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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND ERTUGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORMS

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Abstract:

Another technique was laid out for concurrent assessment of Ertugliflozin and sitagliptin by RP-HPLC strategy. Chromatogram was gone through Inertsil ODS C185 m (4.6 x 250mm). Portable stage containing Phosphate support and Acetonitril in the proportion of 30:70 was siphoned through section at a stream pace of 1ml/min. Support utilized at pH 4.6. Temperature was kept up with at Encompassing. Streamlined frequency for Sitagliptin and Ertugliflozin was 235 nm. Maintenance season of Sitagliptin and Ertugliflozin were viewed as 2.395min and 3.906min. The % virtue of Sitagliptin and Ertugliflozin was viewed as 100.6% and 101.3% individually. The framework appropriateness boundaries for Sitagliptin and Ertugliflozin, for example, hypothetical plates and following variable were viewed as 1.3, 1012.4and 1.2, 1848.2 the goal was viewed as 9.0.The linearity study for Sitagliptin and Ertugliflozin was found in fixation scope of 1µg-5µg and 100µg-500µg and relationship coefficient (r2) was viewed as 0.999 and 0.999, % mean recuperation was viewed as 100.1% and 100.4%, %RSD for repeatability was0.31 and 0.38, % RSD for halfway accuracy was 0.12 and 0.15 separately. The accuracy study was exact, powerful and repeatable. LOD esteem was 2.94 and 3.03, and LOQ esteem was 9.87 and 10.1 separately The consequences of study showed that the proposed RP-HPLC technique is a basic, exact, exact, rough, vigorous, quick and reproducible, which might be valuable for the normal assessment of Ertugliflozin and sitagliptin in drug measurements structure.

Keywords: *Ertugliflozin, sitagliptin, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Ertugliflozin has a place with the class of powerful and particular inhibitors of the sodium-subordinate glucose cotransporters (SGLT), all the more explicitly the sort 2 which is liable for around 90% of the glucose reabsorption from glomerulus. As a feature of an ordinary cycle, the glucose from the blood is separated for discharge and reabsorbed in the glomerulus so short of what one percent of this glucose is discharged in the urine.[1] The reabsorption is intervened by the sodium-subordinate glucose cotransporter (SGLT), primarily the sort 2 which is liable for 90% of the reabsorbed glucose. IUPAC name (1S,2S,3S,4R,5S)- 5-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-1-(hydroxymethyl)-6,8-dioxabicyclo [3.2.1] octane-2,3,4-triol. Atomic recipe C22H25ClO7. Atomic Weight 436.9.

Sitagliptin is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor utilized related to abstain from food and exercise to improve glycemic control in patients with type 2 diabetes mellitus. The impact of this medicine prompts glucose subordinate expansions in insulin and diminishes in glucagon to further develop control of blood sugar.[2] IUPAC Name (3R)- 3-amino-1-[3-(trifluoromethyl)- 5H,6H,7H,8H-[1,2,4] triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phosphoric corrosive hydrate. Sub-atomic Recipe C16H20F6N5O6P. Sub-atomic Weight 523.23.

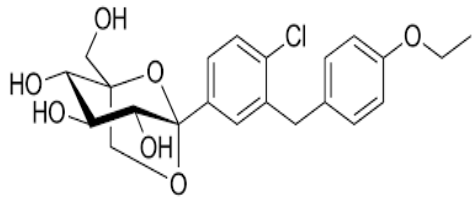

Figure 1: Structure of Ertugliflozin

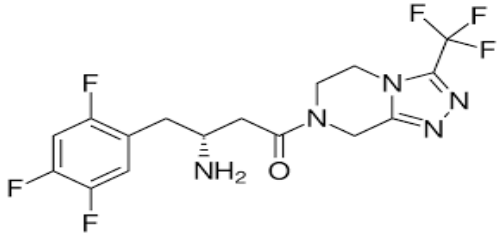

Figure 2: Structure of Sitagliptin

The writing overview uncovered that There are not many strategies detailed in that frame of mind for examination of Ertugliflozin and Sitagliptin alone or in mix with different medications in the unadulterated structure and drugs definitions by RP-HPLC.[3-9] Considering the requirement for a reasonable, savvy RP-HPLC strategy for routine examination of Ertugliflozin and Sitagliptin Concurrent assessment of in drug measurement structure. Endeavors were made to foster straightforward, exact, precise and financially savvy logical strategy for the assessment of Ertugliflozin and Sitagliptin. The proposed technique will be approved according to ICH rules. The target of the proposed work is to foster a new, basic, delicate, precise and conservative scientific technique and approval for the Synchronous assessment of Ertugliflozin and Sitagliptin in drug dose structure by utilizing RP-HPLC. To approve the created strategy as per ICH rules for the planned logical application i.e., to apply the proposed technique for examination of the medication in its measurements structure.

MATERIALS AND METHODS:

Chemicals and Reagents: Ertugliflozin and Sitagliptin were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 235 nm with column Inertsil ODS C185µm (4.6 x 250mm), dimensions at 25^0C temperature. The optimized mobile phase consists of Phosphate buffer and Acetonitril in the ratio of 30:70. Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation of buffer:

Weighed 6.8 grams of KH_2PO_4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase:

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

The diluents:

The Mobile phase was used as the diluent.

Preparation of the individual Sitagliptin standard preparation:

100 mg of working standard was accurately weighed and transferred into a 10 ml clean dry volumetric

flask and about 2 ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of the individual Ertugliflozin standard preparation:
15 mg of Ertugliflozin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant.

Preparation of Sample Solution:
Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 100 mg of Sitagliptin and 15 mg of Ertugliflozin (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a 10 ml volumetric flask and diluted upto the mark with diluant.
Procedure:
20µL of the standard, sample are injected into the chromatographic system and the areas for Sitagliptin and Ertugliflozin peaks are measured and the %Assay are calculated by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 12 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS C185µm (4.6 x 250mm), the mobile phase of composition Sodium Phospahte buffer 3.5 pH and Acetonitrile (30:70) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Ertugliflozin and Sitagliptin in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Ertugliflozin and Sitagliptin and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6,7.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 8,9.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The resulte are shown in table 10,11,12,13

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the

following equations as per ICH guidelines. The resulte are shown in table 14.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION

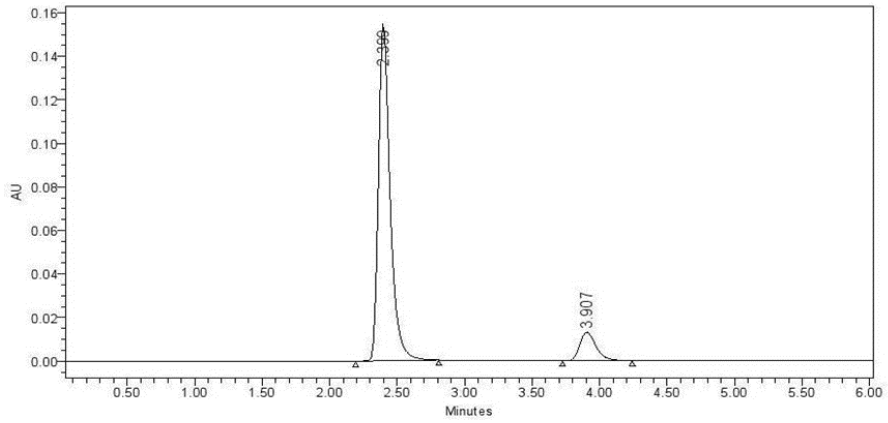

Figure 3: Standard chromatogram

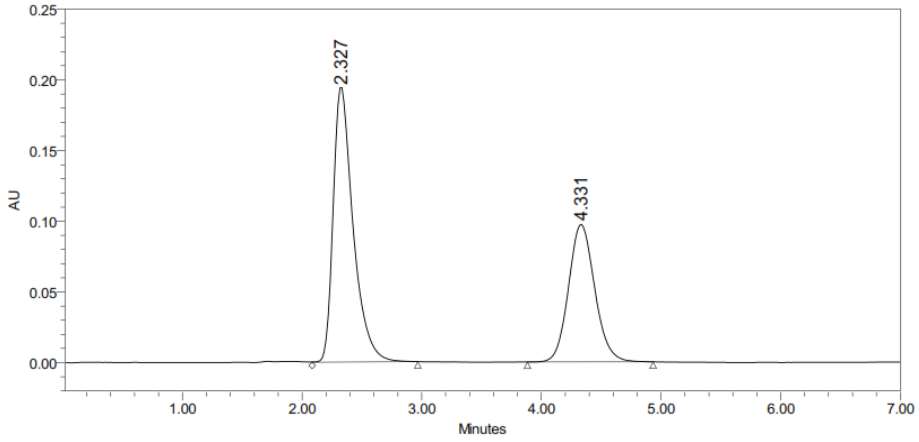

Figure 4: Sample chromatogram

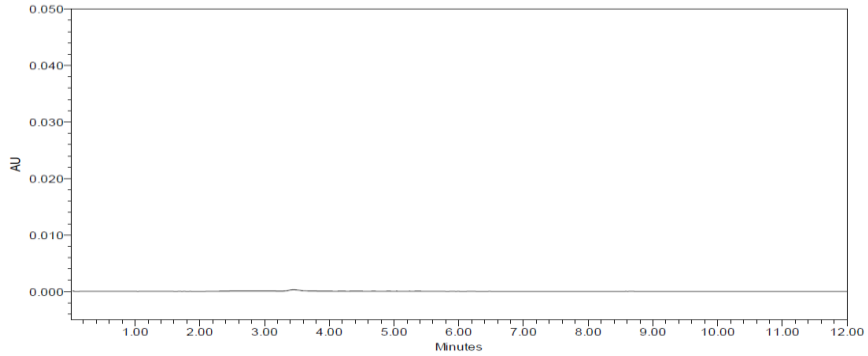

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Ertugliflozin	Sitagliptin
Retention time	4.34	2.23
USP Plate count	2614	2632
USP Tailing	1.6	1.8

Table 2: Assay results for Ertugliflozin and Sitagliptin

	Label Claim (mg)	% Assay
Ertugliflozin	15	101.3
Sitagliptin	100	100.6

Table 3: Linearity results of Sitagliptin and Ertugliflozin

S.NO	SAMPLE NAME	RT	AREA	HEIGHT	SAMPLE NAME	RT	AREA	HEIGHT
1	Linearty 1	2.309	1812101	145867	Linearty 1	4.304	1163273	74586
2	Linearty 2	2.322	2044373	176895	Linearty 2	4.323	1345955	87689
3	Linearty 3	2.324	2366122	206674	Linearty 3	4.214	1556574	101999
4	Linearty 4	2.336	2611248	228475	Linearty 4	4.524	1776565	117084
5	Linearty 5	2.340	2869662	259345	Linearty 5	4.218	1957821	129409

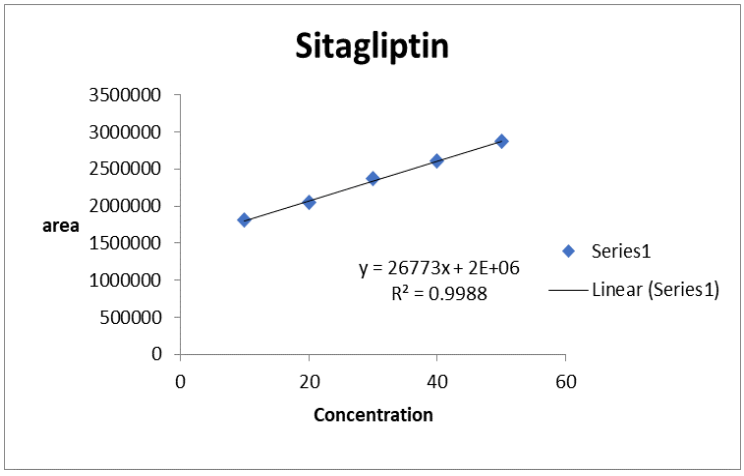

Figure 6: Linearity graph for Sitagliptin

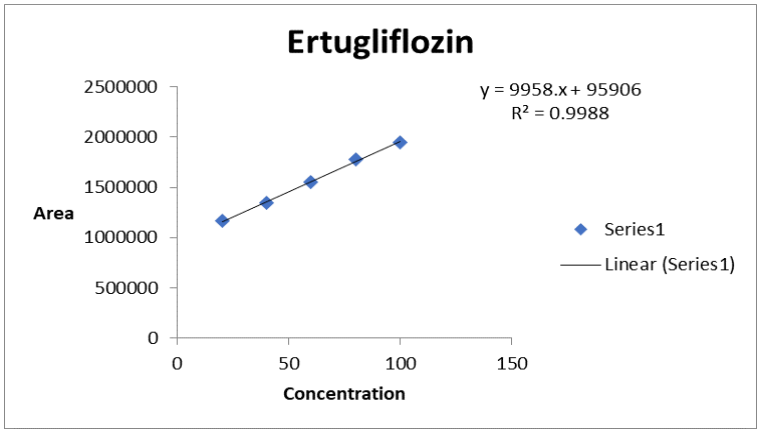

Figure 7: Linearity graph for Ertugliflozin

Table 4: Showing accuracy results for Ertugliflozin

% Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	2331544	7.5	7.60	101.8%	100.4%
100%	3134597	15	14.8	99.9%	
150%	3917897	20	19.4	99.1%	

Table 5: Showing accuracy results for Sitagliptin

%Concentration (at specification level)	Area Area	Amount Added(mg)	Amount Found(mg)	% Recovey % Recovery	Mean Recovery
50%	353757	50	50.8	101.3%	100.1%
100%	4734988	100	99.4	99.4%	
150%	5911698	150	148.9	99.2%	

Table 6: Precision results for Ertugliflozin

S.NO	Name	RT	Area	Height
1	Ertugliflozin	4.302	1401375	100174
2	Ertugliflozin	4.305	1401445	100068
3	Ertugliflozin	4.325	1402315	98415
4	Ertugliflozin	4.315	1404575	98155
5	Ertugliflozin	4.312	1408514	98144
Mean			1491354	
Std.dev			5882.5	
%RSD			0.38	

Table 7: Precision results for Sitagliptin

S.NO	Name	RT	Area	Height
1	Sitagliptin	2.320	2267519	196958
2	Sitagliptin	2.341	2208588	197584
3	Sitagliptin	2.356	2275569	195874
4	Sitagliptin	2.344	2258841	194583
5	Sitagliptin	2.325	2257967	194587
Mean			2254401	
Std.dev			6535.5	
%RSD			0.31	

Table 8. Ruggedness results of Ertugliflozin

S.NO	Name	RT	Area	Height
1	Ertugliflozin	4.302	1401375	95613
2	Ertugliflozin	4.305	1401442	95142
3	Ertugliflozin	4.325	1402312	95158
4	Ertugliflozin	4.315	1404673	95153
5	Ertugliflozin	4.312	1408512	95143
Mean			1455158	
Std.dev			2344.5	
%RSD			0.15	

Table 9. Ruggedness results of Sitagliptin

S.NO	Name	RT	Area	Height
1	Sitagliptin	2.325	2165319	186958
2	Sitagliptin	2.315	2104788	187584
3	Sitagliptin	2.356	2147469	185874
4	Sitagliptin	2.325	2158641	184583
5	Sitagliptin	2.331	218957	184587
Mean			219556	
Std.dev			2559	
%RSD			0.12	

Robustness results

Table 10: Flow variation results for Ertugliflozin

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1778.5	1.23
2	1.0	1547.2	1.2
3	1.2	1938.0	1.2

Table 11: Flow variation results for Sitagliptin

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	882.3	1.56
2	1.0	1244.0	1.1
3	1.2	968.2	1.6

Table 12: System suitability results for Ertugliflozin (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Table 13: System suitability results for Sitagliptin (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	878.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

Table 14: LOD, LOQ of Ertugliflozin and Sitagliptin

Drug	LOD	LOQ
Ertugliflozin	3.03	10.1
Sitagliptin	2.94	9.87

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Ertugliflozin and Sitagliptin in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Sitagliptin and Ertugliflozin in pure and its pharmaceutical dosage forms.

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