AMERICAN JOURNAL OF PHARMTECH RESEARCH

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Stability Indicating UPLC Method Development and Validation for Simultaneous Determination of Ertugliflozin and Sitagliptin

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ABSTRACT

A selective, robust, economic and short method was developed for the simultaneous estimation of the Ertugliflozin (EGZ) and Sitagliptin (SGP) in Tablet dosage form. Chromatogram was run through X-bridge c18 50 x 2.1 mm, 1.7µ. Mobile phase containing Buffer: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.01N KH_2PO_4(3.0PH)buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 240 nm Retention time of EGZ and SGP were found to be 0.883 min and 1.465 min. %RSD of the EGZ and SGP were and found to be 0.3 and 0.8 respectively. %Recovery was obtained as 99.83% and 100.09% for EGZ and SGP respectively. LOD, LOQ values obtained from regression equations of EGZ and SGP were 0.139, 0.421 and 0.18, 0.56 respectively. Regression equation of EGZ is y = 4300x + 253.6, and y = 6814x + 11844 of SGP. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

Keywords: Ertugliflozin, Sitagliptin, UPLC.

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Received 15 December 2018, Accepted 25 December 2018

Please cite this article as: Prasanna D. *et al*., Stability Indicating UPLC Method Development and Validation For Simultaneous Determination of Ertugliflozin and Sitagliptin . American Journal of PharmTech Research 2019.

INTRODUCTION

SGP Chemically it is known as (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. It is a new oral hypoglycemic of the new dipeptidyl peptidase-4 inhibitor class of drugs [1,2]. This enzyme-inhibiting drug is to be used either alone or in combination with metformin or a thiazolidinedione for control of type 2 diabetes mellitus. The drug works to competitively inhibit a protein/enzyme, dipeptidyl peptidase 4, that results in an increased amount of active incretins, reduced amount of release of glucagon (diminishes its release) and increased release of insulin. (figure 1).

EGZ Chemically it is known as **(**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. It is belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters (SGLT), more specifically the type 2 which is responsible for about 90% of the glucose reabsorption from glomerulus [3,4]. (figure 2).

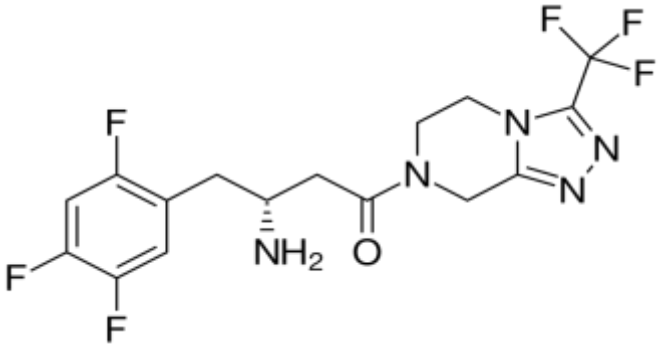

Figure 1: Structures of SGP

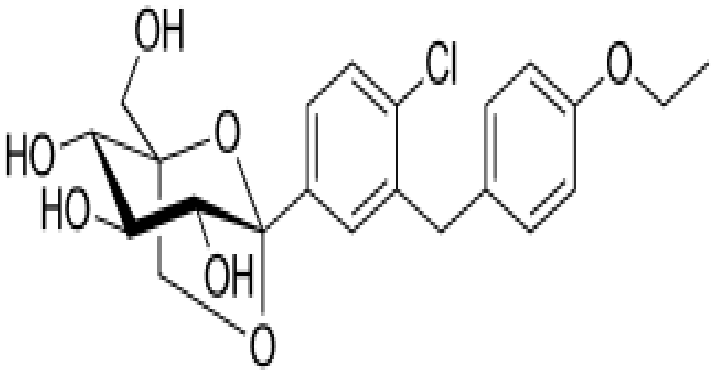

Figure 2: Structures of EGZ

Literature review reveals that different methods RP-HPLC[5-13] , UV[14-19], LCMS[20,21] for its analysis in formulations. Hence our present plan is to develop a new, sensitive, robust& accurate method

for its analysis in formulation, after a detailed study, a new UPLC method was decided to be developed and validated as per ICH norms.

Table 1: Chromatographic conditions

Parameter	Content
Mobile phase	50% 0.01N Kh2po4 (4.8ph) buffer): 50% Acetonitrile
Flow rate	1 ml/min
Column	X-bridge (2.1 x 50mm, 1.7µm)
Detector wave length	240nm
Column temperature	30°C
Injection volume	0.5µL
Run time	3 mi
Diluent	Water and Acetonitrile in the ratio 50:50
Results	Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

MATERIALS AND METHOD

Apparatus and chromatographic parameters:

UPLC WATERS make Acquity, Sartorius Weighing Blance, Metsar pH meter, Labman Sonicator, Crompton Vaccum pump, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of EGZ and SGP solutions.

Drug samples:

EGZ and SGP pure drugs (API), Combination EGZ and SGP tablets (STEGLUJAN), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Reagents and solutions:

Water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid.

Analytical Methodology:

Preparation of Standard stock solutions: Accurately weighed 25mg of SGP 3.75mg of EGZ and transferred to 25ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (150µg/ml of EGZ and 1000µg/ml SGP)

Preparation of Standard working solutions (100% solution):

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15µg/ml of EGZ and 100µg/ml of SGP).

Preparation of Sample stock solutions:

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (150µg/ml of EGZ and 1000µg/ml of SGP)

Preparation of Sample working solutions (100% solution):

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (15µg/ml of EGZ and 100µg/ml of SGP)

Preparation of buffer:

0.01N KH_2PO_4 Buffer:

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Orthophosphoric acid solution.

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water

RESULTS AND DISCUSSION

Method development:

Method development was done by changing various, mobile phase ratios, buffers etc. Initially the mobile phase was pumped for about 3 minutes to saturate the column thereby to set the baseline corrected. Then 0.5 µl of the standard and sample solutions were injected separately. A quantitative determination of the active ingredients was made by comparison of the peak area of the sample injection with the corresponding peak area of the standard injection. The amount of EGZ and SGP present in the sample was calculated through the standard calibration curve.

Optimized method:

Retention times of EGZ and SGP were 0.888 min and 1.469 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific and shown in (figure 3).

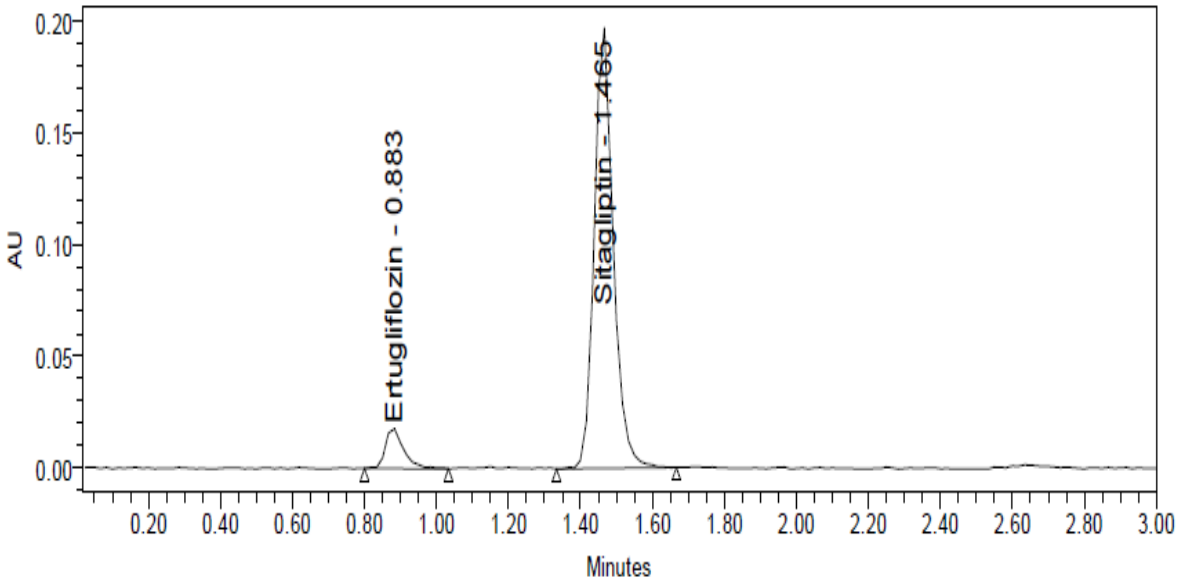

Figure 3: Optimized Chromatogram

Specificity / selectivity:

The responses are distinguished from all other responses and there is no interference were found in the retention of the drugs.

METHOD VALIDATION:

Linearity:

Six linear concentrations of EGZ (3.75-22.5µg/ml) and SGP (25-150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Ertugliflozin was y = 4300x+253.6 and of Sitagliptin was y = 6814x + 11844 Correlation coefficient obtained was 0.999 for the two drugs.(Figure 4 and 5),(Table.3).

Table 2: System suitability parameters for EGZ and SGP

S no	SGP			EGZ			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	0.873	2434	1.38	1.452	3727	1.16	6.0
2	0.883	2486	1.34	1.462	3497	1.13	5.9
3	0.883	2359	1.28	1.463	3465	1.14	5.8
4	0.883	2476	1.25	1.465	3622	1.12	6.1
5	0.887	2411	1.34	1.467	3673	1.14	6.1
6	0.888	2293	1.48	1.469	3652	1.19	6.0

Table 3: Linearity Studies for EGZ and SGP by proposed method

EGZ Conc (µg/mL)	Peak area	SGP Conc (µg/mL)	Peak area
0	0	0	0
3.75	15526	25	179882
7.5	32857	50	372998
11.25	50507	75	526199
15	64415	100	687920
18.75	80398	125	865172
22.5	96760	150	1028107

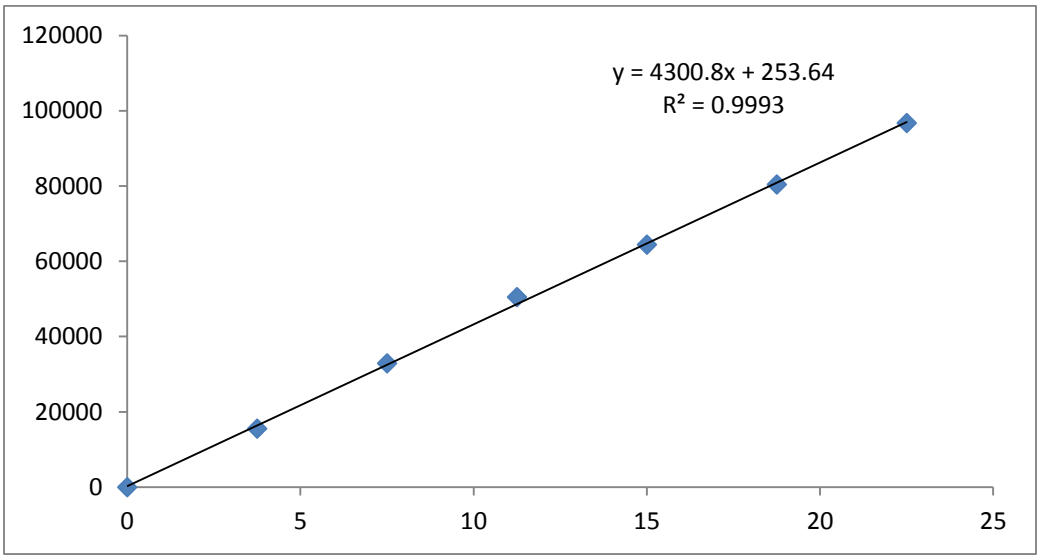

Figure 4: Calibration curves of EGZ

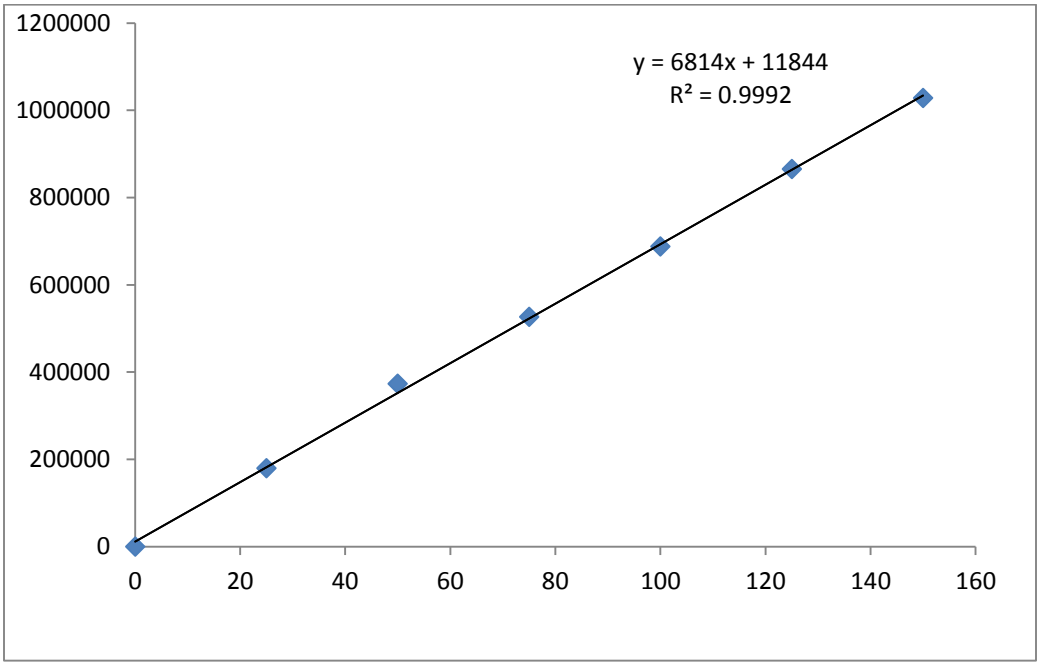

Figure 5: Calibration curves of SGP

Precision:

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 1.0% respectively for EGZ and SGP.As the limit of Precision was less than "2" the system precision was passed in this method.(Table.4).

Table 4: Method Precision (Inter and Intraday) Studies for EGZ and SGP

S.NO	EGZ		SGP	
	Peak area	Day day precision peak area	Peak area	Day day precision peak area
1	64450	62478	684926	665785
2	64263	62448	690546	663671
3	64432	62716	697041	674335
4	64128	63850	693133	664869
5	63679	62834	699376	668298
6	63500	62610	703947	665207
Mean	64075	62823	694828	667028
S.D	398.4	523.7	6752.7	3893.5
%RSD	0.6	0.8	1.0	0.6

Accuracy:

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.83% and 100.09% for EGZ and SGP respectively.(Table.5).

Table 5: Recovery studies for Ertugliflozin and Sitagliptin by proposed method

%Concentration	Ertugliflozin			Sitagliptin		
	50%	100%	150%	50%	100%	150%
Trail-1	100.45	100.80	100.57	99.55	101.60	100.86
Trail-2	99.87	98.49	98.58	100.16	99.06	99.72
Trail-3	99.54	99.47	100.70	99.21	99.57	101.04
AVG(%Recovery	100.0	99.59	99.95	99.64	100.08	100.54
SD	0.46	1.16	1.19	0.481	1.3459	0.7167
%RSD	0.46	1.16	1.19	0.48	1.34	0.71

Robustness:

Robustness conditions like Flow minus (0.25ml/min), Flow plus (0.35ml/min), mobile phase minus (60:40A), mobile phase plus (6=45B:55A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. (Table.6).

Table 6: Robustness studies for EGZ and SGP

Parameter		%RSD	
		Ertugliflozin	Sitagliptin
Flow rate (±0.1ml/min)	0.9mL/min	0.45	0.4
	1.1mL/min	0.4	0.8
Mobile phase(±5ml)	75B:25A	0.94	0.4
	65B:35A	0.47	0.4
Temperature(±5°C)	25°C	0.47	0.9
	35°C	0.80	0.3

Limit of detection and Limit of quantitation:

Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected and Limit of quantitation (LOQ) is the lowest concentration of analyte in a sample. (Table.7).

Table 7: LOD and LOQ studies for EGZ and SGP

Molecule	LOD	LOQ
Ertugliflozin	0.139	0.421
Sitagliptin	0.18	0.56

DEGRADATION

Degradation Studies:

Regarding the pH adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time. (Table.8).

Table 8: Degradation studies of EGZ and SGP

S.NO	Degradation Condition	Ertugliflozin	Sitagliptin
		% Drug Degraded	
1	Acid	8.24	7.73
2	Alkali	5.76	4.45
3	Oxidation	7.03	4.53
4	Thermal	3.92	5.55
5	UV	1.63	1.89
6	Water	0.61	0.73

CONCULSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the EGZ and SGP in Tablet dosage form. Retention time of EGZ and SGP were found to be 0.883 min and 1.465 min. %RSD of the EGZ and SGP were and found to be 0.3 and 0.8 respectively. %Recovery was obtained as 99.83% and 100.09% for EGZ and SGP respectively. LOD, LOQ values obtained from regression equations of EGZ and SGP were and 0.139, 0.421&0.18, 0.56 respectively. Regression equation of EGZ is y = 4300x + 253.6, and y = 6814x + 11844 of SGP. Retention

times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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