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Research Article

DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF EMPAGLIFLOZIN AND LINAGLIPTIN

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Abstract

Simple and reliable first order derivative spectrophotometric method was developed and validated for the simultaneous estimation of empagliflozin and linagliptin in combined dosage form. The quantitative determination of the drugs was carried out using the first order derivative values measured at 221 nm and 238 nm for empagliflozin and linagliptin respectively. The solutions of standard and the sample were prepared in methanol. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of 2.5-30 µg/ml for both empagliflozin and linagliptin. The low relative standard deviation values indicate good precision and high recovery values indicate accuracy of the proposed method. Developed first derivative spectrophotometric method was simple, accurate, precise, specific, sensitive and reproducible which can be directly and easily applied to pharmaceutical dosage forms.

Keywords: Empagliflozin, Linagliptin, First derivative UV-spectrophotometric method, Method validation.

Introduction

Empagliflozin is a new Antidiabetic drug, having SGLT2 inhibitor properties for the treatment of type-2 diabetes. Empagliflozin works by decreasing the amount of sugar that body absorbs and increasing the amount of sugar that leaves the body in the urine. Empagliflozin is chemically (2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S) oxolan-3-yloxy]phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol [1,2]. Literature survey reveals RP-HPLC [3, 4] and spectrophotometric [5] methods for the estimation of empagliflozin in pharmaceutical formulations. Empagliflozin is not yet official in IP, USP, BP, JP and EP; hence no official method is available for the estimation of empagliflozin in pharmaceutical dosage forms.

Linagliptin is an Antidiabetic drug, having DPP-4 inhibitor properties for the treatment of type-2 diabetes. Linagliptin works by increasing hormones that stimulate your pancreas to produce more insulin and stimulate your liver to produce less glucose. Linagliptin is chemically 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 [6, 7]. Literature survey reveals HPLC [8-10], UPLC [11] and spectrophotometric [12-14] methods for the estimation of linagliptin in pharmaceutical formulations. Linagliptin is not yet official in IP, USP, BP, JP and EP; hence no official method is available for the estimation of linagliptin in pharmaceutical dosage forms.

According to the literature survey it was found that few analytical methods such as HPLC[15] and simultaneous equation UV[16] methods were reported for empagliflozin and linagliptin individually as well as in combined dosage form, but no single method has been reported on first derivative spectroscopic method for empagliflozin and linagliptin in combination. The objective of this study

was to develop and validate a simple and specific first derivative spectrophotometric method for the simultaneous determination of empagliflozin and linagliptin in combined dosage form. Derivative spectroscopy has been widely used as a tool for quantitative analysis. This technique offers various advantages over the conventional spectrophotometric methods, such as discrimination of the sharp spectral features over the large bands and the enhancement of the resolution of the overlapping spectra. This method exhibited a precise, accurate and cost effective assay for these drugs in mixture.

MATERIALS & METHODS

Apparatus

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1800, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells, Analytical balance (CP224S, Sartorius, Germany), Ultrasonic cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks and pipettes of borosilicate glass.

Reagents and materials

Empagliflozin and linagliptin bulk powder was kindly supplied as a gift samples from Astron research limited, Ahmedabad, Gujarat, India. The synthetic mixture containing 10 mg empagliflozin and 5 mg linagliptin was prepared in the laboratory using pharmaceutical excipients. Methanol (S. D. Fine Chemicals Ltd., Mumbai, India) used. 0.45 µm filter papers (Gelman Laboratory, Mumbai, India) were used in the study.

Preparation of Solutions

1. Preparation of Standard Stock Solutions

Accurately weighed portion of empagliflozin (10 mg) and linagliptin (10 mg) were transferred in a separate 100 ml of volumetric flasks. Added about 70 ml of diluent (Methanol) and sonicated to dissolved. Final volume was made up to the mark with diluent and mixed.

2. Preparation of Working Standard Solutions

Separately diluted 10.0 ml of each standard stock solution to 100ml with diluent and mixed**.**

First Order Derivative Method

1. Determination of the Zero Crossing Points

The standard solutions of empagliflozin (10 µg/ml) and linagliptin (10 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. At zero crossing point of first drug second drug showed reasonable absorbance, while at zero crossing point of second drug first drug showed reasonable absorbance so these two wavelengths were selected for further measurement.

2. Preparation of Calibration Curves

The calibration curves were plotted over the concentration range of 2.5-30 µg/ml for both, empagliflozin and linagliptin. From standard stock solutions, aliquots of empagliflozin (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 ml) and linagliptin (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 ml) were transferred in a series of 20 ml volumetric flasks. The volume was adjusted to the mark with methanol and mixed. The Absorbance of first derivatised spectra was measured at 221 nm (zero crossing point of linagliptin) and 238 nm (zero crossing point of empagliflozin) against methanol as blank. The analysis was carried out for six replicates. DA/dλ versus concentration were plotted to obtain the calibration curves.

Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines. [17]

1. Linearity and Range (Calibration Curve)

The calibration curves were plotted over the concentration range of 2.5-30 µg/ml for both, empagliflozin and linagliptin. From standard stock solutions, aliquots of empagliflozin (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 ml) and linagliptin (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 ml) were transferred in a series of 20 ml volumetric flasks. The volume was adjusted to the mark with methanol and mixed. The absorbance of first derivatised spectra was measured at 221 nm (zero crossing point of linagliptin) and 238 nm (zero crossing point of empagliflozin) against methanol as blank. The analysis was carried out for six replicates. DA/dλ versus concentration were plotted to obtain the calibration curves.

2. Method Precision (Repeatability)

The precision of the instrument was checked by Repeated scanning and measurement of absorbance of solutions (n = 6) for empagliflozin (10 µg/ml) and linagliptin (10 µg/ml) without changing the parameter of the proposed first order derivative method. The results were reported in terms of relative standard deviation (% RSD).

3. Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of empagliflozin and linagliptin (5, 10, 15 µg/ml for both empagliflozin and linagliptin). The results were reported in terms of relative standard deviation (% RSD).

4. Accuracy (Recovery Study)

The accuracy of the method was determined by calculating recovery of empagliflozin and linagliptin by the standard addition method. Known amounts of standard solutions of empagliflozin and linagliptin were added at 80, 100 and 120% level to placebo solutions. The amounts of empagliflozin and linagliptin were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

5. Limit of Detection and Limit of Quantification

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the residual standard deviation of the y intercept was computed. From these values, the limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the residual standard deviation of the response and S= slope of the calibration curve.

Estimation of empagliflozin and linagliptin in combined dosage form

Taken accurately weighed quantity equivalent to 10 mg of linagliptin (200 mg of synthetic mixture) to 200 ml volumetric flask. Added 140 ml of diluent and sonicated for 30min with intermediate shaking. Cool at room temperature and maked volume up to mark with diluent and mix. From above stock solution diluted 5.0 ml to 50.0 ml with diluent and mix. Filter the solution through 0.45 µ filter paper. The response of the sample solution was measured at 221 nm and 238 nm for estimation of empagliflozin and linagliptin respectively. The amounts of both the drugs, present in the sample solution were calculated by fitting the responses into their respective regression equations for both the drugs in the proposed method.

RESULTS AND DISCUSSION

From the optical characteristics obtained with the proposed method it was found that the drug obeys linearity within the concentration range of 2.5-30 µg/ml for both empagliflozin and linagliptin. From the precision studies, it was found that the percent relative standard deviation (% RSD) is less than 2% which indicates that the method has good reproducibility. From the results of recovery studies, it was found that the percent recovery values of empagliflozin and linagliptin were in between 99.8-100.5% and 100.3-100.6% respectively, which indicates that the method is accurate and reveals that commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method. The proposed method was simple, sensitive and reliable with good precision and accuracy.

Table 1 Recovery data for the proposed method

Drug	Level	Amount of placebo taken (mg)	Amount of standard spiked (%)	Mean % Recovery ± % RSD (n=3)
Empagliflozin	I	85	80 %	100.3 ± 0.50
	II	85	100 %	100.5 ± 0.75
	III	85	120 %	99.8 ± 0.55
Linagliptin	I	85	80%	100.6 ± 1.15
	II	85	100%	100.3 ± 0.99
	III	85	120%	100.5 ± 0.62

Table 2 Analysis of synthetic mixture of empagliflozin and linagliptin by First derivative spectrophotometry (n = 3)

Formulation	Drug	Label claim (mg)	Amount found (mg)	% Label claim ± % RSD (n=3)
Synthetic mixture	Empagliflozin	10	9.98	99.8 ± 0.74
	Linagliptin	5	4.97	99.4 ± 0.92

Table 3 Regression analysis data and summary of validation parameter for the proposed First derivative UV-Spectrophotometric method

PARAMETERS		First Derivative Method	
		Empagliflozin	**Linagliptin**
Detection wavelength(nm)		221	238
Beer's law limit (µg/ml)		2.5 – 30	2.5 – 30
Regression equation (y = mx + c)		y = -0.0063x - 0.0007	y = -0.0055x - 0.0009
Slope(m)		-0.0063	-0.0055
Intercept(c)		0.0007	0.0009
Correlation coefficient (r^2)		0.9995	0.9996
Repeatability (% RSD, n = 6)		1.11	1.02
Precision (%RSD)	Intraday(%RSD)	0.88 – 1.31	0.64 – 1.54
	Interday(%RSD)	0.79 – 1.58	0.53 – 1.26
LOD(µg/ml)		0.748	0.716
LOQ(µg/ml)		2.268	2.168
Accuracy (% Recovery ± SD,(n = 3)		100.2 ± 0.34	100.5 ± 0.13
%Assay ± %RSD. (n=3)		99.8 ± 0.74	99.4 ± 0.92

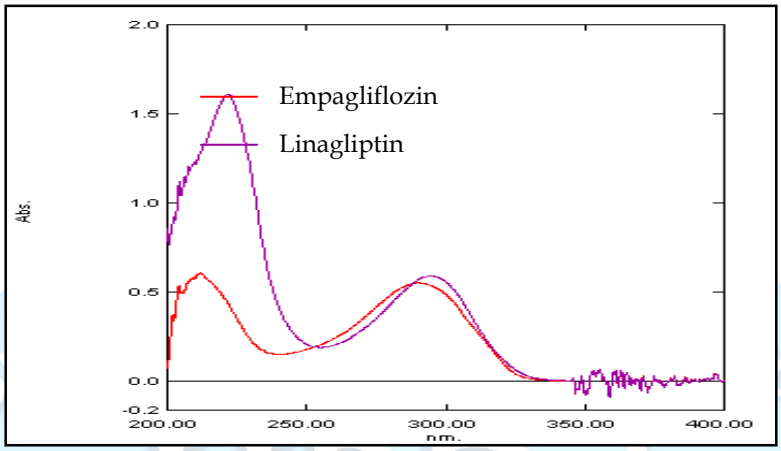

Figure 1. Overlain Zero Order UV absorption spectra of empagliflozin (10 µg/ml) and linagliptin (10 µg/ml)

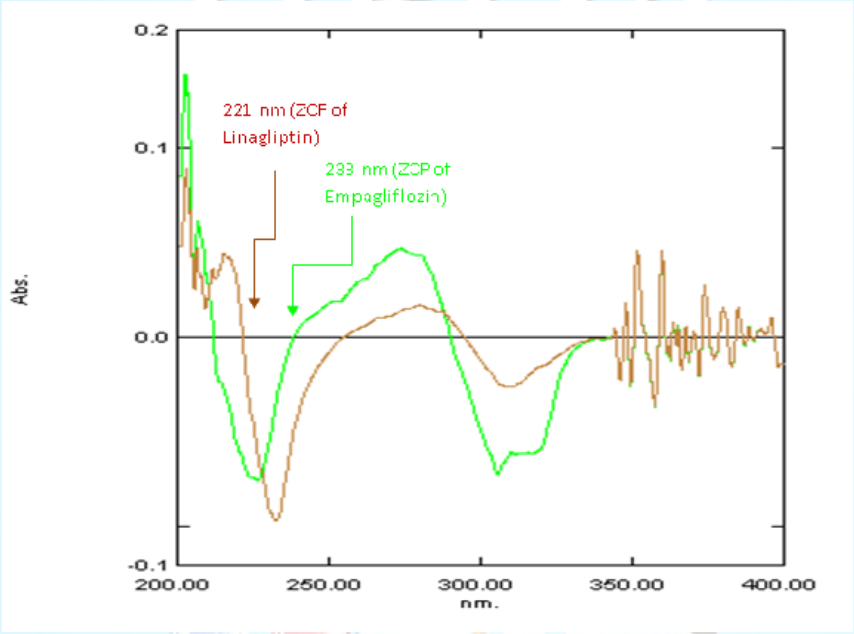

Figure 2. Overlain First Order Derivative UV absorption spectra of empagliflozin (10 µg/ml) and linagliptin (10 µg/ml)

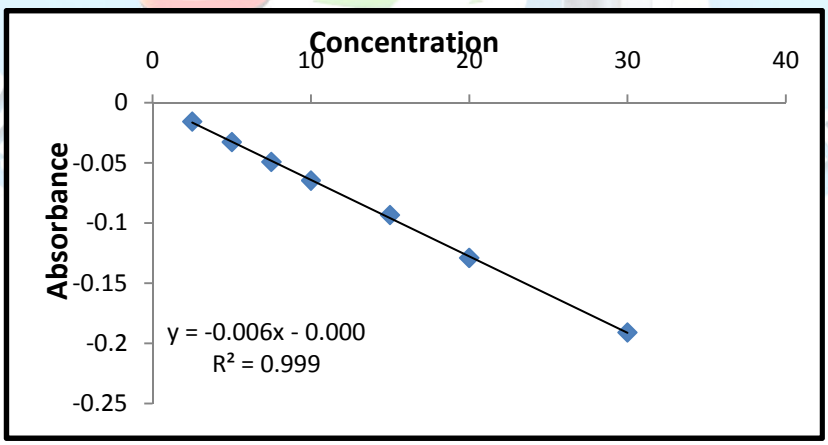

Figure 3. Calibration curve of empagliflozin at 221 nm

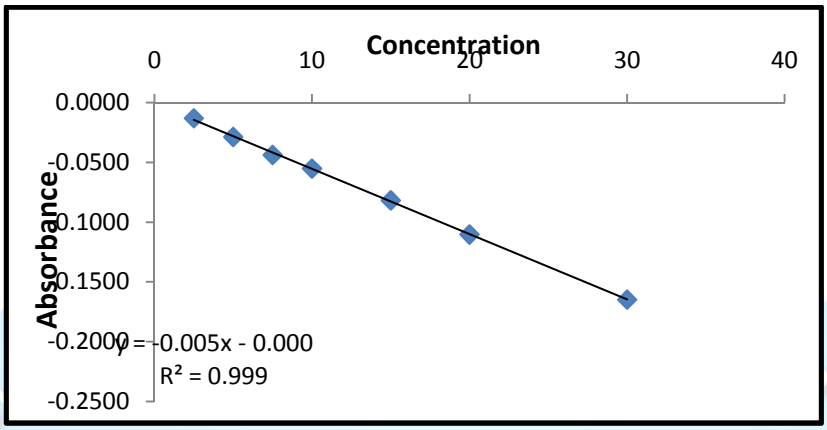

Figure 4. Calibration curve of linagliptin at 238 nm

CONCLUSION

A convenient and rapid UV method has been developed for simultaneous estimation of empagliflozin and linagliptin in available dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and Inter-day (% RSD) coupled with excellent recoveries. Hence, this method can be easily and conveniently adopted for routine analysis of empagliflozin and linagliptin in combined dosage form.

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