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Simultaneous Method Development and Validation of UVSpectroscopic Method for the Estimation of Empagliflozin and Sitagliptin in Bulk DosageForm

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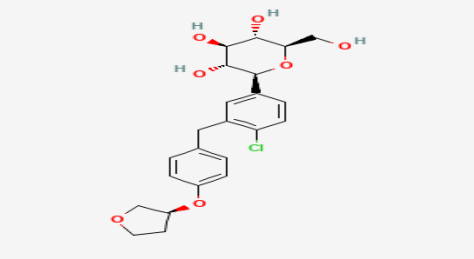
ABSTRACT

Two simple, Precise and economical UV spectrophotometric methods have been developed for the Simultaneous estimation of Empagliflozin and Sitagliptin in bulk and Pharmaceutical dosage forms. Method A is simultaneous equation method (Vierodt's Method), which is based on measurement of absorption at 224nm and 267nm i.e.λmax of Empagliflozin and sitagliptin respectively. Method B is Absorbance ratio (Q analysis method) which is based on measurement of absorption at wavelength of 233nm and 265nm i.e. iso absorptive point of Empagliflozin and sitagliptin and λmax of Empagliflozin respectively. Linearity was observed in the concentration range of 5-25µg/ml for Empagliflozin and 5-25µg/ml for sitagliptin. The accuracy of methods was assessed by recovery studies and was found to be within range of 98.97-102.14% for both Empagliflozin and sitagliptin. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The results were validated statistically as per ICH Q2 R1 guideline and results were found to be satisfactory.

Keywords: Empagliflozin ,Sitagliptin , UV Spectrophotometric , Linearity , Precision.

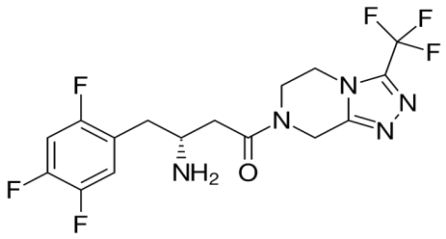
INTRODUCTION

Empagliflozin works by inhibiting the sodium-glucose co-transporter-2 (SGLT-2) found in the proximal tubules in the kidneys. Through SGLT2 inhibition, empagliflozin reduces renal reabsorption of glucose and increases urinary excretion of glucose. The glucose-lowering effect of the drug is independent of insulin.chemical formula of empagliflozin is C23H27C1O7 molecular weight 450.91 g/mol and its chemical name is D-Glucitol,1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3furanyl]oxy]phenyl]methyl]phenyl]In type 2 diabetes patients, urinary glucose excretion increased by approximately 64 grams per day with 10 mg of empagliflozin and 78 grams per day with 25 mg.Empagliflozin reduces sodium and volume load, causing intravascular contraction through its diuretic and natriuretic properties. Moreover, empagliflozin is associated with weight loss, with reductions in blood pressure without increasing heart rate.



Structure of Empagliflozin

Sitagliptin slows DPP-4 mediated inactivation of incretins like GLP-1 and GIP. Incretins are released throughout the day and upregulated in response to meals as part of glucose homeostasis. Sitagliptin chemical name is 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-[3-(trifluoromethyl)-1,2,4- triazolo[4,3-a]pyrazine phosphate and its molecular formula is C16H15F6N5O its molecular weight is 407.31g/mol Reduced inhibition of incretins increase insulin synthesis and decrease glucagon release in a manner dependant on glucose concentrations. These effects lead to an overall increase in blood glucose control which is demonstrated by reduced glycosylated hemoglobin (HbA1c).



Structure of sitagliptin

MATERIALS AND METHODS

REAGENTS

Analytical grade reagents were used methanol and purified water were used.

GLASSWARE

Volumetric flask, funnel, beakers, Measuring cylinder, pipette, used were of Pyrex type and were washed with chromic acid followed by thorough washing with water and finally rinsed with double distilled or deionizedwater which was freshly prepared in the laboratory.

INSTRUMENTS

Spectrophometer:PG Instrument (T80 UV/vi's spectrometer) along with a pair of 5 cm quartz
Corvettes
Weighing Balance: Pioneer OHAIUS (Item PA214C)
Water Bath: DT; Digital constant temperature tank HH-4
UV Lamp: Power: 8N, LF-204.LS,Serial N 045571, 4W254 nm,4W-365 nm. UV, visible 1601 Shimadzu double beam spectrophotometer was used for measurement of spectra. The solvent, which is used for the assay was distill water.

WAVELENGTH SELECTION

Sample About 250 ppm of empagliflozin and sitagliptin was accurately prepared in distill water. The wavelength maxima(λmax) was observed at 237 nm for empagliflozin and 267 nm for sitagliptin this wavelength was adopted for absorbance measurement.

PREPARATION OF STOCK SOLUTION AND SELECTION OF WAVELENGTH FOR ANALYSIS

Standard stock solutions of Empagliflozin and sitagliptin were prepared separately by adding 10mg of drug to methanol (only for empagliflozin) taken in 10ml volumetric flasks and then sonicated for five minutes and the volume was made up with water. The resulting solutions contain 1mg/ml of the drug. The stock solutions of Empagliflozin and sitagliptin both solutions are diluted to five concentrations 2 mg/ml, 4 mg/ml 6 mg/ml 8 mg/ml 10mg/ml The resulting solutions were then scanned in UV-Spectrophotometer from 400 to 200 nm. From the resulting spectra λmax for Empagliflozin and Sitagliptin were calculated separately .The overlay spectra of Empagliflozin and Sitagliptin was also recorded. From the overlay spectra iso absorptive point of Empagliflozin and Sitagliptin was calculated.

PERFORMANCECHARACTERISTICS EXAMINED WHEN CARRYOUT METHOD VALIDATION ARE:

Validation:
The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the accuracy, linearity, sensitivity, precision, range, robustness and ruggedness ofa analyte.
Accuracy:
To ascertain the accuracy of theproposed methods, recovery studies were carried at three different levels (80%,100% and 120%). Percent recovery for EMPAGLIFLOZIN AND SITA by this method, was found in the range of [Table 1 & 2]
Precision:
To access the repeatability of the present study the Intra-day and inter-day precision of the method was evaluated for EMPAGLIFLOZIN AND SITA at FIVE different independent concentrations by determining their assay.[Table 4&5]
Linearity and Range:
Linearity is the ability of the method to obtain test results that

are directly proportional to the analyte concentration within a given rang.

The range of analytical procedure is internal between the upper and lower concentration of the analyte in the sample (including concentration) for which it has demonstrated that the analytical procedure has a suitable level of precision, accuracy for linearity.The linearity data of EMPAGLIFLOZIN AND SITA w was shown in [Table-3]

RUGGEDNESS

Degree of reproducibility of test results obtained by analyzing the same sample under a variety of normal test conditions and the data of ruggedness is shown in [table 6&7]

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal.

Table 1: Standard curve of empagliflozin

CONCENTRATION	ABSORBANCE
0	0
2	0.015
4	0.032
6	0.048
8	0.064
10	0.082

Table2:Standard curve of sitagliptin

CONCENTRATION	ABSORBANCE
5	0.2765
10	0.3654
15	0.5492
20	0.5967
25	0.78964

Table 3: Optical characteristics and linearity data

PARAMETERS	EMPAGLIFLOZIN	SITAGLIPTIN
Absorption maximum (nm	233	267
Beer's law limit(µg/ml)	5-25	2-10
Correlation coefficient	0.999	0.9995
Regression equation Y=mX+C	0.0207x + 0.018	0.008
Intercept	0.08	0.005
Slope(m)	0.0207	0.0209

Table4: Precision data of empagliflozin

PRECISION	INTRA DAY	INTER DAY		
		Day 1	Day 2	Day 3
%recovery	0.047	0.05666	0.06866	0.72566
Sd	0.001	0.00057	0.00057	0.00115
% Rsd	1.2197	1.2197	1.01885	0.15912

Table 5: precision data of sitagliptin

TIME PERIOD	CONCENTRATION TAKEN (µG/ML)	% FOUND	%RSD
Intra day	5	98.16 ±0.452	0.46
	10	99.11 ±0.8221	0.82
	15	100.1±0.8121	0.82
Inter day	5	100.23±0.6152	0.61
	10	99.86±0.6121	0.62
	15	100.75 ±0.4759	0.48

Table 6: Ruggedness results of Empagliflozin

PARAMETER	ANALYST 1	ANALYST 2
Mean	96.20	93.52
Sd	0.0104	0.0106
% RSD	1.040194	1.065018

Table 7: Ruggedness results of sitagliptin

Parameter	Analyst 1	Analyst 2
Mean	0.6315	0.6258
Sd	0.0007	0.0038
% RSD	0.110	0.616

RESULTS AND DISCUSSION

Development and optimization of the spectrophotometric method: Proper wavelength selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of empagliflozin, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, and other chromatographic conditions.Our preliminary trials were by using different compositions of diluents consisting of methanol best result was obtained and degassed in an ultrasonic bath. Below figure represent the spectrum.Selection of wavelength: Scan standard stock solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using Methanol as a blank. Empagliflozin shows λmax at 224nm and Sitagliptin showsλmax at 267nm.The combine overlay of both drugs shows λmax at 227nm. The proposed analytical method is simple, accurate and reproducible.

CONCLUSION

Based on the result obtained new, simple, rapid; precise UV spectrophotometric method was developed for the simultaneous estimation of Empagliflozin and sitagliptin. Hence, this method can be applied for the estimation of Empagliflozin and sitagliptin in drug testing laboratories and pharmaceutical industries.

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