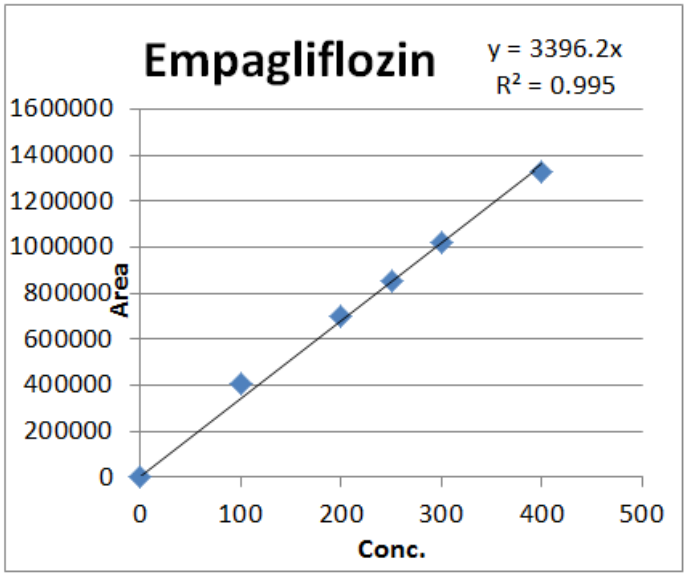

Fig-6: Empagliflozin Linearity

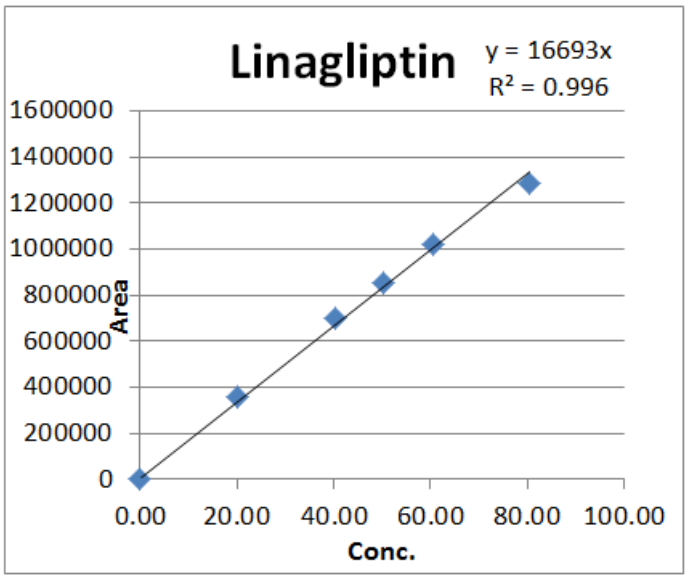

Fig-7: Linagliptin Linearity

ACCURACY

Performed the accuracy of test method using Linagliptin & Empagliflozin API and placebo at 50 %, 100 %, 150 % spike levels in triplicate. Calculated the % Recovery and recorded the results.

Table-11: Accuracy Linagliptin

METHOD VALIDATION_ASSAY_ACCURACY_LINAGLIPTIN							
Linagliptin-Empagliflozin IR tablets		Std. Wt. in mg	25.00	Std. Response	689347	Potency	99.8
		Volume (mL)	100	Sample Volume	250	M/F	1
		Dil. Rate	5	Dil. Rate	4	Strength	5
Spike Level	**Wt. of Sample Taken in mg**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**Average**	**% RSD**
50%_01	25.32	355784	25.3	25.8	101.9	101.8	0.32
50%_02	25.68	360982	25.6	26.1	102.0		
50%_03	25.75	359872	25.7	26.1	101.4		
100%_01	50.12	689345	50.0	49.9	99.8	99.7	0.70
100%_02	50.43	697789	50.3	50.5	100.4		
100%_03	50.36	687630	50.3	49.8	99.0		
150%_01	75.43	1043782	75.3	75.6	100.4	99.4	0.92
150%_02	74.92	1018436	74.8	73.7	98.6		
150%_03	75.23	1029338	75.1	74.5	99.2		

Table-12: Accuracy Empagliflozin

METHOD VALIDATION_ASSAY_ACCURACY_EMPAGLIFLOZIN							
Linagliptin-Empagliflozin IR tablets		Std. Wt. in mg	24.69	Std. Response	859345	Potency	99.98
		Volume(mL) :	25	Sample Volume	250	M/F	1
		Dil. Rate	4	Dil. Rate	4	Strength	25
Spike Level	**Wt. of Sample Taken in mg**	**Sample Area**	**µg/ml added**	**µg/ml found**	**% Recovery**	**Average**	**% RSD**
50%_01	125.32	440983	125.3	126.7	101.1	101.8	1.13
50%_02	125.01	439820	125.0	126.3	101.1		
50%_03	124.32	446029	124.3	128.1	103.1		
100%_01	250.21	867345	250.2	249.1	99.6	99.1	0.50
100%_02	250.32	859356	250.3	246.9	98.6		
100%_03	250.08	862985	250.0	247.9	99.1		
150%_01	375.12	1287094	375.0	369.7	98.6	98.5	0.12
150%_02	375.45	1286454	375.4	369.5	98.4		
150%_03	376.21	1290928	376.1	370.8	98.6		

BENCH TOP STABILITY OF STANDARD AND TEST PREPARATION

Performed the assay of Linagliptin-Empagliflozin tablets as per the test method for 5-25 mg and kept on bench top for 48 Hrs. after analysing the initial amount. Injected the samples at initial, 24 hours and 48 hours. Calculated the assay against the freshly prepared standard solution and checked the difference in assay of the samples between the initial and bench top stability samples.

Table-13: Solution Stability Empagliflozin

METHOD VALIDATION_ASSAY_BENCH TOP STABILITY_EMPAGLIFLOZIN								
Sample Name		Weight of tablets						
Precision test Solution-1(Initial)		2412.34		M/F	1	Ave. Wt.(mg)		241.5
		Strength		Vol.(m	250			
		25 mg		Dil.	4			
Std. Wt.(Initial)	25.0	m	Vol.(m	25	Dil. Rate	4	**Purity**	99.98
Fresh Std. Wt.(24	25.1	m	Vol.(m	25	Dil. Rate	4	**Purity**	99.98
Fresh Std. Wt.	25.4	m	Vol.(m	25	Dil. Rate	4	**Purity**	99.98
Sample Name	**STD**		**Sample Area**		**Assay**	**Differen**	Acceptance Criteria: Difference in % assay results of initial, 24 hours and 48 hours shall be NMT 2.0 %.	
Precision test Solution-1	856007		846734		99.01	NA		
Precision test Solution-1	861728		848734		99.05	-0.05		
Precision test Solution-1	869231		849736		99.41	-0.40		

Table-14: Solution Stability Linagliptin

METHOD VALIDATION_ASSAY_BENCH TOP STABILITY_LINAGLIPTIN								
Sample Name		Weight of tablets						
Precision test Solution-1(Initial)		2412.34		M/F	1	Ave. Wt.(mg)		241.5
		Strength		Vol.(mL)	250			
		5 mg		Dil. Rate	4			
Std. Wt. (Initial)	25.00	mg	Vol.(mL)	100	Dil. Rate	5	Purity	99.8
Fresh Std. Wt. (24 Hrs.)	25.21	mg	Vol.(mL)	100	Dil. Rate	5	Purity	96.7
Fresh Std. Wt. (48Hrs.)	25.13	mg	Vol.(mL)	100	Dil. Rate	5	Purity	96.7
Sample Name	**STD Area**		**Sample Area**		**Assay (%)**	**Difference**	Acceptance Criteria: Difference in % assay results of initial, 24 hours and 48 hours shall be NMT 2.0 %.	
Precision test Solution-1 (Initial)	680091		693475		101.88	NA		
Precision test Solution-1 (24Hrs.)	691234		695672		101.40	0.48		
Precision test Solution-1 (48Hrs.)	697865		700012		100.74	1.14		

ROBUSTNESS

Performed the robustness by altering the flow rate by ± 0.1mL/min from 1.0 mL/min, column oven temperature by ± 5℃ from 30℃ and buffer pH by ± 0.1 from 2.20.

Prepared the standard solution and checked the system suitability criteria by altering the above mentioned parameters. System Suitability criteria was within the limits for all the altered parameters**.**

Table-15: Change In Flow Rate 0.9ml/Min

ROBUSTNESS_CHANGE IN FLOW RATE_0.9mL/min						
S. No.	**LINAGLIPTIN**			**EMPAGLIFLOZIN**		
			Theoretical			**Theoretical**
1	693428	1.32	10234	867390	1.12	13928
2	689978	1.34	10879	867453	1.14	13478
3	690897	1.34	11009	867391	1.20	13467
4	691789	1.35	10865	868673	1.19	13987
5	692452	1.33	10289	878282	1.18	14007
AVERAGE	**691709**	**1.34**	**10655**	**869838**	**1.17**	**13773**
STDEV	**1338.76**	**0.01**	**364.28**	**4752.03**	**0.03**	**276.24**
% RSD	**0.19**	**0.85**	**3.42**	**0.55**	**2.95**	**2.01**

Table-16: Change in Flow Rate 1.1 mL/min

ROBUSTNESS_CHANGE IN FLOW RATE_1.1 mL/m						
S. No.	**LINAGLIPTIN**			**EMPAGLIFLOZIN**		
			Theoretical			**Theoretical**
1	673456	0.98	9832	846321	1.10	11233
2	679834	0.99	9789	842371	1.11	11223
3	674592	0.98	9921	856324	1.12	12029
4	680021	0.96	9846	860123	1.10	12902
5	681203	1.01	9874	857843	1.11	12129
AVERAGE	677821	0.98	9852	852596	1.11	11903
STDEV	3528.80	0.02	49.12	7778.35	0.01	702.90
% RSD	0.52	1.85	0.50	0.91	0.76	5.91

Table-17: Change in Buffer pH 2.1

ROBUSTNESS_CHANGE IN BUFFER pH_2.1						
S. No.	**LINAGLIPTIN**			**EMPAGLIFLOZIN**		
			Theoretical			**Theoretical**
1	687575	1.12	11897	842345	1.52	14289
2	679867	1.14	11237	850877	1.54	14278
3	680982	1.12	11343	849326	1.53	14330
4	682347	1.15	12009	852340	1.52	14310
5	679999	1.16	11234	853490	1.5	14329
AVERAGE	**682154**	**1.14**	**11544**	**849676**	**1.52**	**14307**
STDEV	**3188.75**	**0.02**	**378.02**	**4386.11**	**0.01**	**23.38**
% RSD	**0.47**	**1.57**	**3.27**	**0.52**	**0.97**	**0.16**

Table-18: Change in Buffer pH 2.3

ROBUSTNESS_CHANGE IN BUFFER pH_2.3						
S. No.	**LINAGLIPTIN**			**EMPAGLIFLOZIN**		
			Theoretical			**Theoretical**
1	689233	1.45	10787	856320	1.51	14567
2	683453	1.45	10983	857642	1.52	14500
3	689345	1.47	10485	860932	1.51	14602
4	689324	1.46	10463	861234	1.50	14678
5	678934	1.44	10347	862759	1.55	14587
AVERAGE	**686058**	**1.45**	**10613**	**859777**	**1.52**	**14587**
STDEV	**4719.35**	**0.01**	**263.07**	**2685.99**	**0.02**	**64.18**
% RSD	**0.69**	**0.78**	**2.48**	**0.31**	**1.27**	**0.44**

Table-19: Change in Column Oven Temperature 25℃

ROBUSTNESS_CHANGE IN COLUMN OVEN TEMPERATURE_25℃						
S. No.	**LINAGLIPTIN**			**EMPAGLIFLOZIN**		
	Area	**Tailing**	**Theoretical Plates**	**Area**	**Tailing**	**Theoretical Plates**
1	689760	1.21	10234	875234	1.61	12897
2	699875	1.23	10783	889340	1.62	12874
3	689340	1.25	10275	887452	1.69	12784
4	699347	1.24	10852	885322	1.68	12783
5	698734	1.24	11002	885609	1.73	12746
AVERAGE	695411	1.23	10629	884591	1.67	12817
STDEV	5367.79	0.02	351.40	5474.09	0.05	65.07
% RSD	0.77	1.23	3.31	0.62	3.02	0.51

Table-20: Change in Column Oven Temperature 35℃

ROBUSTNESS_CHANGE IN COLUMN OVEN TEMPERATURE_35℃						
S. No.	**LINAGLIPTIN**			**EMPAGLIFLOZIN**		
	Area	**Tailing**	**Theoretical Plates**	**Area**	**Tailing**	**Theoretical Plates**
1	667834	1.32	10289	867453	1.60	13800
2	668934	1.34	10378	867453	1.62	13856
3	669768	1.35	10389	863453	1.62	13895
4	668395	1.36	11845	866782	1.64	13876
5	670933	1.34	11892	870231	1.62	13826
AVERAGE	**669173**	**1.34**	**10959**	**867074**	**1.62**	**13851**
STDEV	**1215.21**	**0.01**	**831.69**	**2421.30**	**0.01**	**38.12**
% RSD	**0.18**	**1.11**	**7.59**	**0.28**	**0.87**	**0.28**

FORCED DEGRADATION STUDY

Performed the forced degradation of test method to demonstrate the non-interference of impurities, degradation products in quantification of analyte by various stress conditions like acid, base peroxide and thermal.

Table-21: Forced Degradation Linagliptin & Empagliflozin

S. No.	Stress condition	Linagliptin	Empagliflozin	Acceptance criteria
1	Acid degradation	Passes	Passes	Peak purity shall pass
2	Base degradation	Passes	Passes	
3	Peroxide degradation	Passes	Passes	
4	Thermal degradation	Passes	Passes	

RESULTS AND DISCUSSION

The method was specific as no peaks were observed at the RT's of Empagliflozin & Linagliptin from blank, placebo and impurity spiked solutions. The overall % RSD of method and intermediate precision was below 2 for Empagliflozin (1.19%) & Linagliptin (0.77%) by which we can say that the method is precise. R^2 was 0.995 for empagliflozin and 0.996 for Linagliptin when performed the linearity from 40% to 160 % against the standard concentration. This shows that the method is linear from 100.09 ppm to 400.37ppm for empagliflozin and 20.14 to 80.54 for Linagliptin. Recovery was 101.8, 99.1 & 98.5 for empagliflozin and 101.8, 99.7 & 99.4 for Linagliptin when performed the accuracy at 50 %, 100% and 150 %. These results were within the limits and the method is accurate. Difference in % assay results of initial, 24 hours and 48 hours are well within the limits, so the test solutions are stable up to 24 Hrs. System suitability criteria is met for both the analytes when performed the robustness by altering the flow rate by ± 0.1mL/min from 1.0 mL/min, column oven temperature by ± 5℃ from 30℃ and buffer pH by ± 0.1 from 2.20 by which we can say that the method is robust. Peak purity passes for both the analytes when subjected to acid, base, peroxide and thermal stress conditions, which shows that the method developed is stability indicating.

CONCLUSION

A new RP-HPLC method has been developed for simultaneous estimation of Linagliptin and Empagliflozin in marketed formulation. The method showed good resolution between the two drugs and also with degradants in forced degradation study. The two analyte peaks were well separated from the impurities of Linagliptin and Empagliflozin. The developed method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. It proved to be stability indicating, specific, novel, simple, accurate, precise and cost effective. Hence the proposed RP-HPLC method is suitable for routine assay of Linagliptin and Empagliflozin in pharmaceutical dosage forms in quality control laboratories.

REFERENCES

1. Padmaja, N., & Veerabhadram, G. (2015). Development and validation of analytical method for Simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy. *Der Pharmacia Lettre*, *7*(12), 306-312.
2. Padmaja, N., Babu, M. S., & Veerabhadram, G. (2016). Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs and combined dosage forms. *Der Pharmacia Lettre*, *8*(13), 207-213.

3. Naazneen, S., & Sridevi, A. (2016). Development and validation of stability indicating RP-HPLC method for simultaneous estimation of empagliflozine and linagliptin in tablet formulation. *Der Pharmacia Lettre*, *8*(17), 57-65.
4. Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology Q2 (R1). In *International Conference on Harmonization, Geneva, Switzerland* (pp. 11-12).