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Development and validation of stability indicating RP-HPLC method for simultaneous estimation of empagliflozine and linagliptin in tablet formulation

S. Naazneen[1*] and A. Sridevi[2]

[1]*Assistant Professor, St Mary's College of Pharmacy, Secunderabad, Andhra Pradesh, India*
[2]*Assosciate Professor, IPT, Sri Padmawathi Mahila VishwaVidyalayam, Tirupathi, Andhra Pradesh, India*

ABSTRACT

A stability indicating high Performance Liquid Chromatographic (HPLC) method was developed and validated for the estimation of combined tablet formulation of Empagliflozine and Linagliptin. Chromatographic separation was optimized by isocratic HPLC on a C18 column [BDS 250mm x 4.6 mm, 5µ.] utilizing a mobile phase consisting a mixture of 0.1% Perchloric acid and Acetonitrile in the ratio of 60:40 v/v at a flow rate of 1ml/min with UV detection at 230nm. The retention time of Empagliflozine and Linagliptin was 2.05 min and 4.10 min respectively. Good linearity obtained over the range of 12.5µg/ml to 75µg/ml for Empagliflozine and Linagliptin. Correlation coefficient was found to be 0.999&0.999 for Empagliflozine& Linagliptin respectively. The % RSD of precision for Empagliflozine and Linagliptin was found to be 0.33and 0.22respectively. The % mean recovery was found to be 100.96-101.48% for Empagliflozine and 100.09-101.13% for Linagliptin. The results obtained for accuracy, precision, LOD, LOQ and Ruggedness were within the limits. Thus the validated economical method was applied for forced degradation study of Empagliflozine and Linagliptin tablet.

Keywords: Empagliflozine and Linagliptin, stress study, HPLC method

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INTRODUCTION

The combination of linagliptin and Empagliflozin is available as tablets formulation for oral use in the management of type 2 diabetes. Empagliflozin is an orally-active inhibitor of the sodium-glucose co-transporter (SGLT2).The chemical name of empagliflozin is D-Glucitol,1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro- 3-furanyl]oxy]phenyl]methyl]phenyl]-,(1S). Linagliptin is an orally-active inhibitor of the dipeptidyl peptidase-4 (DPP-4) enzyme.The chemical name of linagliptin is 1H-Purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1- yl)-3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl)methyl] [1-5]. Several HPLC methods are rported for estimation of linagliptin and empagliflozine individually or in combination with other drugs from pharmaceutical dosage forms and in human plasma [6-10]. The stability indicating HPLC and HPTLC methods are reported for estimation of lingliptin individually or in combination with other agents[11-14]. One UPLC method was reported for simultaneous determination of empagliflozin, linagliptin and metformin[15]. However, no stability indicating method is reported for simultaneous determination of linagliptin and empagliflozin in pharmaceutical dosage form by RP-HPLC in any literature. In the present investigation, a specific stability indicating RP-HPLC method is described for the simultaneous determination of linagliptin and metformin drugs.

MATERIALS AND METHODS

Chemicals and reagents
HPLC grade ,acetonitrile and analytical grade perchloric acid were purchased from Merck (Mumbai, India). Linagliptin working standard was obtained as a gift sample from Natco Pharma Ltd., Hyderabad., and Empagliflozine working standard from Hetero drugs Ltd, Hyderabad, India.

Instrumentation:
The HPLC system consisted of Alliance waters 2695 with dual λ Absorbance UV detector. HPLC column BDS 250mm x 4.6 mm, 5µ. Mobile phase filtration unit (Pall Life sciences, Mumbai, India), LAB-INDIA U.V with UV Win software, Sonicator, P^H meter (LAB-INDIA), digital balance (Denver).

Preparation of standard solutions
Standard Preparation: (50µg/ml Linagliptin & 100µg/ml Empagliflozine)
Accurately Weighed and transferred 12.5mg&25mg of Linagliptin and Empagliflozine working Standards into a 25ml and 25ml clean dry volumetric flask respectively, add 20ml and 20ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

RESULTS AND DISCUSSION

Chromatographic Conditions:
Chromatographic Conditions: The HPLC system consisted of Alliance waters 2695 with dual λ Absorbance UV detector. The wavelength of detection as set at 230nm. Separation was carried out in isocratic mode on BDS C18 column (4.6x250mmx5µm) and the retention time of Empagliflozine and Linagliptin was found to be 2.05 min and 4.10 min respectively. (figure 1), using mobile phase consisting a a mixture of 0.1% Perchloric acid and Acetonitrile in the ratio of 60:40 v/v at a flow rate of 1ml/min with UV detection at 230nm. The mobile phase filtered through nylon milli pore (0.2µm) membrane filter, purchased from pall life sciences, Mumbai and degassed with Ultra sonicator prior to use. Chromatography was carried out at room temperature 25°C and maintains the column temperature at 30°C.

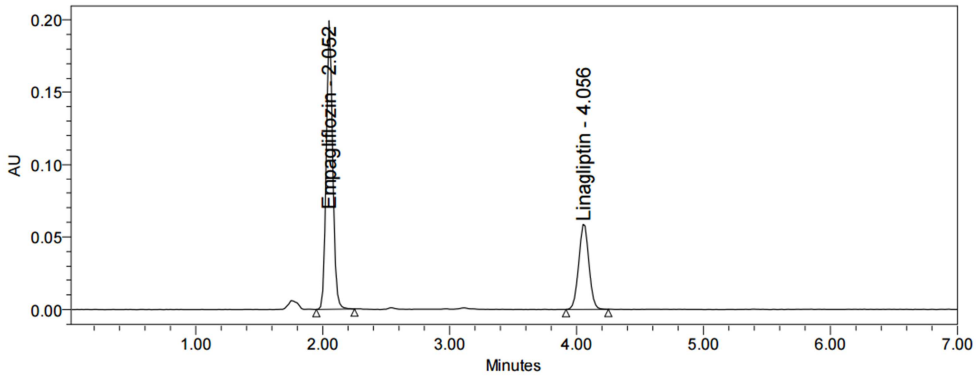

Figure 1: Chromatogram Showing Empagliflozine and Linagliptin

METHOD VALIDATION:
The developed Method was validated for linearity, precision, accuracy, ruggedness and is applied for forced degradation studies as per the ICH guidelines [16-17].

Linearity:
Linear concentrations of both drugs were prepared and the best fit line was calculated. Wide range calibration was determined by solutions containing 25µg/ml to 150µg/ml for Empagliflozine and 12.5µg/ml to 75µg/ml Linagliptin. Correlation coefficient was found to be 0.999&0.999 for Empagliflozine & Linagliptin respectively (fig 2&3).

Table 1: Linearity data for Empagliflozine& Linagliptin

S.no	Empagliflozine		Linagliptin	
	Concentration(µg/ml)	Peak area	Concentration(µg/ml)	Peak area
1	25	456801	12.5	235592
2	50	950080	25	445338
3	75	1490729	37.5	677762
4	100	1966013	50	890059
5	125	2397570	62.5	1092101
6	150	2863491	75	1353505

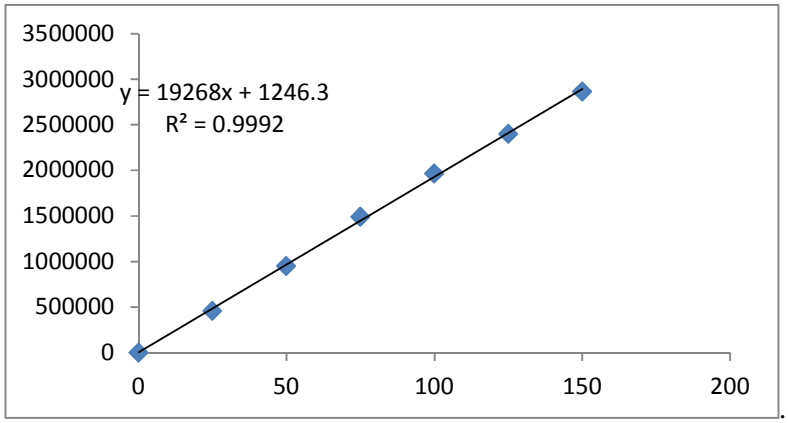

Figure 2: Showing calibration curve of Empagliflozine

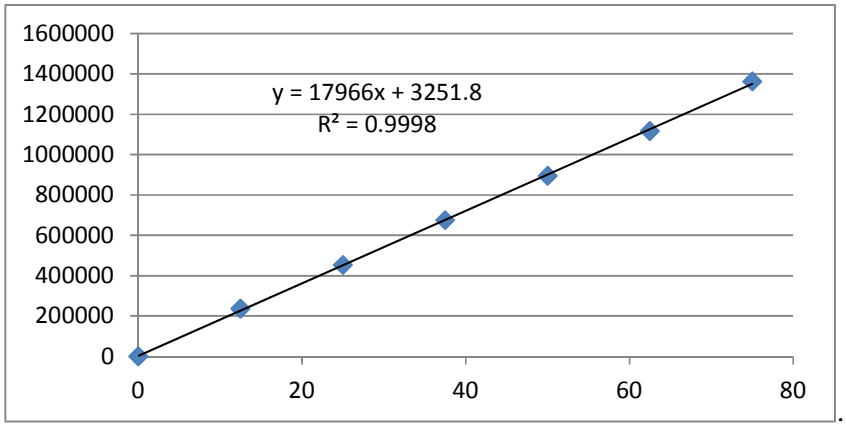

Figure 3: Showing calibration curve of Linagliptin

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

The LOD is calculated using the formula 3.3 times σ/s where "σ" is standard deviation of the intercept obtained for calibration curve and "s" is the slope of the calibration curve. Similarly LOQ is calculated using the formula 10 times σ/s. The calculated LOD and LOQ are shown in table 2 &3

Table 2: Showing LOD & LOQ Results of Empagliflozine

Conc (µg/ml)	Area 1	Area 2	Area 3	Avg Area
25	459227	452382	458793	456801
50	952951	947693	949595	950080
75	1493832	1487160	1491194	1490729
100	1993278	1968338	1936422	1966013
125	2408715	2391128	2392867	2397570
150	2876214	2850396	2863864	2863491
Intercept	1179	1443	1115	1246
slope	19383	19213	19209	19268
Intercept Standard Deviation				173.9
LOD (µg/ml)				**0.03**
LOQ(µg/ml)				**0.09**

Table 3: Showing LOD & LOQ Results of Linagliptin

Conc (µg/ml)	Area 1	Area 2	Area 3	Avg Area
12.5	235592	237271	235362	236075
25	445338	457617	457576	453510
37.5	677762	679083	670439	675761
50	890059	894655	899345	894686
62.5	1092101	1132198	1127290	1117196
75	1353505	1357424	1373638	1361522
Intercept	4380	4839	535.1	3251
Slope	17766	17998	18133	17966
Intercept Standard Deviation				**2363.5**
LOD (µg/ml)				**0.43**
LOQ(µg/ml)				**1.32**

Precision:

The intraday precision was demonstrated by injecting standard solutions of Empagliflozine and Linagliptin with 100 µg/ml and 50µg/ml respectively as per the test procedure (Table 4) & recording the chromatograms of six standard solutioins. The % RSD of Empagliflozine and Linagliptin was found to be 0.24 and 0.1 respectively.

Table 4: Method Precision data of Empagliflozine and Linagliptin

Empagliflozine (100µg/ml)		**Linagliptin (50µg/ml)**
S.No	**Area**	**Area**
1	1919332	876871
2	1927659	876313
3	1923605	877356
4	1923801	878790
5	1919096	875829
6	1930615	878666
Mean	1924018	877304
SD	4543	1217
%RSD	0.24	0.1

Intermediate Precision:

Intermediate precision of the analytical method was determined by performing method precision on in three successive days by different analysts under same experimental condition by injecting six replicate standards preparations was determined and the mean % RSD of Empagliflozine(100µg/ml) and Linagliptin (50µg/ml) was found to be 0.33 and 0.22 respectively (Table 5).

Table 5: Precision Data for Empagliflozine& Linagliptin

Empagliflozine Area for 100µg/ml					Linagliptin Area for 50µg/ml			
S.No	**Day-1**	**Day-2**	**Day-3**	**Avg**	**Day-1**	**Day-2**	**Day-3**	**Avg**
1	1919332	1925927	1913079	1919446	876871	881647	875509	878009
2	1927659	1939848	1927659	1931722	876313	871857	876418	874862.7
3	1923605	1928785	1923605	1925332	877356	879224	876938	877839.3
4	1923801	1923727	1923801	1923776	878790	881018	877241	879016.3
5	1919096	1913246	1911305	1914549	875829	889705	876136	880556.7
6	1930615	1929975	1929104	1929898	878666	882459	878210	879778.3
Mean	1924018	1926918	1921426	1924121	877304	880985	876742	878343.7
SD	4543	8696	7487	6422.028	1217	5745.8	942	1994.584
%RSD	0.24	0.45	0.39	0.333764	0.1	0.7	0.1	0.227085

Accuracy:

Accuracy of the method was established by performing recovery studies according to the ICH guidelines. Spiked samples were prepared by spiking pre-analyzed sample solutions with pure drug at three different concentration levels each in triplicate. Mean percentage recovery values at three different concentrations of the two drugs was calculated. The % mean recovery of Empagliflozine(100.96-101.48%) & Linagliptin (100.09-101.13.%) at each level was within the limits of 98% and 102% (Table 6)

Table-6: Accuracy of Empagliflozine & Linagliptin

Accuracy of Empagliflozine						
S.N0.	**Conc.**	**Calculated Concn.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1	50	50.46284	100.93			
2	50	51.03815	102.08	101.48	0.57	0.56
3	50	50.72348	101.45			
1	100	101.5953	101.60			
2	100	101.3888	101.39	100.96	0.93	0.92
3	100	99.8865	99.89			
1	150	153.9187	102.61			
2	150	150.8636	100.58	101.44	1.05	1.03
3	150	151.69	101.13			
Accuracy of Linagliptin						
S.N0.	**Conc.**	**Calculated concn.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1	25	24.91829	99.67			
2	25	25.42586	101.70	100.33	1.19	1.18
3	25	24.90076	99.60			
1	50	49.91723	99.83			
2	50	50.03317	100.07	100.09	0.27	0.27
3	50	50.18813	100.38			
1	75	75.71852	100.96			
2	75	76.69787	102.26	101.13	1.05	1.04
3	75	75.13693	100.18			

Ruggedness:

The ruggedness of method for Empagliflozine(150µg/ml) and Linagliptin(75µg/ml) was calculated with six injections of in two batches using two different columns. The % CV of ruggedness for Empagliflozine was 0.24 with column-1 and 0.04 with column-2 and the % CV of ruggedness for Linagliptin was 0.1 with column-1 and 0.07 with column-2 (Table-7), which is within acceptance limits

Table 7: Showing the results of Ruggedness

	Empagliflozine 150µg/ml		Linagliptin 75µg/ml	
S.NO	**Column 1**	**Column 2**	**Column 1**	**Column 2**
1	148.06	148.12	74.18	74.14
2	149.06	148.2	74.21	74.01
3	148.34	148.09	74.14	74.04
4	148.54	148.22	74.02	74.02
5	148.15	148.11	74.15	74.09
6	148.55	148.24	74.04	74.11
Mean	148.45	148.1633	74.1233	74.0683
± SD	0.359	0.064083	0.07659	0.05269
% CV	0.24183	0.043252	0.10333	0.07114
% Accuracy	98.9667	98.77556	98.8311	98.7578

Results of Stress Degradation Studies:

Stress degradation studies were performed as per the ICH guidelinesQ1A (R2) Stability Testing of New Drug Substances and Products, using the proposed validated analytical method.(Table 10&11)

Acid Degradation studies:

To 1ml of stock solution Empagliflozine and Linagliptin, 1ml of 2N HCl was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 2)

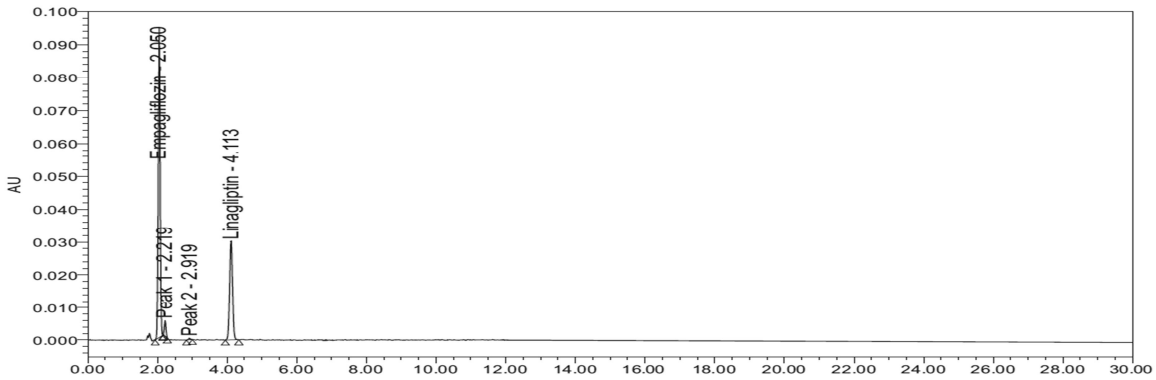

Figure 4: Showing Chromatogram of Acid Degradation

Alkali Degradation Studies:

To 1ml of stock solution of of standard drug and sample Empagliflozine and Linagliptin, 1ml of 2N NaOH was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 5)

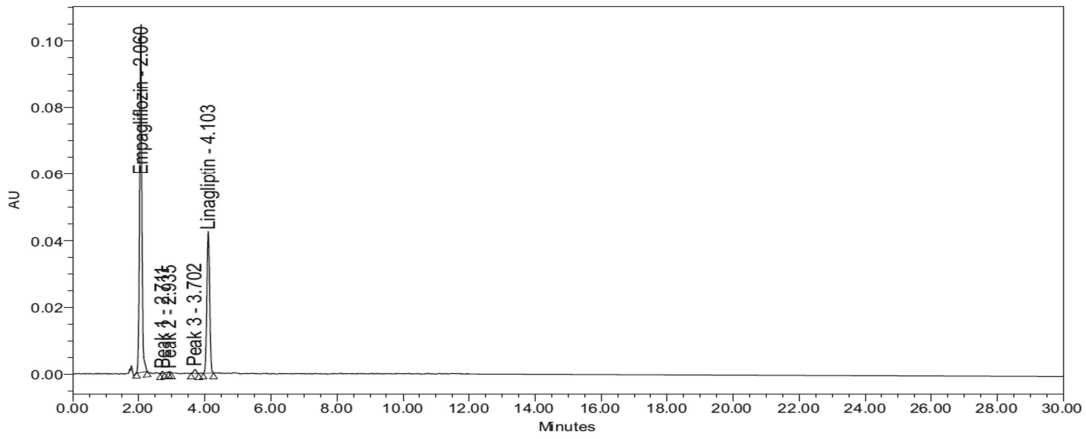

Figure 5: Showing Chromatogram of Base Degradation

Oxidative Degradation:

To 1ml of stock solution of standard drug and sample of Empagliflozineand Linagliptin, 1ml of 20% $H_2O_2$was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 6)

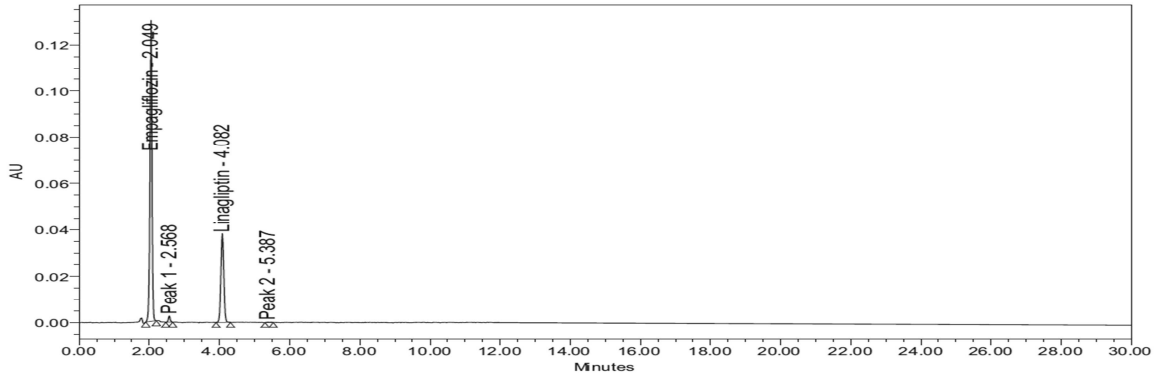

Figure 6: Showing Chromatogram of Oxidative Degradation

Photo Stability Studies:

The photochemical stability of the drug was also studied by exposing the 50 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber . For HPLC study, from the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Figure 7)

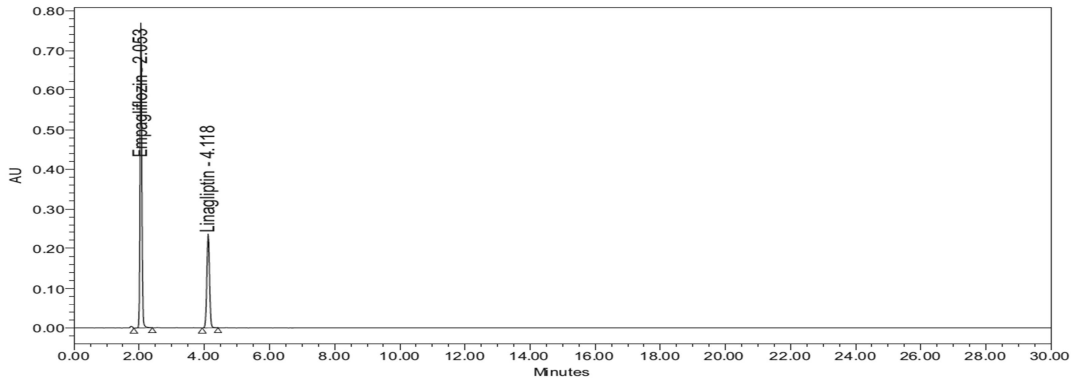

Figure 7: Showing Chromatogram of UV Degradation

Thermal degradation studies:

The 1ml of stock solution of standard drug and sample of Empagliflozine and Linagliptin was exposed to temperature 105^{o}C for 24hrs for HPLC study, from the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample.(Figure: 8)

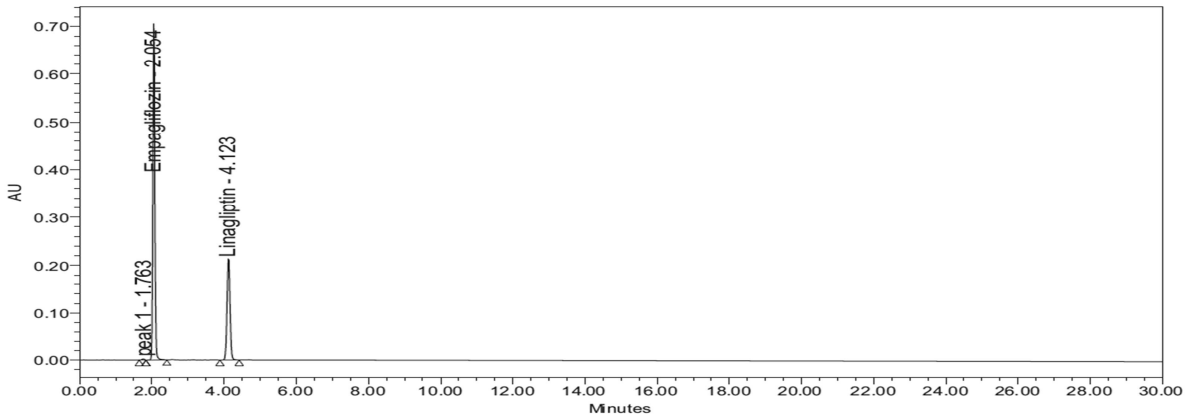

Figure 8: Showing Chromatogram of Thermal Degradation Study

Table 10: Results of stress degradation studies of Empagliflozine

Sno	Stress conditions	Time	% Assay	% Degradation	Purity angle	Purity threshold
1	Acid Degradation	30 min	97.07	2.93	0.95	1.2
2	Base Degradation	30 min	98.20	1.80	0.5	0.8
3	Peroxide Degradation	30 min	96.50	3.50	0.77	0.9
4	UV Degradation	7 days	99.73	0.27	0.14	0.3
5	Thermal Degradation	24hrs	99.72	0.28	0.16	0.3

Table 11: Results of stress degradation studies of Linagliptin

Sno	Stress conditions	Time	% Assay	% Degradation	Purity angle	Purity threshold
1	Acid Degradation	30 min	96.89	3.11	0.405	0.542
2	Base Degradation	30 min	98.02	1.98	0.39	0.46
3	Peroxide Degradation	30 min	95.14	4.86	0.03	0.48
4	UV Degradation	7 days	99.36	0.64	0.44	0.62
5	Thermal degradation	24hrs	99.23	0.77	0.45	0.54

Empagliflozine and Linagliptin undergoes significant degradation in acidic, oxidation and alkaline Comparatively More degradation was found with peroxide for Linagliptin and Empagliflozine. As per ICH guidelines peak purity angle should be less than peak purity threshold. Hence, method of the analysis of Empagliflozineand Linagliptin in tablet dosage form shows that the degradation product doesn't interfere with the analytical determination. hence the proposed analytical method is also useful for the determination of Empagliflozineand Linagliptin stability in sample of pharmaceutical dosage form.

CONCLUSION

A simple, precise, accurate, robust & cost-effective method was developed for the routine analysis. The method was successfully validated in terms of linearity, precision, accuracy as per ICH guidelines. The method provides a linear response across a wide range of concentrations. Moreover, the method is fast with respect to analysis time compared to sophisticated chromatographic techniques. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results & found to be suitable for the routine analysis and quality control and percentage degradation of pharmaceutical preparations containing these drugs either individually or in combination.

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