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Research Article

Stability Indicating RP-HPLC Method Development and Validation for 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 the Sitagliptin and Ertugliflozin in API and Tablet dosage form. Chromatogram was run through Std Ascentis C18 150 x 4.6 mm, 2.7µ. Mobile phase containing Buffer 0.01N Ammonium acetate: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 0.9ml/min. Buffer used in this method was 0.01N Ammonium acetate. Temperature was maintained at 30°C. Optimized wavelength selected was 220nm. Retention time of Sitagliptin and Ertugliflozin were found to be 2.151min and 2.722min. %RSD of the Sitagliptin and Ertugliflozin were found to be 1.2 and 0.8 respectively. %Recovery was obtained as 99.99% and 100.61% for Sitagliptin and Ertugliflozin respectively. LOD, LOQ values obtained from regression equations of Sitagliptin and Ertugliflozin were 0.69, 0.05 and 2.10, 0.15 respectively. Regression equation of Sitagliptin is y = 28297x + 20644, and y = 74691x + 2292.1 of Ertugliflozin. 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.

Key Words: Ertugliflozin, Sitagliptin, RP-HPLC

INTRODUCTION

Ertugliflozin 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. Ertugliflozin is a small inhibitor of the SGLT2 and its activity increases glucose excretion, reducing hyperglycemia without the requirement of excessive insulin secretion. Sitagliptin is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs.

This enzyme-inhibiting drug is to be used either alone or in combination with metformin or

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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 (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin. A simple, precise, accurate RP-HPLC method was developed for simultaneous estimation of Ertugliflozin and Sitagliptin.

MATERIALS AND METHODS

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

Instruments and chromatographic conditions
Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 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 absorbances of Ertugliflozin and Sitagliptin solutions. The mobile phase used was 0.01NAmmonium acetate:Acetonitrile(60:40), at a flow rate of 0.9ml/min, samples were analyzed at 220nm detector wavelength and at an injection volume of 10µL using Ascentis C18(4.6 × 150mm, 2.7µm) with run time of 8 min.

Methods:

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

Buffer: 0.1% OPA Buffer (1ml of Conc Ortho Phosphoric acid was diluted to 1000mlwith water).

0.01N Ammonium acetate
Accurately weighed 0.77gm of Ammonium acetate 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 to get 0.01N Ammonium acetate buffer.

Preparation of Standard stock solutions: Accurately weighed 2.5mg of Ertugliflozin, 50mg of Sitagliptin and transferred to individual 50ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (50µg/ml of Ertugliflozin and 1000µg/ml of Sitagliptin)

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. (5µg/ml Ertugliflozin of and 100µg/ml of Sitagliptin)

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 10ml 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 (50µg/ml of Ertugliflozin and 1000µg/ml of Sitagliptin)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(5µg/ml of Ertugliflozin and 100µg/ml of Sitagliptin)

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 interferences 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 Sitagliptin is taken in to 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, 50mg of Sitagliptin and transferred to individual 50ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (50µg/ml of Ertugliflozin and 1000µg/ml of Sitagliptin)

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 flask, 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 diluents.

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 (0.8ml/min), Flow plus (1.0ml/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 affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Ertugliflozin, Sitagliptin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Ertugliflozin, Sitagliptin, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters:
The system suitability parameters were determined by preparing standard solutions of Ertugliflozin (5ppm) and Sitagliptin (100ppm) 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: Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system.

RESULTS AND DISCUSSION

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 0.01NAmmonium acetate: Acetonitrile (60:40), at a flow rate of 0.9ml/min, samples were analyzed at 220nm detector wavelength and at an injection volume of 10µL using Ascentis C18 (4.6 × 150mm, 2.7µm) with run time of 8 min. The proposed method was optimized to give sharp peaks with good resolution, minimum tailing effect for Ertugliflozin and Sitagliptin, the optimized chromatogram was obtained as shown in (figure-7).

Validation:
Linearity was established for Sitagliptin (25-150µg/ml) and Ertugliflozin (1.25-7.5µg/ml) at six different concentrations each were injected in a duplicate manner. Average areas were mentioned and linearity equations obtained for Sitagliptin was y = 28297x + 20644.and of Ertugliflozin was y = 74691x + 2292.1. Correlation coefficient obtained was 0.999 for the two drugs. The Linearity calibration curves were plotted as shown in (Figure-3,4). Retention times of Sitagliptin and Ertugliflozin were 2.151 min and 2.722 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. Three levels of Accuracy samples 50%, 100%, 150% were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.61% and 99.99% for Ertugliflozin and Sitagliptin respectively (Table-3). From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 1.2%and 0.8% respectively for Sitagliptin and Ertugliflozin. %RSD for Repeatability for both Sitagliptin and Ertugliflozin was obtained as 0.7%. In Intermediate precision %RSD were calculated for two drugs and obtained as 0.9% and 1.0% respectively for Sitagliptin and Ertugliflozin. As the limit of Precision was less than “2” the system precision was passed in this method. The LOD and LOQ values obtained from regression equations of Sitagliptin and Ertugliflozin were 0.69, 0.05 and 2.10, 0.15(Table-4). Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner (Table-5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. Merck Sharp & Dohme Corp

(Steglujan), bearing the label claim Ertugliflozin 100mg, Sitagliptin5mg. Assay was performed with the above formulation. Average % Assay for Ertugliflozin and Sitagliptin obtained was 100.01% and 99.20% respectively (Table-7). The chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-5,6).

Degradation studies:

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table-8).

CONCLUSION

Chromatographic conditions used are stationary phase Ascentis C18 (4.6 x 150mm, 2.7µm) Mobile phase 0.01N Ammonium acetate: Acetonitrile in the ratio of 60:40 and flow rate were maintained at 0.9ml/min, detection wave length was 220 nm, column temperature was set to 30ºC. Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be 0.999 for both Ertugliflozin and Sitagliptin. Precision was found to be 1.2 and 0.8 for Sitagliptin and Ertugliflozin. LOD, LOQ values obtained from regression equations of Sitagliptin and Ertugliflozin were 0.69, 0.05 and 2.10, 0.15 respectively. By using above method assay of marketed formulations was carried out and average % Assay for Ertugliflozin and Sitagliptin obtained was 100.01% and 99.20%respectively. Degradation studies of Ertugliflozin and Sitagliptin were done, in all condition purity threshold was more than purity angle and within acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Ertugliflozin and Sitagliptin. 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.

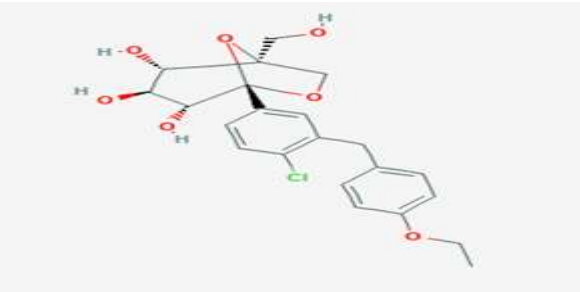

Figure-1: Structure of Ertugliflozin

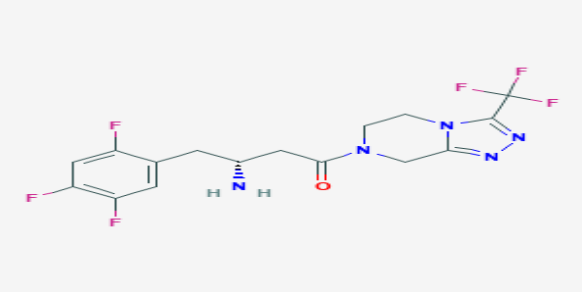

Figure-2: Structure of Sitagliptin

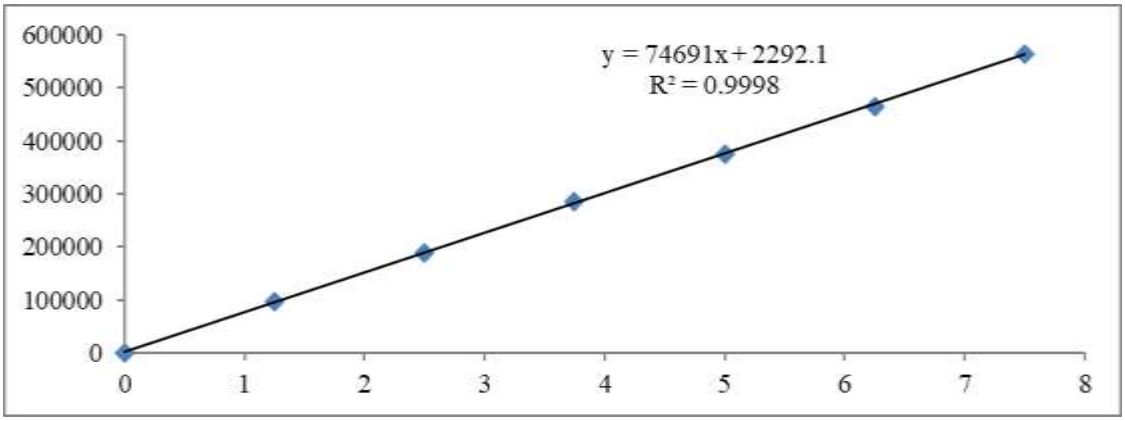

Figure-3: Calibration curve of Ertugliflozin

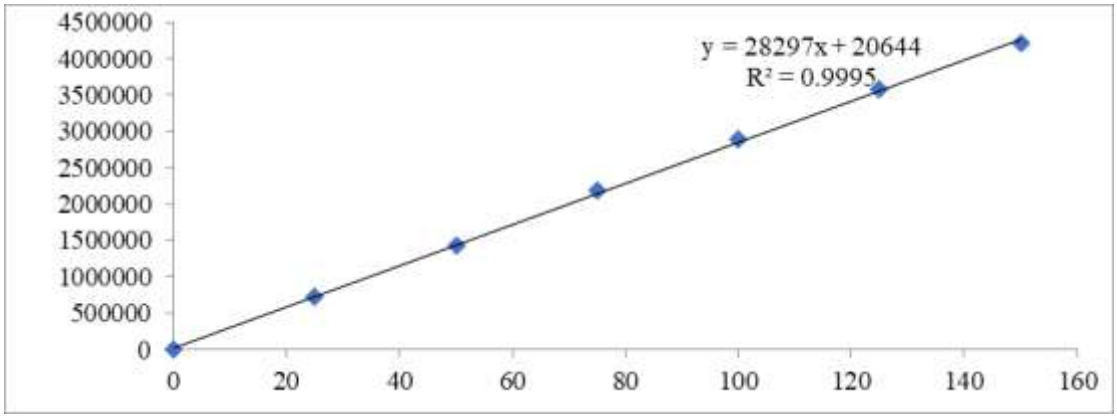

Figure-4: Calibration curve of Sitagliptin

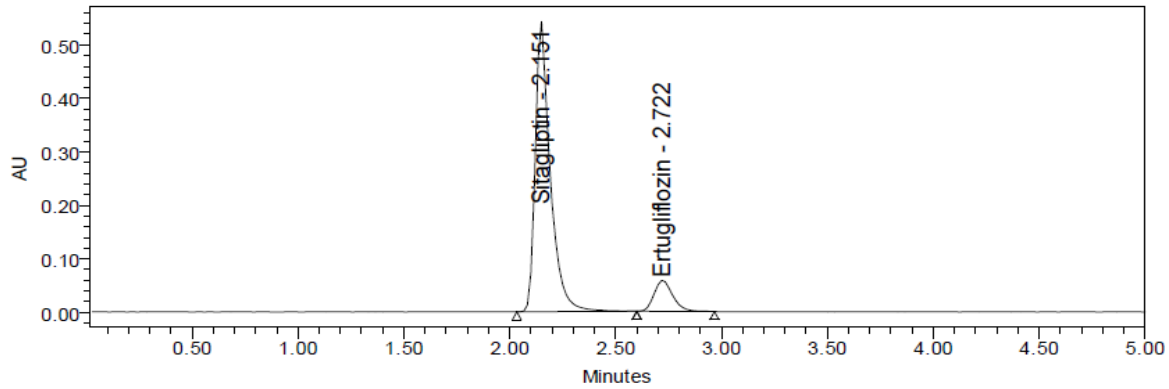

Figure-5: Chromatogram of working standard solution

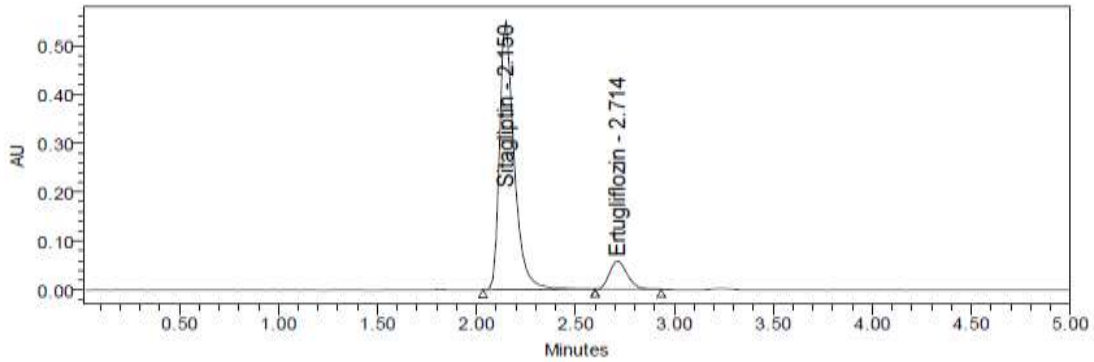

Figure-6: Chromatogram of working sample solution

Table-1: Optimized method Chromatographic conditions:

Parameter	Condition
RP-HPLC	WATERSHPLC2695 SYSTEM equipped with quarternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 software
Mobile phase	0.01N Ammonium acetate:Acetonitrile (60:40)
Flow rate	0.9ml/min
Column	Std Ascentis C18 (4.6 x 150mm, 2.7µm)
Detector wavelength	220nm
Column temperature	30°C
Injection volume	10µL
Run time	8min
Diluents	Water and Acetonitrile in the ratio 50:50
Results	In this trail Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

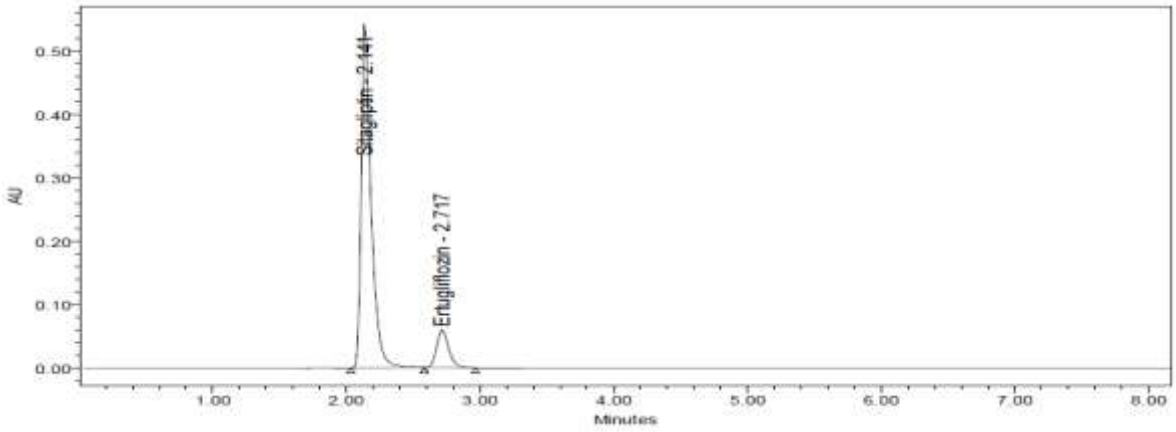

Figure-7: Optimized Chromatogram

Table-2: Precision Results of Sitagliptin and Ertugliflozin

s.no	System precision		Repeatability		Intermediate precision	
	Sitagliptin	Ertugliflozin	Sitagliptin	Ertugliflozin	Sitagliptin	Ertugliflozin
1	2825809	374775	2825268	375393	2808616	378860
2	2801560	373393	2830088	373699	2785129	369315
3	2879932	378963	2796786	380403	2819380	372387
4	2832040	379692	2820903	376372	2798389	375833
5	2869835	375306	2792868	378549	2750301	378156
6	2795873	372335	2840886	379275	2772945	377431
mean	2834775	375744	2817800	377282	2789127	375330
S.D	33901.7	2974.2	19039.7	2551.6	25164.1	3740.5
%RSD	1.2	0.8	0.7	0.7	0.9	1.0

Table-3: Accuracy results of Ertugliflozin (Drug1) and Sitagliptin (Drug2)

%Level	Amount Spiked(µg/ml)		Amount Recovered(µg/ml)		% Recovery		Mean % Recovery	
	Drug 1	Drug 2	Drug 1	Drug 2	Drug 1	Drug 2	Drug 1	Drug 2
50%	2.5	50	2.521	49.701	100.82	99.40	100.61%	99.99%
	2.5	50	2.509	50.528	100.38	101.06		
	2.5	50	2.538	50.161	101.53	100.32		
100%	5	100	5.041	99.703	100.83	99.70		
	5	100	5.025	100.809	100.51	100.81		
	5	100	5.009	100.769	100.17	100.77		
150%	7.5	150	7.568	147.918	100.91	98.61		
	7.5	150	7.541	149.677	100.19	99.78		
	7.5	150	7.512	149.237	100.16	99.49		

Table4: LOD and LOQ Values of Sitagliptin and Ertugliflozin

Molecule	LOD	LOQ
Sitagliptin	0.69	2.10
Ertugliflozin	0.05	0.15

Table-5: Robustness Data of Ertugliflozin and Sitagliptin

S.no	Condition	%RSD of Ertugliflozin	%RSD of Sitagliptin
1	Flow rate (-) 0.8ml/min	1.0	0.2
2	Flow rate (+) 1.0ml/min	1.7	0.8
3	Mobile phase (-) 65B:35A	1.1	1.6
4	Mobile phase (+) 55B:45A	0.6	0.6
5	Temperature (-) 25°C	0.9	0.5
6	Temperature (+) 35°C	1.1	0.9

Table-6: System Suitability Parameters for Sitagliptin and Ertugliflozin

S.no	Sitagliptin			Ertugliflozin			
Inj	**RT(min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**Resolution**
1	2.139	4296	1.52	2.712	4466	1.22	3.9
2	2.140	4266	1.52	2.714	4489	1.22	3.9
3	2.141	4395	1.50	2.715	4627	1.22	4.0
4	2.150	4675	1.47	2.719	4540	1.24	3.7
5	2.150	4677	1.47	2.719	4516	1.23	3.7
6	2.151	4957	1.46	2.722	4599	1.23	3.8

Table-7: Assay Results of Sitagliptin and Ertugliflozin

S.no	% Assay Sitagliptin	%Assay Ertugliflozin
1	99.47	99.51
2	99.63	99.06
3	98.46	100.83
4	99.31	99.77
5	98.32	100.34
6	100.02	100.54
Avg	99.20	100.01
Stdev	0.7	0.68
%RSD	0.7	0.7

Table-8: Degradation data for Sitagliptin and Ertugliflozin

Type of degradation	Sitagliptin			Ertugliflozin		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	2665162	93.83	6.17	354181	93.88	6.12
Base	2708437	95.35	4.65	359368	95.26	4.74
Peroxide	2727122	96.01	3.99	361816	95.91	4.09
Thermal	2768901	97.48	2.52	366383	97.12	2.88
Uv	2784928	98.05	1.95	370129	98.11	1.89
Water	2806313	98.80	1.20	374029	99.15	0.85

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