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Stability Indicating RP-HPLC Method for Simultaneous Determination of Diabetes Mellitus in Tablet Dosage Form

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Linagliptin and Empagliflozin in Tablet dosage form. The retention time of Linagliptin and Empagliflozin was found to be 1.920 min and 3.699 min. %RSD of the Linagliptin and Empagliflozin was and found to be 1.0 and 0.94 respectively. % assay was obtained as 100.63% and 100.20% for Linagliptin and Empagliflozin respectively. LOD, LOQ values are obtained from regression equations of Linagliptin and Empagliflozin were 0.24ppm, 0.72ppm and 0.17ppm, 0.51ppm respectively. Regression equation of Linagliptin and Empagliflozin is y = 9531.x + 4618, and y = 37150x + 745.2 Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION

Pharmaceutical Analysis is the core branch of pharmacy education and research, which is advancing very fast. It can be categorized as the synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making a quantitative and qualitative evaluation. In practice, quantifying an analyte in a complex sample becomes an exercise in problem resolving. To be efficient and effective, the analytical chemist must know the tools that are available to tackle a wide variety of problems [1,2]. Analytical chemistry is divided into two branches qualitative and quantitative. In this qualitative method provides information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method provides numerical information as to the relative amount of one or more of the components. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations, and bioanalytical samples. In the pharma industry, spectrophotometric and chromatographic methods have gained significance in recent studies. Spectrophotometric methods [2-6] is defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. Their electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into two main types,

DRUG PROFILE

Linagliptin[8]

Linagliptin is a DPP-4 inhibitor developed by Boehringer Ingelheim for the treatment of type II diabetes [Wikipedia]. Two pharmacological characteristics that set linagliptin apart from other DPP-4 inhibitors is that it has a non-linear pharmacokinetic profile and is not primarily eliminated by the renal system.

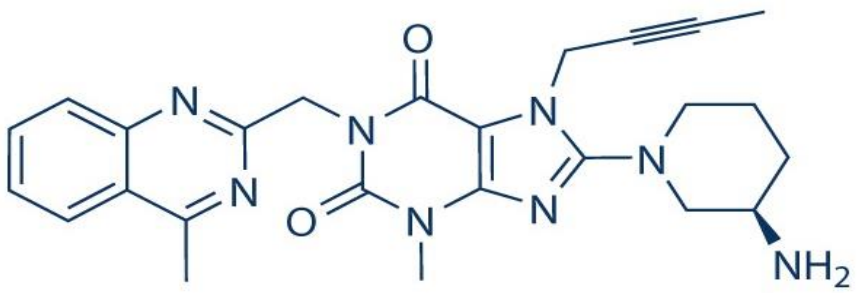

Figure No: 1 Structure of Linagliptin

Appearance: Solid

Solubility : Soluble in DMSO (17 mg/ml at 25 °C), water (<1 mg/ml at 25 °C), ethanol (1 mg/ml at 25 °C), 0.5% hydroxyethyl cellulose (30 mg/ml at 25 °C), and methanol.

Molecular Weight: 472.54

Molecular Formula: $C_{25}H_{28}N_8O_2$

IUPAC Name: 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methyl quinazoline-2-yl) methyl]-2, 3, 6, 7-tetrahydro-1H-purine-2, 6-Dione

Empagliflozin[9]

Empagliflozin is a sodium-glucose co-transporter-2 (SGLT-2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes. SGLT2 co-transporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore resulting in increased glucose excretion. Additionally, it contributes to reduced hyperglycemia and also assists in weight loss and blood pressure reduction.

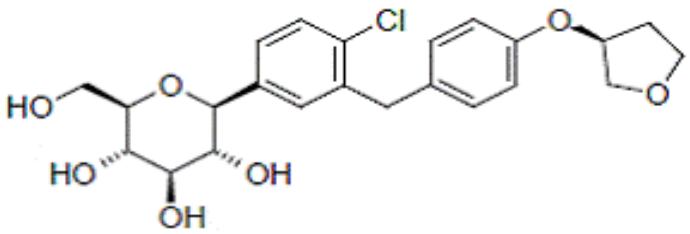

Figure No: 2 Structure of Empagliflozin

Appearance: Empagliflozin is a white to yellowish, non-hygroscopic powder.

Solubility: It is very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol and acetonitrile; soluble in 50% acetonitrile/water; and practically insoluble in toluene.

Weight Average: 450.91

Chemical Formula: $C_{23}H_{27}ClO_7$

IUPAC Name: (2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy]phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol

Indication: Empagliflozin is indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes.

1. System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Table No. 1: System suitability studies of Linagliptin and Empagliflozine method

Property	Linagliptin	Empagliflozin
Retention time (t_R)	1.920min	3.690min
Theoretical plates (N)	7217 ± 63.48	8554 ± 63.48
Tailing factor (T)	1.08 ± 0.117	1.22± 0.117

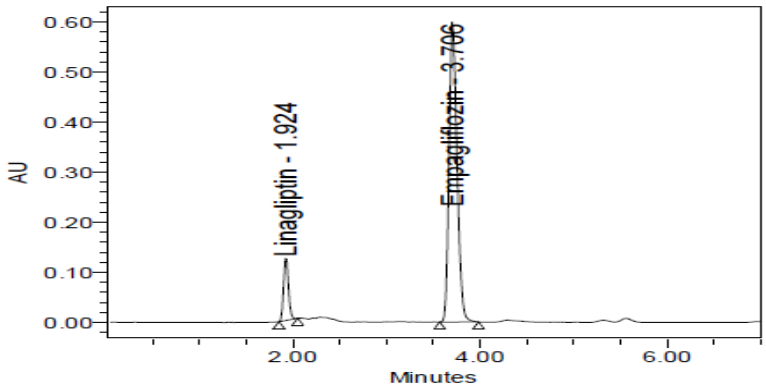

Figure No: 3 Typical chromatogram of Linagliptin and Empagliflozin.

2. Linearity: Six Linear concentrations of Linagliptin (12.5-75µg/ml) and Empagliflozin (25-150 µg/ml) are prepared and Injected. Regression equation of the Linagliptin and Empagliflozin are found to be, y = 9531.x + 4618, and y = 37150x + 745.2. And regression co-efficient was 0.999.

Table No: 2 Calibration data of Linagliptin and Empagliflozin method.

S. No.	Concentration Linagliptin	Response	Concentration Empagliflozin	Response
1	0	0	0	0
2	25%	126420	25%	905911
3	50%	245671	50%	1934386
4	75%	367825	75%	2778580
5	100%	477424	100%	3645445
6	125%	593849	125%	4628418
7	150%	723119	150%	5616375

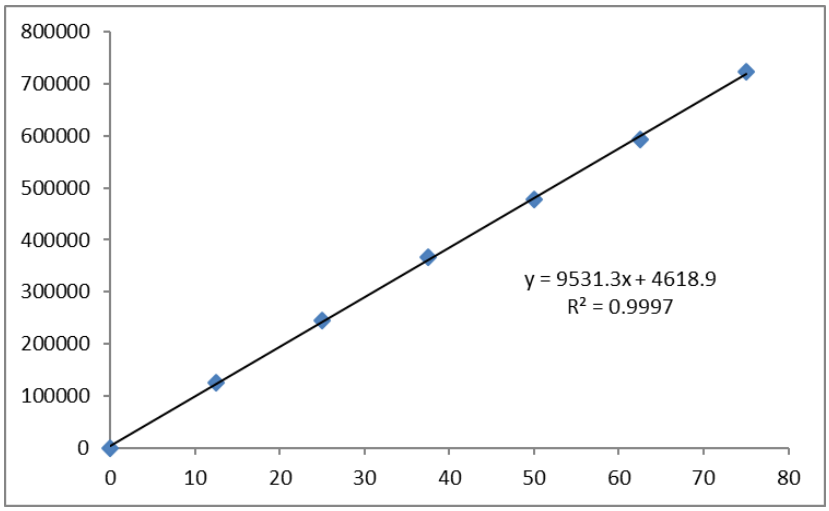

Figure no: 4 Calibration curve of Linagliptin

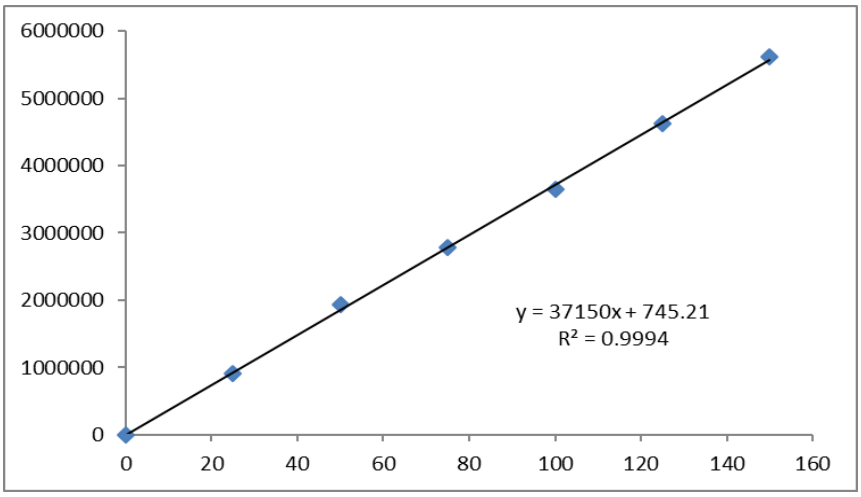

Figure No. 5: Calibration curve of Empagliflozin

3. Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Linagliptin and Empagliflozin were found to be 1.0% and 0.94% respectively.

Table No. 3: Repeatability results for Linagliptin and Empagliflozin.

Sr. No.	Linagliptin	Empagliflozin
1	462762	3598346
2	463892	3598711
3	467252	3587845
4	469414	3565861
5	473880	3656485
6	472737	3563766
Mean	468323	3595169
Std. Dev.	4544.4	33708
%RSD	1.0	0.94

*Average of six determinations

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Linagliptin and Empagliflozin were 0.60% and 0.43%.

Table No. 4: Inter day precision results for Linagliptin and Empagliflozin.

Sr. No.	Linagliptin	Empagliflozin
1	457175	3611613
2	457484	3572397
3	459261	3587699
4	464175	3598528
5	459196	3574390
Mean	459155	3590575
Std. Dev.	2616	15326
%RSD	0.6	0.43

4. Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and the amount Recovered and % Recovery was displayed in Table 5.

Table No. 5: Table of Accuracy

Sample	Concentration (%) (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Linagliptin	25	25.28	101.11	0.94
	50	49.89	99.79	0.54
	75	75.15	100.20	0.25
Empagliflozin	50	50.56	101.12	0.57
	100	100.58	100.58	0.85
	150	151.06	100.71	0.34

5. LOD: Limit of detection was calculated by the std deviation method Linagliptin and Empagliflozin and LOD for Linagliptin and Empagliflozin were found to be 0.24and 0.17respectively.

6. LOQ: Limit of Quantification was calculated by std deviation method Linagliptin and Empagliflozin and LOQ for Linagliptin and Empagliflozin were found to be 0.72 and 0.51 respectively.

7. Robustness: Small deliberate changes in a method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized changes in the result and are within range as per ICH Guidelines.

Table No. 6: Robustness data of Linagliptin and Empagliflozin

Sr. No.	Robustness condition	Linagliptin % RSD	Empagliflozin % RSD
1	Flow minus	1.34	0.65
2	Flow Plus	0.75	0.20
3	Mobile phase minus	0.23	0.26
4	Mobile phase Plus	0.02	0.15
5	Temperature minus	0.94	0.66
6	Temperature Plus	1.36	0.06

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both samples and standards are injected into six homogeneous samples. The drug in the formulation was estimated by taking the standard as the reference. The Average

%Assay was calculated and found to be 100.63% and 100.20% for Linagliptin and Empagliflozin respectively.

Table No. 7: Assay of Tablet

S. No.	Linagliptin %Assay	Empagliflozin %Assay
1	99.44	100.29
2	99.68	100.30
3	100.40	100.00
4	100.87	99.38
5	101.83	101.91
6	101.58	99.33
AVG	100.63	100.20
STDEV	0.9765	0.9395
% RSD	.0	0.94

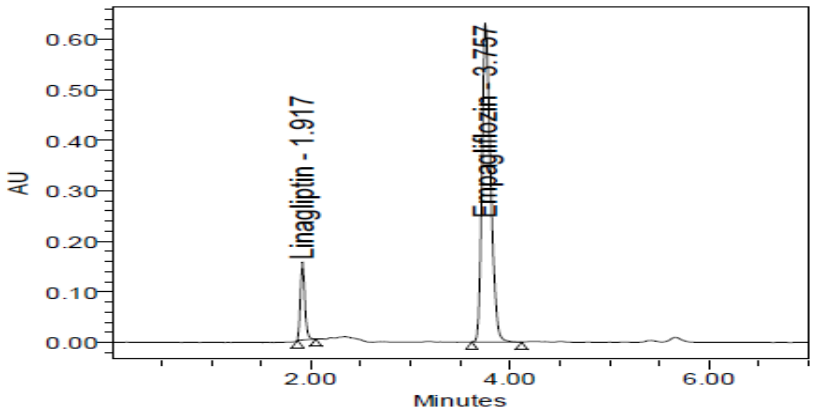

Figure No. 6: Assay of Tablet

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table No. 8: Degradation Data of LINAGLIPTIN

S. No.	Degradation Condition	Area	% Asaay	Amount Degraded %
1	Acid	452252	97.18	2.82
2	Alkali	459880	98.82	1.18
3	Oxidation	449176	96.52	3.48
4	Thermal	462236	99.32	0.68
5	UV	461745	99.22	0.78
6	Water	463578	99.61	0.39

Table No. 9: Degradation Data of EMPAGLIFLOZIN

S. No.	Degradation Condition	Area	%Assay	Amount Degraded %
1	Acid	3503766	97.65	2.35
2	Alkali	3528711	98.35	1.65
3	Oxidation	3486053	97.16	2.84
4	Thermal	3561306	99.26	0.74
5	UV	3551594	98.99	1.01
6	Water	3564912	99.36	0.64

SUMMARY AND CONCLUSION

Table No. 10: Summary

Parameters	Linagliptin	Empagliflozin
Calibration range (mcg / ml)	12.5-75 ppm	25-150 ppm
Optimized wavelength	210nm	210nm
Retention time	1.920min	3.699 min
Regression equation (Y*)	y = 9531.x + 4618.	y = 37150x + 745.2
Correlation coefficient (r^2)	0.999	0.999
Precision (% RSD*)	1.0	0.94
% Assay	100.63%	100.20%
Limit of Detection (mcg / ml)	0.24ppm	0.17ppm
Limit of Quantization (mcg / ml)	0.72ppm	0.51ppm

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Linagliptin and Empagliflozin in Tablet dosage form. The retention time of Linagliptin and Empagliflozin was found to be 1.920min and 3.699 min. %RSD of the Linagliptin and Empagliflozin was and found to be 1.0 and 0.94 respectively. %assay was obtained as 100.63% and 100.20% for Linagliptin and Empagliflozin respectively. LOD, LOQ values are obtained from regression equations of Linagliptin and Empagliflozin were 0.24ppm, 0.72ppm and 0.17ppm, 0.51ppm respectively. Regression equation of Linagliptin and Empagliflozin is y = 9531.x + 4618, and y = 37150x + 745.2 Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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