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Research Article

Stability Indicating RP -HPLC Method Development and validation for the simultaneous estimation of Ertugliflozin and Sitagliptin in Bulk and Pharmaceutical Dosage form

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ABSTRACT

A simple, Accurate, Precise method was developed for the simultaneous estimation of sitagliptin and ertugliflozin in tablet dosage form. Chromatogram was run through std zorbac C18 150x4.6 mm, 5 µ. Mobile phase containing buffer 0.01N Na_2HPO_4: Acetonitrile taken in the ratio 55:45 was pumped through column at flow rate of 1ml/min. Buffer used in this method was 0.01N Na_2HPO_4. Temperature was maintained at 30°C. Optimized wavelength selected was 265nm. Retention time of sitagliptin and ertugliflozin were found to be 2.156 min and 3.057 mins. %RSD of the sitagliptin and ertugliflozin were found to be 0.7 and 0.6 respectively. %Recovery was obtained as 101.06% and 99.03% for sitagliptin and ertugliflozin respectively. LOD, LOQ values obtained from regression equations of sitagliptin and ertugliflozin were 1.55,0.04 and 4071,0.12 respectively. Regression equation of sitagliptin is Y=20989x +10319 and Y=86906x +1104.2 of Ertugliflozin. Retention times were decreased and that rub time was decreased, so the method developed was simple and economical that can be adopted in regular quality control test in industries.

Keywords: RP-HPLC, Ertugliflozin, Sitagliptin, Method Development

INTRODUCTION

Ertugliflozin and sitagliptin are anti-diabetic drugs mainly used to treat type 2 diabetic patients. administration of ertugliflozin in combination with sitagliptin is indicated to improve glycemic control in adult patients with type 2 diabetic patients. Literature survey revealed that there were few analytical methods reported for ertugliflozin and sitagliptin in RP -HPLC.

However, an extensive literature search didn't reveal any estimation method for Ertugliflozin and sitagliptin in API and Pharmaceutical dosage form. Therefore, an attempt has been made to develop and validate simple, precise, accurate economical RP-HPLC method as per ICH guidelines for the estimation of Ertugliflozin and Sitagliptin in API and Pharmaceutical dosage form.

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MATERIALS AND METHODS

Chemicals and Reagents: Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), water (HPLC grade) were purchased from Merck (India) Ltd, Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) of Ertugliflozin and Sitagliptin reference standards were procured from Spectrum Pharma labs, Hyderabad, India.

Instruments and Chromatographic Conditions Electronics Balance-Denver, PHmeter - BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695SYSTEM equipped with quaternary pumps, Photo diode array detector and Auto sampler integrated with Empower2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV- win 6 Software was used for measuring absorbance of Ertugliflozin and Sitagliptin solutions. The mobile phase used was Acetonitrile: Water (50:50) at a flow rate of 1.1ml/min, samples were analyzed at 265nm detector wavelength and at an injection volume of 10 µL using discovery ZorbaxC18(4.6 x 150mm,5µm) with run time of 6 mins.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Buffer: 0.01N OPA Buffer:1ml orthophosphoric acid was diluted with 1000ml water. 0.01N Sodium dihydrogen phosphate in 1000ml volumetric flask add about 900ml of water added and degas to sonicate and finally make up with water.

Standard Preparation: 1 ml of Ertugliflozin and Sitagliptin from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.

Sample Preparation: 5tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric fl ask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out and made up to the mark with diluent.

Method Validation
As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method.so this method was said to be specific.

Linearity: Stock solutions of Ertugliflozin and Sitaglitin is taken into 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy: Preparation of Standard stock solutions: Accurately weighed 2.5mg of Ertugliflozin and Siatagliptin transferred to two separately 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained, and samples were injected in duplicate manner. System suitability parameters were not much effected, and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.1ml Ertugliflozin and Sitagliptin, were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml of Standard stock solution was pipetted out and transferred to 10ml volumetric flasks and made up with diluents. From the above solution 0.3ml Ertugliflozin and

Sitagliptin were transferred to 10ml volumetric flasks and made up with the same diluents

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Ertugligflozin and Sitagliptin (5ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Assay of Ertugliflozin and Sitagliptin: Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for estimation of Ertugliflozin and Sitagliptin in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table- 1.The final analysis was performed by using 60% Orthophosphoric acid:40% Acetonitrile at a flow rate of 1ml/min, samples were analyzed at 265nm detector wave length and at an injection volume of 10µL using Discovery ZorbaxC18 4.6 x 150mm, 5µm with run time of 6min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Ertugliflozin and Sitagliptin, the optimized chromatogram was obtained as shown in (Figure-2).

Validation:

Linearity was established for Ertugliflozin (25-150µg/ml) and Sitagliptin (1.25-7.5µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y = 86906x + 1104.2 for Ertugliflozin and y =20989x +10319 for Sitagliptin, correlation coefficient (R^2) was determined as 0.999 for two drugs. The Linearity calibration curves were plotted as shown in (Figure-3, 4). Retention times of Ertugliflozin was2.156min and Sitagliptin was 3.057 where no interfering peaks in blank and placebo were found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean% Recovery was obtained as 99.03% for Ertugliflozin and 101.06% for Sitagliptin was shown in (Table-2,3).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Sitagliptin and Ertugliflozin was obtained as 0.7% and 0.6% and the % RSD for Repeatability was obtained as 0.8% and 1.1% , Low % RSD values indicates that the method developed was precise as shown in (Table- 4). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Ertugliflozin and Sitagliptin. The detection limit value of Ertugliflozin and Sitagliptin was obtained as 0.04 and 1.55 and Quantitation limit of Ertugliflozin and Sitagliptin was found to be 0.12 and 4.71 as given in (Table-5). Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60:40), mobile phase plus (50:50), temperature minus (25°C) and temperature plus (35°C) were

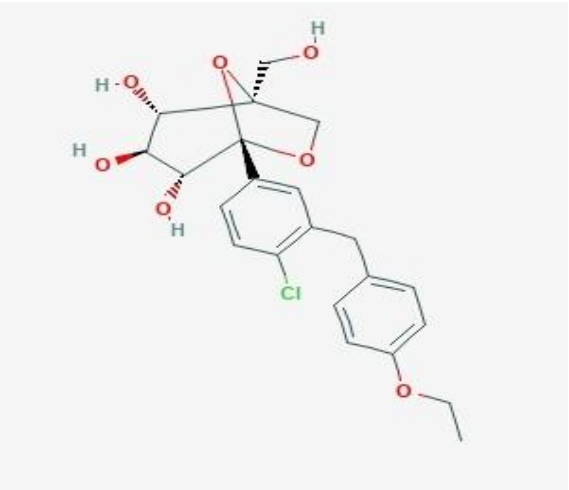

Figure1: Chemical Structure of Ertugliflozin

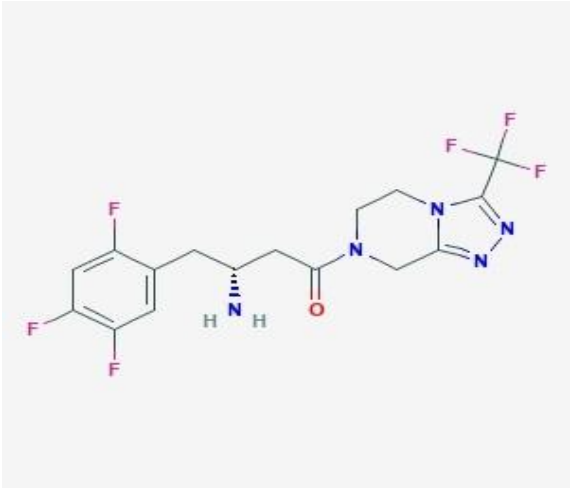

Figure2: Chemical Structure of Sitagliptin

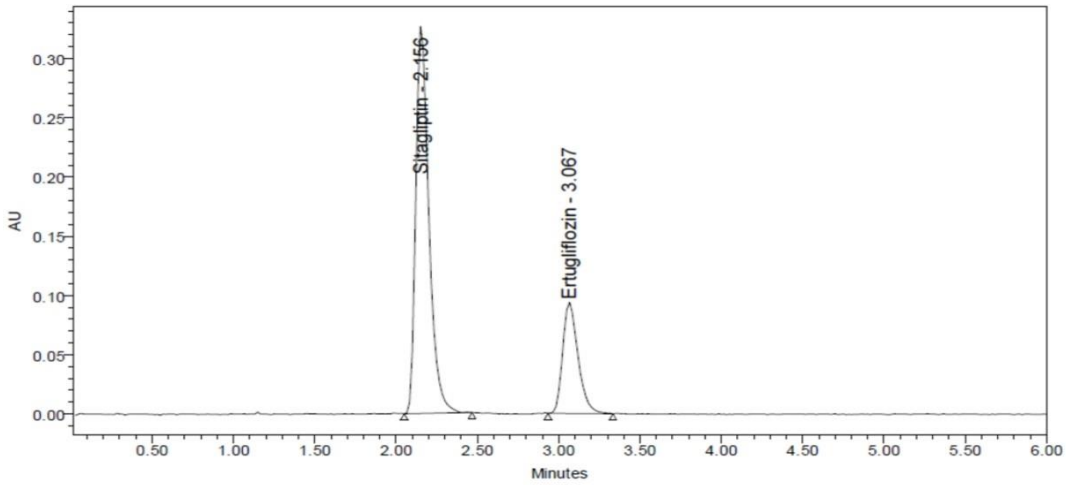

Figure-3: Optimized Chromatogram of Ertugliflozin and Sitaglipin

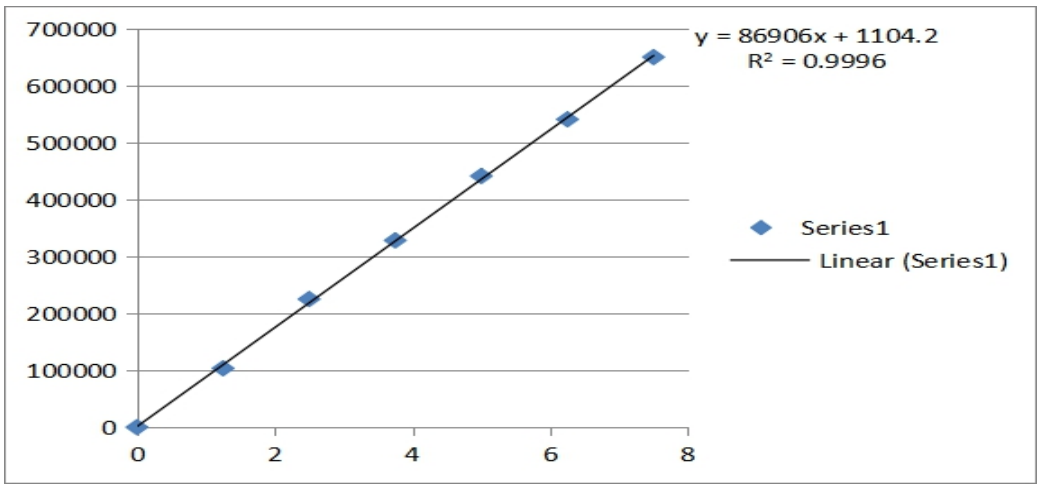

Figure4: Linearity curve of Ertugliflozin

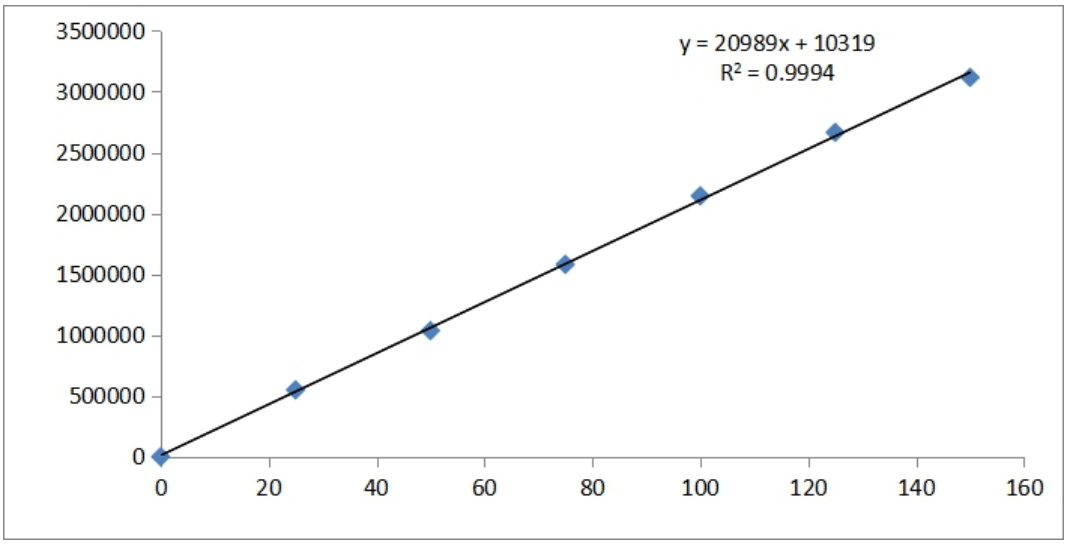

Figure5: Linearity curve of Sitagliptin

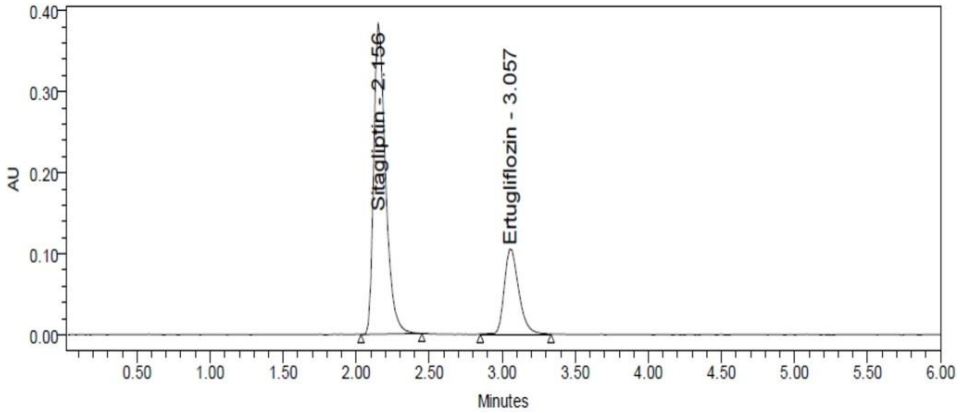

Figure 5: Standard Chromatrogram of Standard Solution

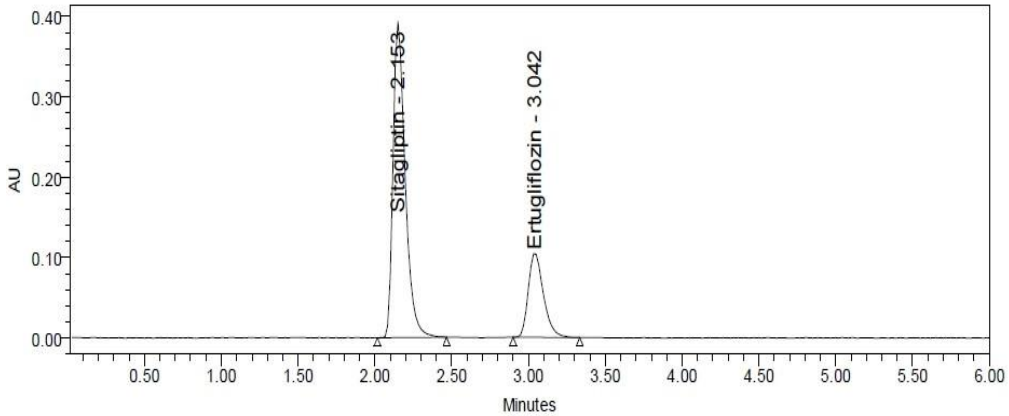

Figure6: Standard Chromatogram of Sample Solution

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	60%0.1N OPA:40%Acetonitrile
Flow rate	1ml/min
Column	Zorbax C18(4.6x150mm,5µm)
Detector wave length	265nm
Column temperature	30°C
Injection volume	10µl
Run time	**6** mins
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Sitagliptin:2.156mins;Ertugliflozin:3.067mins
Theoretical Plates	Sitagliptin:3551.5;Ertugliflozin:5085.8

Table-2: Accuracy results of Ertugliflozin and Sitagliptin

%Level	Amount Spiked (µg/ml)		Amount Recovery (µg/ml)		%Recovery		Mean %Recovery	
	Ertugliflozin	Sitagliptin	Ertugliflozin	Sitagliptin	Ertugliflozin	Sitagliptin	Ertugliflozin	Sitagliptin
50%	2.5	50	2.4713	50.092	98.85	100.18	99.03%	101.06%
	2.5	50	2.5018	50152	100.07	100.30		
	2.5	50	2.4937	49.883	99.75	99.77		
100%	5	100	4.9247	101.522	98.49	101.52	99.03%	101.06%
	5	100	4.9341	101.861	98.68	101.86		
	5	100	4.9225	103.068	98.45	103.07		
150%	7.5	150	7.4511	150.196	99.35	100.13	99.03%	101.06%
	7.5	150	7.3720	152.011	98.29	101.34		
	7.5	150	7.4471	152.048	99.30	101.37		

Table-3: Precision Results of Ertugliflozin and Sitagliptin

S.NO	Precision		Intermediate Precision	
	Area of Ertugliflozin	Area of Sitagliptin	Area of Ertugliflozin	Area of Sitagliptin
1.	440508	2159234	433193	2154770
2.	434658	2145994	434699	2155910
3.	438775	2160249	436845	2133628
4.	436085	2122010	434658	2145994
5.	440781	2146307	431775	2110249

6.	435551	2127837	436085	2122010
7.	437726	2143605	434543	2137094
8	2646.1	15806.8	1854.7	18471.0
9.	0.6	0.7	0.4	0.9

Table-4: LOD and LOQ values of Ertugliflozin and Sitagliptin

Molecule	**LOD**	**LOQ**
Ertugliflozin	**0.04**	**0.12**
Sitagliptin	**1.55**	**4.71**

Table-5 Robustness Data of Ertugliflozin and Sitagliptin

S.no	**Condition**	**%RSD of Ertugliflozin**	**%RSD of Sitagliptin**
1.	Flow rate(-)0.9ml/min	0.4	0.2
2.	Flow rate(+)1.1ml/min	0.3	0.7
3.	Mobile phase (-)60B:40A	0.7	0.1
4.	Mobile phase (+)50B:50A	0.2	1.1
5.	Temperature(-)25°C	0.2	0.7
6.	Temperature(+)35°C	0.4	0.4

Table6: Robutness Results of Ertugliflozin and Sitagliptin

S.no	Ertugliflozin			Sitagliptin			
Injection volume	RT(min)	USP Plate count	Tailing	RT(min)	USP Plate count	Tailing	Resolution
1	3.038	4759	1.31	2.149	3366	1.38	5.5
2	3.056	4695	1.31	2.154	3485	1.40	5.5
3	3.057	4599	1.30	2.155	3479	1.40	5.4
4	3.057	4641	1.29	2.155	3487	1.40	5.5
5	3.057	4597	1.30	2.156	3505	1.39	5.4
6	3.060	4693	1.28	2.156	3531	1.40	5.4

Table7:Degradation of Ertugliflozin and Sitagliptin

Type of Degradation	**Ertugliflozin**			**Sitagliptin**		
	Area	**% Recovered**	**% Degrade d**	**Area**	**% Recovered**	**% Degraded**
Acid	413505	94.09	5.91	2065429	96.16	3.84
Base	419843	95.53	4.47	2021318	94.11	5.89
Peroxide	421575	95.92	4.08	2039341	94.95	5.05
Thermal	422018	96.03	3.97	2090943	97.35	2.65
Uv	432833	98.49	1.51	2131008	99.21	0.79

Water	436005	99.21	0.79	2146900	99.95	0.05

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