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Development and validation of analytical method for Simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy

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ABSTRACT

Simple, precise and economical UV Spectrophotometric methods have been developed for the simultaneous estimation of Empagliflozin and Linagliptin in bulk and pharmaceutical dosage forms. The simultaneous equation (Vierodt's Method), which is based on measurement of absorption at 233nm and 277nm i.e. λmax of Empagliflozin and Linagliptin respectively. Linearity was observed in the concentration range of 5-15µg/ml for Empagliflozin and 2-6µg/ml for Linagliptin. The accuracy of methods was assessed by recovery studies and was found to be within range of 98-101% for both Empagliflozin and Linagliptin. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents. The results were validated statistically as per ICH Q2 R1 guidelines and were found to be satisfactory.

INTRODUCTION

Empagliflozin

Empagliflozin chemically,(1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran-3-yl-oxy) benzyl]-benzene (Fig.1) is an orally administered selective sodium glucose cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type 2 diabetes by blocking the reabsorption of glucose in the kidneys and promoting excretion of excess glucose in the urine

[1-5].The sodium glucose cotransporter 2 (SGLT2), located in the proximal tubule of the nephron, is estimated to facilitate-90% of this reabsorption [3-5]. In addition to glucose control, SGLT2 inhibitors are associated with weight loss and blood pressure reductions, and do not increase the risk of hypoglycemia [6-10].

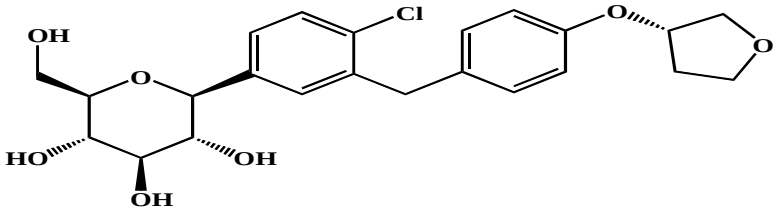

Fig. 1: Structure of Empagliflozin

Linagliptin

Linagliptin chemically8-[(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 (Fig .1) is an inhibitor of DPP-4, an enzyme that degrades the incretion hormones glucagon-like Peptide (GLP-1) and glucose-dependent insulin tropic polypeptide (GLP)[11-13].BothGLP-1 and GIP-1 increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose levels. GLP-1 also reduces glucagon secretion from pancreatic alpha cells, resulting in a reduction in hepatic glucose output [14].

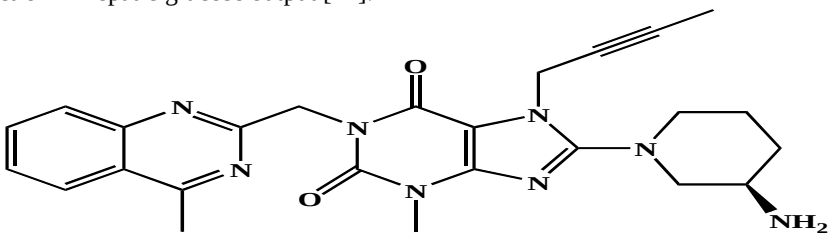

Fig. 2: Structure of Linagliptin

Literature survey revealed that few analytical methods are reported for analysis of both the drugs alone as well as in combination using UV Spectrophotometry [15-16], HPLC [17-25] and HPTLC [26] UPLC[27],LC-MS [28]. On the other hand simultaneous equation (SE) or Vierordt's method was not reported for this new combination. Simultaneous equation (SE) or Vierordt's method is typically applied to estimate drug combinations that contain two drugs or more than two drugs in combined dosage form. Technical hitches involved in this method is very less when compared to other UV methods. Hence an attempt has been made to develop a simple and a reproducible SE method to ensure the safety and efficacy of this selected combination. This developed method was fully validated and applied successfully for the simultaneous estimation of Empagliflozin and Linagliptin in pure and pharmaceutical dosage form. The developed method was validated as per ICH guidelines [29].

MATERIALS AND METHODS

Instrument

Shimadzu 2600 double beam UV-Visible spectrophotometer was used to record the spectra of reference and sample solutions using matched pair of Quartz cells of 10 mm path length. All the weighing was carried out on the ER 200A weighing balance.

Chemicals

Empagliflozin and Linagliptin were supplied by Mylon laboratories, Hyderabad, India. The commercial formulation Glyxambi ® tablets (25 mg) were purchased from Indian pharma network, Noida, Delhi. The analytical grade methanol was procured from Loba Chemie Pvt. Ltd. India.

Preparation of stock solution and selection of wavelength for analysis:

Standard stock solutions of Empagliflozin and Linagliptin were prepared separately by adding 10 mg of drug to methanol taken in 10 ml volumetric flasks and then sonicated for five minutes and the volume was made up with methanol. The resulting solutions contain 1mg/ml of the drug. The stock solutions of Empagliflozin and Linagliptin were further diluted with water to obtain the concentration of 30µg/ml. The resulting solutions were then scanned in UV spectrophotometer from 400 to 200nm. From the resulting spectra λmax for Empagliflozin and Linagliptin were calculated separately (Fig.3, 4). The overlay spectra of Empagliflozin and Linagliptin was also recorded (Fig.5). From the overlay spectra the absorptive point of Empagliflozin and Linagliptin was calculated.

Method: Simultaneous equation method (Vierodt's Method)

If a sample contains two drugs having aborbance atleast at one wavelength, then it is possible to determine the drugs by simultaneous equation method (Vierodt's Method). Two equations are constructed based on the fact that the absorbance at a particular λmax is sum of individual absorbance of two components.

The scanning spectra of 30µg/ml solution of Empagliflozin and Linagliptin show clear peaks at 233nm and 277nm respectively for Empagliflozin and Linagliptin .The λmax of each drug was selected for analysis.

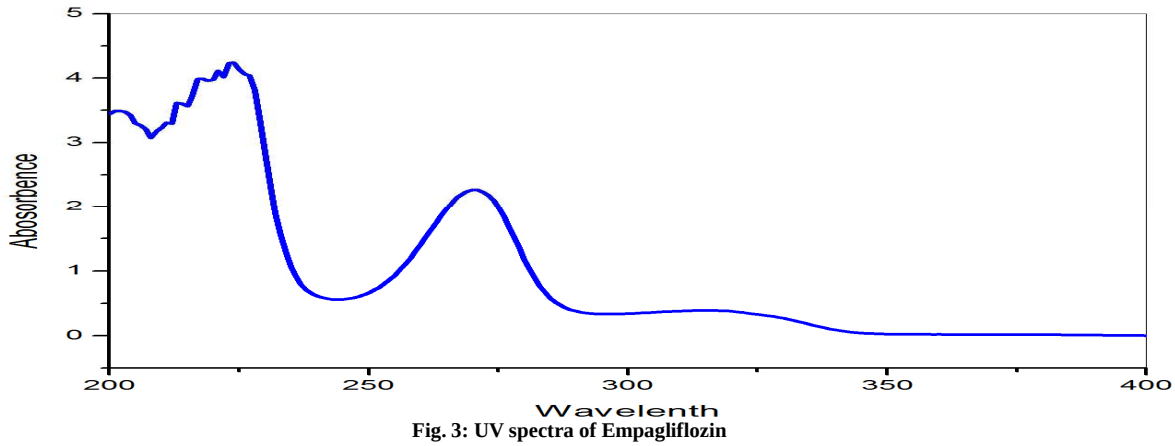

Fig. 3: UV spectra of Empagliflozin

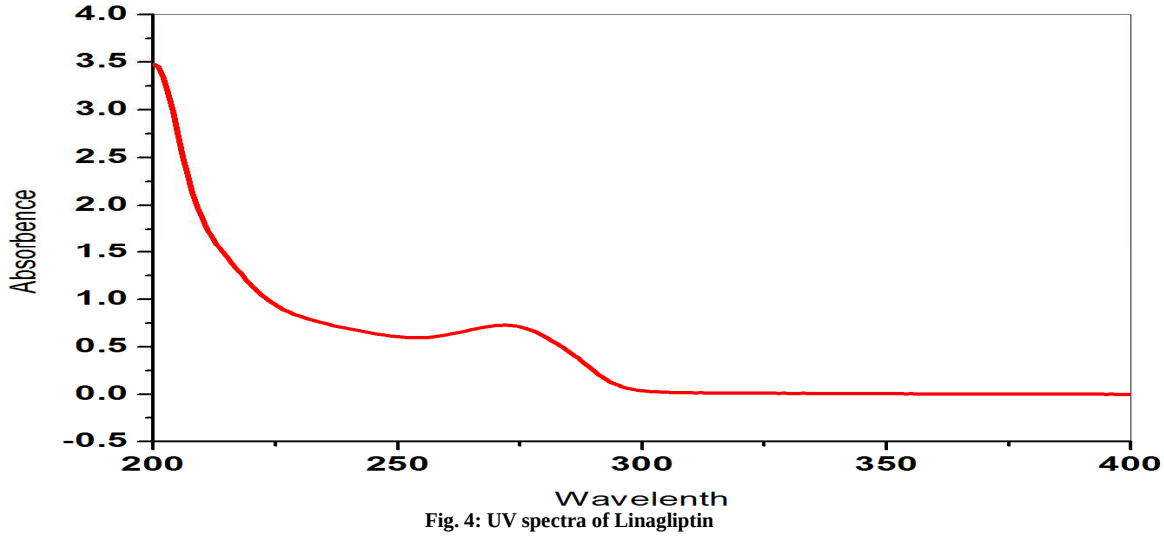

Fig. 4: UV spectra of Linagliptin

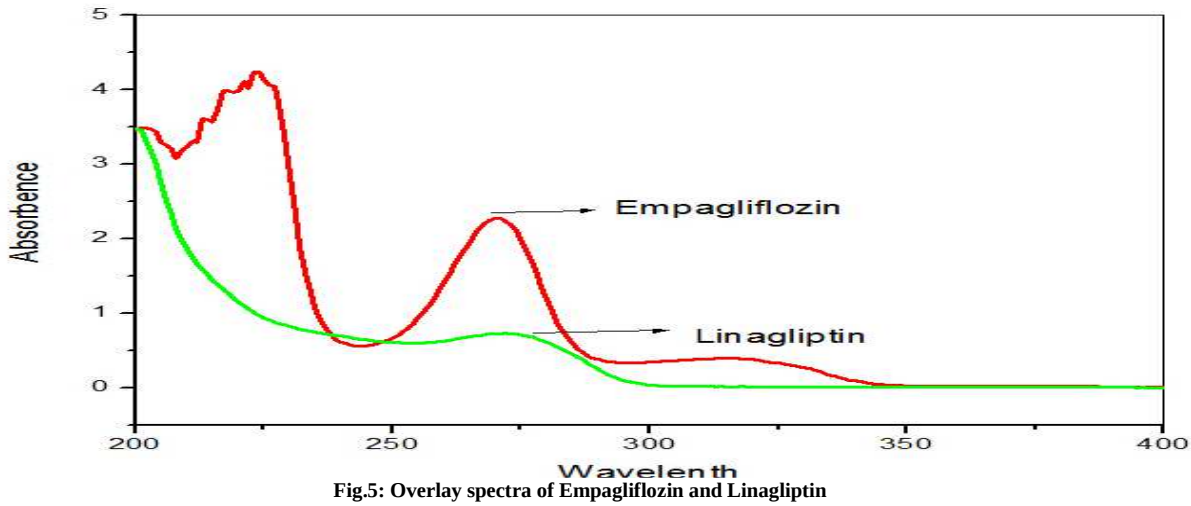

Fig.5: Overlay spectra of Empagliflozin and Linagliptin

The stock solution of Empagliflozin and Linagliptin was then diluted and the absorbance of these solutions was measured at 277nm to verify the Beer's law and the absorptivity values. Two simultaneous equations as given

$A_1 = ax_1C_1 + ay_1C_2$

$A_2=ax_2C_1 + ay_2C_2$
Where
$A_1= (52.16)\ C_1+ (84.54)\ C_2$ (I)
$A_2= (49.21)\ C_1+ (92.34)$ (II)

C_1 and C_2 are the concentrations of Empagliflozin and Linagliptin in gm/100ml respectively in sample solution. A_1 and A_2 are the absorbances of mixture at 233nm and 277nm respectively. Solving equation 1 and 2, C_1 and C_2 are calculated as absorbances of mixture at 233nm and 277nm.

$C_1 = A_2\ ay_1 - A_1\ ay_2 / ax_2\ ay_1 – ax_1\ ay_2$
$C_2= A_1\ ax_2 – A_2\ ax_1 / ax_2\ ay_1 –ax_1ay_2$
$C_1= A_2\ (49.21) - A_1\ (92.34)/-656.241$ (III)
$C_2= A_1\ (84.54) - A_2(52.16)/ -656.241$ (IV)

Preparation and assay of tablet formulation
Fixed dose combination of Empagliflozin and Linagliptin is approved for marketing in USA (Glyxambi ® tablets) containing Empagliflozin, Linagliptin. 20 Glyxambi ®tablets were weighed and triturated in a mortar pestle and powder equivalent to 50mg of Empagliflozin was taken. To this powder 25mg of Linagliptin was added, to make concentration of Empagliflozin/ Linagliptin in ratio of 1:2. A quantiy of sample equivalent to 25 mg of Linagliptin and 50 mg of Empagliflozin was transferred into 100 ml volumetric flask containing 40 ml of methanol and sonicated for 10 min Final volume was made up to the mark and filtered through whatman filter paper (No. 41). 0.1 ml of resulting solution was diluted with methanol to 100ml. 1ml of the resulting solution was again transferred to 100 ml volumetric flask diluted with methanol and the volume was adjusted up to the mark. The absorbance was taken at 233nm and 272 nm against blank .The concentrations of Empagliflozin and Linagliptin was calculated by equation III, IV. The results are reported in the Table 1.

Method validation
The UV Spectrophotometric method was validated as per ICH guidelines for method validation. The performance parameters like linearity, precision and accuracy were evaluated.

Linearity:
Linearity was studied by diluting standard stock solution of Empagliflozin to 5-25µg/ml and Linagliptin 2-12µg/ml concentrations (n=3). Calibration curves with concentration verses absorbance were plotted at their respective wavelengths and the obtained data was subjected to regression analysis using the least square method. The standard curves for Empagliflozin and Linagliptin are shown in (Fig. 6, 7) respectively and data is presented in Table 2.

Accuracy:
To check the accuracy of the developed methods and to study interference of formulation additives, analytical recovery experiments were carried out by using standard addition method. Reference standard solution of each drug was added to tablet samples at three different concentrations level (50, 100 and 150%). At each level, samples were prepared in triplicate and the mean percentage recoveries and % RSD value were calculated. Table .6 shows the result for accuracy of the method.

Precision:
Repeatability: A mixture containing 10µg/ml each of Empagliflozin and Linagliptin was prepared and analyzed both by method A and B (n=6). The data is represented in Table 3. Intermediate precision: intermediate precision is studied in terms of intraday and inter-day precision. Three concentrations of Empagliflozin and Linagliptin was selected in a mixture and analyzed by method A and B (n=3). For intraday, the analysis was carried out at different intervals on the same day and for inter day, the analysis was carried on different days. Table 4 and 5 give the results for intraday and inter-day studies respectively.

Ruggedness
Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slots by different analysts using similar operational and environmental conditions. The results are shown in table.

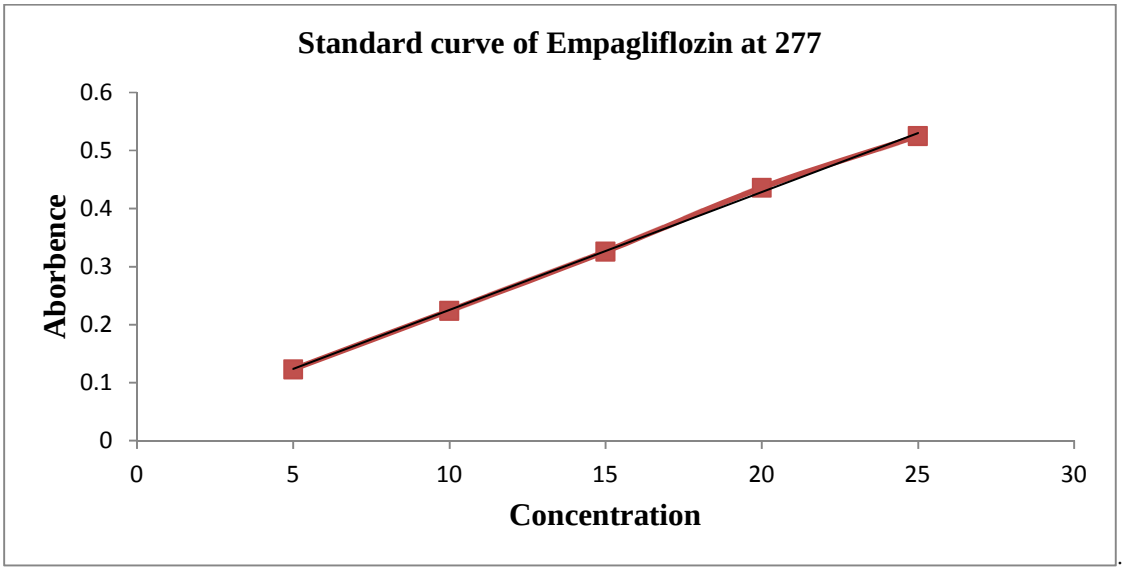

Fig.6. Standard curve of Empagliflozin at 277

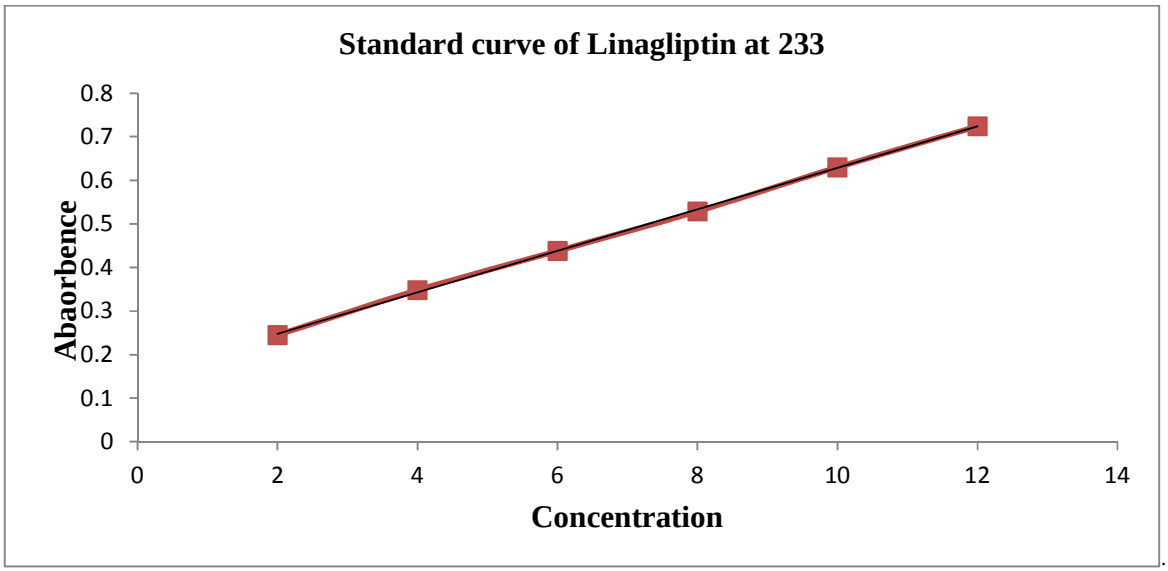

Fig.7. Standard curve of Linagliptin at 233

Table 1: Optical characteristics and linearity data

Parameter	Empagliflozin	Linagliptin
Absorption maximum (nm)	233	277
Beer's law limit(ug/ml)	5-25	2-12
Correlation coefficient	0.999	0.999
Regression equation Y=mX+C	0.020x + 0.022	0.047x + 0.15
Intercept	0.022	0.15
Slope(m)	0.020	0.047

Table 2: *E (1%, 1 cm) for Empagliflozin and Linagliptin

*E(1%,1 cm)at 233nm±SD		*E(1%,1 cm)at 277nm±SD	
Empagliflozin	Linagliptin	Empagliflozin	Linagliptin
Ax_1=52.16±0.46	Ay_1=84.54±0.71	Ax_2=49.21±0.83	Ay_2=92.34±0.54

Table 3: Assay of formulation (n=6)

Brand (Glyxambi)		*%Amount found ±SD	% RSD
Linagliptin + Empagliflozin	Empagliflozin	100.83± 0.58	0.57
	Linagliptin	100.28± 0.42	0.42

Table 4: Repeatability study data for mixture of Empagliflozin and Linagliptin (n=6)

Drug	Concentration taken (ug/ml)	% Found	% RSD
Empagliflozin	10	99.28± 1008	1.01
Linagliptin	10	99.05± 0.8577	0.86

Table 5: Intraday precision data for mixture of Empagliflozin and Linagliptin (n=3)

Drug	Concentration taken (ug/ml)	% Found	% RSD
Empagliflozin	5	99.08± 0.7968	0.8
	10	99.07± 0.8021	0.8
	15	99.04± 0.8001	0.8
Linagliptin	2	98.71± 0.5559	0.5
	4	98.38± 0.3728	0.3
	6	99.61± 0.4336	0.4

Table 6: Interday precision data for mixture of Empagliflozin and Linagliptin (n=3)

Drug	Concentration taken (ug/ml)	% Found	% RSD
Empagliflozin	5	99.610± 0.6229	0.15
	10	99.41± 0.6261	0.62
	15	99.75± 0.4782	0.47
Linagliptin	2	99.65± 0.4557	0.45
	4	99.31± 0.9904	0.9
	6	99.98± 0.7148	0.75

Table 7: Recovery study data for Empagliflozin and Linagliptin (n=3)

Drug	Pre-analyzed sample solution	Drug added	% Recovery	% RSD
Empagliflozin	8	0		0.8
		4	99.86±0.1258	0.12
		5	99.77±0.2753	0.27
		6	99.77±0.3356	0.33
Linagliptin	4	0		0.5
		8	99.44±0.8770	0.88
		10	99.10±0.8902	0.89
		12	98.77±0.356	0.33

Table 8: Ruggedness data for Empagliflozin and Linagliptin (n=3)

Drug	Parameter	% Found	%RSD
Empagliflozin	Analyst 1	101.25± 0.87	0.86
Linagliptin	Analyst 2	99.98± 0.7148	0.75

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient, precise and accurate way for simultaneous analysis of Empagliflozin and Linagliptin in its bulk and pharmaceutical dosage form. Absorbance maxima of Empagliflozin at 277nm and Linagliptin at 233nm were selected for the analysis. Regression analysis shows linearity over the concentration range of 5-15µg/ml for Empagliflozin and 2-6µg/ml for Linagliptin with respective correlation coefficients of 0.999 and 0.9998 respectively. The % RSD for repeatability (n=3), intraday and interday (n=3) precision was found to be less than 2% indicating the precision of method. The amount of drugs estimated by the proposed methods was in good agreement with the label claim.The proposed methods were validated. The Accuracy of the proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Recovery experiments indicated the absence of interference from commonly encounterd pharmaceutical additives. % Recovery for A Empagliflozin and Linagliptin was found within the range of 99.77 % and 99.86%. Values of standard deviation and coefficient of variation were satisfactorily low indicating the accuracy of both the methods. The assay for Linagliptin and Empagliflozin was found to be 99.31 ± and 99.68. The % RSD value for both

Empagliflozin and Linagliptin was found to be less than 2%. The results did not show any statistical difference between operators suggesting that methods developed were rugged. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulations containing both these drugs.

CONCLUSION

Based on the results obtained, it is found that the developed UV-Spectrophotometric technique is quite simple, accurate, precise, reproducible, sensitive and economical. They can become effective analytical tools for routine quality control of Empagliflozin and Linagliptin bulk drug combinations and their combined pharmaceutical dosage form without any prior separation of components.

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