Research Article

Analytical Method Development and Validation for Simultaneous Estimation of Empagliflozin and Linagliptin in Bulk Drug and in Pharmaceutical Dosage Formulation by HPLC

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ABSTRACT

The purpose of this study was to develop a novel, sensitive and accurate analytical method for the for simultaneous estimation of empagliflozin and linagliptin in bulk drug and in pharmaceutical dosage formulation by HPLC Separation was achieved on waters acquity C18 (100 X 2.1mm id) by using methanol and water in the ratio of 60:40 eluted at a flow rate of 0.5ml/min. The retention time of Empagliflozin and Linagliptin was found to 1.320 and 2.343 mins. Quantification was achieved with photo diode array detector at the wavelength of 270nm. The developed method was validated as per the international conference on harmonization (ICH) guidelines. Based on the peak area with the linear calibration curve at the concentration of 50-150 µg/ml for Empagliflozin and 25-75 µg/ml for Linagliptin % recovery was found to be 99.2% and 99.47% for Empagliflozin and Linagliptin respectively. The LOD were found to be 0.24 µg/ml and 0.734 µg/ml. and LOQ was found to be 0.090 µg/ml and 0.701 µg/ml for Empagliflozin and Linagliptin. The method was found to be specific and cab be employed for the routine quality control analysis of both the drugs individually and in combined dosage form.

Keywords: Method development, Empagliflozin, Linagliptin, analytical validation.

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INTRODUCTION

In the current Indian scenario, most commonly attacking disease to a common man has been found to be diabetes. Recent studies indicate that prevalence of type-2 diabetes is rapidly increasing in the society. Type-2 diabetes is a progressive disorder with a consistent and steady increase in glycosylated hemoglobin (HbA1C) overtime associated with enhanced risk of micro- and macrovascular complications and a substantial reduction in life expectancy. There are three major pathophysiologic abnormalities associated with type-2 diabetes: (i) impaired insulin secretion, (ii) excessive hepatic glucose output, and (iii) insulin resistance in skeletal muscles, liver and adipose tissue. These defects have been treated by use of oral insulin secretagogues (sulphonyl ureas/glinides) or insulin, biguanides, and thiazolidinediones, respectively.

A combination of linagliptin and empagliflozin is used and marketed as tablets for oral use for the treatment of type 2 diabetes and cardiovascular risk. Empagliflozin (EMPA) is used as a sodium glucose cotransporter-2 (SGLT-2) inhibitor to improve blood glucose control in adult patients with type 2 diabetes. SGLT-2 co-transporters reabsorb glucose from the glomerular filtrate and the renal glucoretic substance action due to SGLT-2 inhibition, which reduces renal absorption and reduces renal glucose threshold, which increases glucose secretion, which decreases hyperglycemia and also helps lower blood pressure. Linagliptin (LINA) is a competitive, reversible DPP-4 inhibitor responsible for reducing the degradation of GLP-1 and the glucose-dependent insulinotropic polypeptide (GIP). GLP-1 and GIP stimulate insulin release from pancreatic beta cells inhibition of glucagon release from pancreatic beta cells. Together, these effects are reduced breakdown of glycogen in the liver and increases insulin release in response to glucose[1].

Keeping the medical importance in mind, a group of drugs used for treating/maintaining diabetes, namely, Empagliflozin and Linagliptin has been selected for method development and validation. The two drugs are antidiabetic drugs. These drugs are very potent and are normally prescribed either individually or in combinations as per the demand of the situation. These two drugs are also available in the market as a combination, dosage forms.

Empagliflozin[3]

Empagliflozin is an inhibitor of sodium-glucose co-transporter-2 (SGLT2), the transporters primarily responsible for the reabsorption of glucose in the kidney. It is used clinically as an adjunct to diet and exercise, often in combination with other drug therapies, for the management of type 2 diabetes mellitus.

Linagliptin[4]

Linagliptin is a DPP-4 inhibitor developed by Boehringer Ingelheim for the treatment of type II diabetes. Linagliptin differs from other DPP-4 inhibitors in that it has a non-

linear pharmacokinetic profile, is not primarily eliminated by the renal system, and obeys concentration dependant protein binding. Linagliptin was approved by the FDA on May 2, 2011[2].

A detailed literature survey revealed that few analytical methods were reported for analysis of simultaneous quantification of empagliflozin, linagliptin, metformin HCL in bulk and synthetic mixtures by RP-LC method, However only a few RP-HPLC methods were reported for the simultaneous estimation of Empagliflozin and Linagliptin and thus there is a need to develop rapid, sensitive and cost effective method for the simultaneous estimation of Empagliflozin and Linagliptin in fixed dosage combinations. Hence the present study was aimed to develop a simple, sensitive and a precise RP-HPLC method for the simultaneous determination of Empagliflozin and Linagliptin in their bulk and combined dosage form.[7-14]

MATERIALS AND METHODS

Chemicals and reagents

The reference standards of Empagliflozin and Linagliptin (more than 99 % purity) were procured from Madras Pharmaceuticals, Chennai and the tablet dosage forms were purchased from the local pharmacy. HPLC grade acetonitrile and water were purchased from Bhiwandi, Maharashtra and analytical grade chemicals such as potassium dihydrogen ortho phosphate, orthophosphoric acid (OPA), were purchased from E. Merck Limited, Mumbai.

Method development

Apparatus and instrumentation

An Agilent technologies model 1290 infinity series HPLC equipped with quaternary pumps and photodiode array (PDA) detector was employed in this study. The output signal was monitored and integrated by SHIMADZU, model-LC 2010CHT, LC solutions of HPLC- software. SHIMADZU electrical balance. PH meter- thermo, ultra sonicator, citizen digital ultrasonic cleaner along with UV visible spectrophotometer of thermos technology was used.

Chromatographic conditions

Separation was performed using water acquity C 18(100X2.1MM id) 1.8µ as a column with mobile phase methanol:water (60:40 %v/v). The samples were analyzed using 20 µl injection volume, maintaining the flow rate at 0.5ml/min with a runtime of 5 min, and the temperature was maintained at ambient conditions. Detection and purity establishment of the drugs was achieved using a PDA detector at 260 nm wavelength.

Mobile phase

Mobile phase used for the optimized trial was methanol and water in the ration of 60:40

Preparation of standard solution

About 100 mg of EMPAFLIFLOZIN and 50 mg of LINAGLIPTIN were weighed onto 100 ml volumetric flask, to this 70 ml of mobile phase was added, sonicated and the volume was made up with mobile phase. Pipette 5 ml of clear solution into 50 ml volumetric flask and make up the volume with mobile phase. (100 µg/ ml and 50 µg/ ml of Empagliflozin and Linagliptin respectively.)

Preparation of sample solutions

Crush more than 20 tablets then weigh a quantity of powder equivalent to 100mg of EMPAGLIFLOZIN and 50 mg of LINAGLIPTIN in 100 ml volumetric flask add 70 ml of mobile phase. Pipette 5 ml of the clear solution in 50 ml in a volumetric flask and makeup volume with mobile phase. Filter the solution through 0.45 mu m filter paper. The resulting solution is used to record the chromatogram.

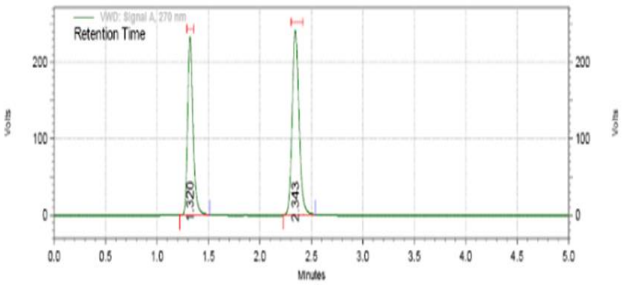

Figure 1: Optimized chromatogram of the proposed method

RESULTS AND DISCUSSIONS

Specificity

Capacity of the method to measure the analyte peak response in the presence of other components is termed as specificity. The specificity of the method was evaluated after performing the analysis of blank solution, and placebo solution to examine the blank chromatogram for any interfering peaks within the retention time of the analyte peaks. The chromatogram for the blank is given in the figure 2.

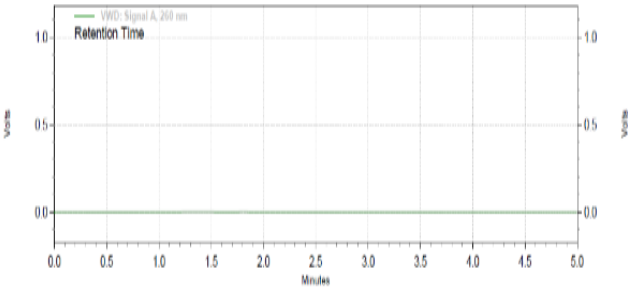

Figure 2: Chromatogram of Blank

System Suitability and System Precision

The system suitability test was carried out by performing six replicate injections of a working standard solution containing 100 µg/ ml and 50 µg/ ml of empagliflozin and linagliptin respectively. The results for system suitability of empagliflozin and linagliptin is given in the table 1.

Table 1: Results for System Suitability of Empagliflozin and Linagliptin

Empagliflozin					Linagliptin				
Injection	**RT**	**Peak area**	**TP**	**TF**	**RT**	**Peak area**	**TP**	**TF**	**Rs**
1	1.323	14561651	3151	1.26	2.360	18999753	6087	1.14	9.3
2	1.320	14256145	3145	1.24	2.350	18792158	6045	1.16	9.6
3	1.320	14328763	3166	1.25	2.343	18681457	6025	1.15	9.5
4	1.320	14370761	3130	1.30	2.337	18743845	6037	1.16	9.3
5	1.320	14303452	3152	1.27	2.330	18841274	6147	1.14	9.4
6	1.320	14303935	3146	1.27	2.330	18841425	6255	1.18	9.3
Mean	1.321	14353924	-	-	2.342	18816674	-	-	-
SD	0.001	108334	-	-	0.012	108557	-	-	-
%RSD	0.1	0.8	-	-	0.5	0.6	-	-	-

TF- Theoretical plates.TP-Tailing factor Rs-Resolution

Linearity

An appropriate volume of aliquots from standard empagliflozin, linagliptin stock solutions were transferred to different volumetric flasks. The volumes were adjusted to the mark with diluent to give a solution containing concentration of 50, 80, 100, 120, 150µg/ml of empagliflozin and 25, 40, 50, 60, 75µg/ml of linagliptin. The linearity graphs for empagliflozin and linagliptin is shown in figure 3 and 4.

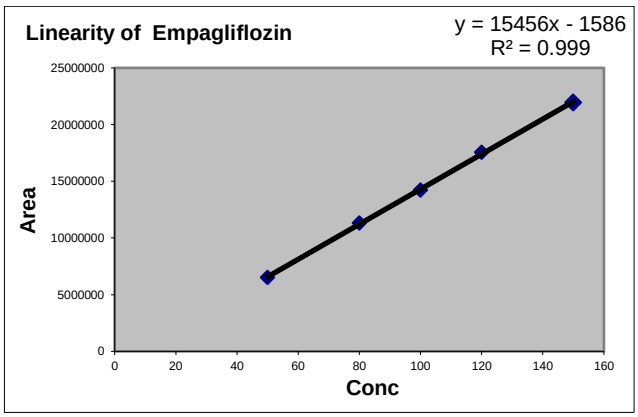

Figure 3: Linearity graph for Empagliflozin

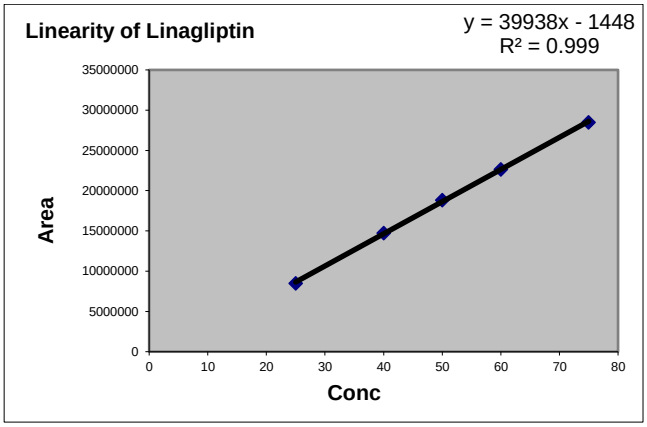

Figure 4: Linearity graph for Linagliptin

Precision

The method precision (repeatability) was performed by carrying out six independent assays of the test sample at 100 µg/ml of Empagliflozin, 50 µg/ml of linagliptin. Against the reference standard. The intermediate precision was Evaluated by carrying out six independent assays of test samples on Different days at 100 µg/ml of empagliflozin, 50 µg/ml of linagliptin, against the reference Standard.

Table 2: Precision data for Empagliflozin and Linagliptin

Injection	Empagliflozin			Linagliptin		
	Rt	**Area**	**%Assay**	**Rt**	**Area**	**%Assay**
1	1.320	14107654	98.9	2.323	18518473	98.2
2	1,323	14255777	98.9	2.320	18575390	98.5
3	1.323	14223044	98.7	2.313	18674178	99.0
4	1.323	14398647	99.9	2.307	18783295	99.6
5	1.320	14176918	98.4	2.300	18539666	98.3
6	1.320	1427862	99.0	2.923	18896466	100.2
Average	1.3215	-	98.8	2.414	-	99.0
SD	0.001643	-	0.7	0.024763	-	0.8
%RSD	0.12	-	0.7	1.0	-	0.8

Accuracy

Concentrations of drugs at 50%, 100%, and 150 % levels were spiked to the pre-analyzed sample solution and were injected into the HPLC system each in triplicate. The % mean recovery at each of the concentration levels was calculated to determine the accuracy. The accuracy data for empagliflozin and linagliptin is given in the table 3 & 4.

Ruggedness

The ruggedness of the method was studied by determining the analyst to analyst variation by performing the assay by two different analyst. The ruggedness of the proposed method is expressed in terms of %RSD. Ruggedness data for Empagliflozin and Linagliptin is given in the table 5.

Robustness

The robustness study was performed by slight modification in the flow rate of the mobile phase and change in the temperature. Robustness data for Empagliflozin and Linagliptin is given in table 6.

Table 3: Accuracy data for Empagliflozin

% Recovered	Area	Concentration Added	Concentration Recovered	% Recovery	Average
50% _01	7132194	50	49.69	99.4	
50% _02	7152899	50	49.83	99.7	
50% _03	7171079	50	49.96	99.9	
100% _01	14269044	100	99.41	99.4	
100% _02	14286707	100	99.53	99.5	99.2%
100% _03	14229355	100	99.13	99.1	
150% _01	21284249	150	148.28	98.9	
150% _02	21205193	150	147.73	98.5	
150% _03	21280624	150	148.26	98.8	

Table 4: Accuracy data for Linagliptin

% Recovered	Area	Concentration Added	Concentration Recovered	% Recovery	Average
50% _01	9374038	25	24.91	99.6	
50% _02	9371143	25	24.90	99.6	
50% _03	9329351	25	24.79	99.16	
100% _01	18765131	50	49.86	99.7	
100% _02	18740887	50	49.80	99.6	99.47%
100% _03	18687222	50	49.66	99.3	
150% _01	28045467	75	74.52	99.3	
150% _02	28083994	75	74.63	99.5	
150% _03	28097082	75	74.66	99.5	

Table 5: Ruggedness data for Empagliflozin and Linagliptin

Empagliflozin	% Assay	Linagliptin	% Assay
Analyst 01	99.18	Analyst 01	100.29
Analyst 02	99.84	Analyst 02	100.51
%RSD	1.25	%RSD	0.78

Table 6: Robustness data for Empagliflozin and Linagliptin

Chromatographic changes		Theoretical Plates		Tailing factor		Resolution
		Empagliflozin	Linagliptin	Empagliflozin	Linagliptin	Between Empagliflozin & Linagliptin
Flow rate (mL/min)	0.4	4250	7229	1.39	1.22	9.9
	0.6	2508	5083	1.20	1.12	8.9
Temperature (°C)	25	3116	5901	1.17	1.24	8.6
	35	3053	7027	1.28	1.10	12.3

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the developed method was calculated based on the standard deviation of the response and the slope of the linearity curve. The LOD and LOQ was found to be 0.24 µg/ml and 0.734 µg/ml respectively for empagliflozin. The LOD and LOQ was found to be 0.090 µg/ml and 0.701 µg/ml respectively for linagliptin.

Analysis of tablets

The proposed method was used for the assay of commercially available tablets containing combinations of empagliflozin and Linagliptin. The assay was performed in triplicates. The assay percentage of empagliflozin and linagliptin was found to be within the limits of 99.82 and 100.036% respectively.

CONCLUSION

The proposed HPLC method was validated as per the ICH guidelines and was found to be applicable for routine quantitative analysis of empagliflozin and linagliptin by HPLC in bulk drug and in pharmaceutical dosage form. The results of linearity, precision, accuracy, specificity, LOQ, LOD were proved to be within limits. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and a run time of less than 10 mins allows its application in its routine determination of empagliflozin and linagliptin in the pharmaceutical dosage form.

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