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A Novel Stress Indicating RP-HPLC Method for Estimation of Ertugliflozin and Sitagliptin in Combined Dosage Form

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ABSTRACT

A Sensitive, fast, linear, and accurate technique was developed for the simultaneous estimation of the Ertugliflozin and Sitagliptin in the tablet dosage form. The column used as X-bridge C18(50 x 2.1 mm, 1.7µ) with Buffer: Acetonitrile took in the ratio 50:50 as mobile phase and pumped through the column at a flow rate of 1 ml/min at 30^0C.The optimized wavelength selected was 240 nm. Retention times of Ertugliflozin and Sitagliptin were found to be 0.883 min and 1.465 min. %Recovery was obtained as 99.94% and 100.08% for Ertugliflozin and Sitagliptin respectively. LOD and LOQ values obtained from regression equations of Ertugliflozin and Sitagliptin were 0.139, 0.421, and 0.18, 0.56 respectively. Regression equation of Ertugliflozin is y = 4269.5x + 853.43, and y = 6890x + 5729.8 of Sitagliptin. Retention times were decreased and that run time was decreased, so the method developed was low cost, easy, sensitive, and fast that can be adopted in regular Quality control tests in companies.

INTRODUCTION

Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters (SGLT), more specifically type 2 which is responsible for about 90% of the glucose reabsorption from the glomerulus. Gliflozins are novel SGLT2 inhibitors, which inhibit glucose reabsorption into plasma by inhibiting SGLT2 located at S1 and S2 segments of proximal renal tubules. Ertugliflozin, chemically is (1S,2S,3S,4R,5S)-5-[4-Chloro-3-(4-ethoxy benzyl) phenyl]-1-hydroxy methyl 6,8 dioxabicyclo octane-2,3,4-triol.Its molecular formula is C22H25ClO7 and its molar mass is 436.9 g/mol. which inhibit SGLT2 and are used for the treatment of T2DM. It also lowers S.B.P. and D.B.P. The novel combination of SIT and ERT, along with diet and exercise, is adopted for the management of T2DM.

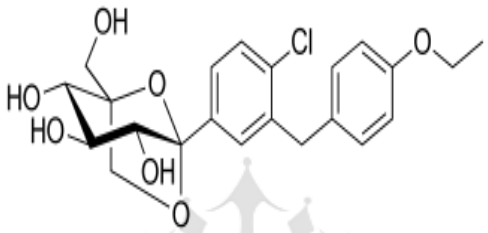

Figure No.1: Chemical Structure of Ertugliflozin

Sitagliptin is chemically called as (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine, is the first DPP-4 (Dipeptidyl peptidase) inhibitor. Its molecular formula is $C_{16}H_{15}F_6N_5O$ and its molar mass is 407.320 g/mol. 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. This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing the breakdown of GLP-1 and GIP, they can increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas.

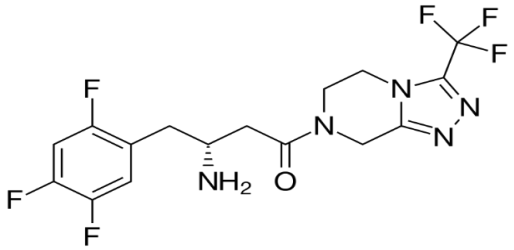

Figure No.2: Chemical structure of Sitagliptin

Materials and Reagents: API of Ertugliflozin and Sitagliptin were kindly gifted from Spectrum labs Hyderabad. HPLC grade Potassium dihydrogen phosphate (KH_2PO_4), acetonitrile procured from Merck, India. Pure water was prepared by using Millipore Milli Q plus a purification system.

METHODS

Diluent

Water: acetonitrile has taken in the ratio 50:50% v/v.

Mobile Phase

0.01N KH_2PO_4: Acetonitrile (50:50, %v/v).

Preparation of buffer

0.01N KH_2PO_4Buffer: weigh accurately 1.36gm of Potassium dihydrogen Ortho phosphate transferred into a 1000ml of Volumetric flask add about 900ml of milli-Q water, sonicate to degas and finally make up the volume with water then adjust $P^H$to 4.8 with dilute Orthophosphoric acid solution.

Preparation of standard solution

Weigh Accurately about 7.5 mg of Ertugliflozin, 50 mg of Sitagliptin and transferred to 50ml volumetric flasks diluted with 3/4 th of diluent, sonicated for 10 minutes, and made up to the mark with diluent. Working standards were prepared by serial dilution of stock solution of ERT and SIT 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, and 1.5ml with diluent in a 10-ml volumetric flask to produce concentration about 3.75, 7.5, 11.25, 15, 18.75, and 22.5µg/ml of ERT and 25, 50, 75, 100, 125, and 150µg/ml of SIT.

Preparation of Sample s solutions

20 tablets were weighed accurately and grinded in a mortar with a pestle into a fine powder. Accurately weighed tablet powder equivalent to 7.5 mg of ERT and 50 mg of SIT were transferred into a 50 ml volumetric flask, diluted with diluent, and sonicated for 25 min, further the volume was made up to the mark with diluent and filtered by 0.45 µ membrane filter. Take 1ml of the above-filtered solution, diluted to 10 ml with diluent to produce a concentration of 15 µg/ml and 100 µg/ml of ERT and SIT respectively.

Chromatographic conditions

Analysis was carried out by using WATERS HPLC 2695 SYSTEM fitted with quaternary pumps, Photo Diode Array Detector, and Auto Sampler integrated with Empower 2 software. The wavelength of detection was set at 240nm. Separation was mode on X-bridge C18(50 x 2.1 mm, 1.7µ) using a mobile phase consisting of a mixture of 0.01N $KH_2PO_4$and Acetonitrile in the ratio of 50:50 v/v at a flow rate of1ml/min and the column temperature was maintained at 30^oC.

Method development

A series of trials were conducted with different columns with different mobile phase ratios to develop a suitable RP-HPLC method for the estimation of Ertugliflozin and Sitagliptin in bulk and tablet dosage forms. X-bridge column was found to be satisfactory for better separation and good resolution, and analytes were checked with a PDA detector at 240nm was considered satisfactory for detecting both the drugs with adequate sensitivity.

Method validation

The developed method was validated as per ICH guidelines The validation covers system suitability, linearity, precision, accuracy, ruggedness LOD, LOQ, and forced degradation studies.

System suitability

To ensure the system suitability parameters, the standard solutions were prepared as per the test method and injected into the chromatographic system. The parameters such as theoretical plates, resolution, and asymmetric factor were evaluated. The system suitability parameters were tabulated in Table No.1. All the parameters were found to be within the limits.

Specificity

To check the specificity of the developed analytical method, blank and placebo injections were prepared as per the test method and injected into the chromatographic system. From the results, it was found that there were no interfering peaks at the retention times of analytes. Hence the results manifest that the developed method was said to be specific for the estimation of Ertigliflozin and sitagliptin.

Precision

The precision of analysis was accomplished in terms of inter-day precision and intra-day precision. Precision is expressed in terms of% RSD (Table No:3).

Linearity

Linear concentrations of both drugs were prepared and the best fit line was calculated. Wide range calibration was determined by solutions containing 3.75µg/ml to 22.5µg/ml for Ertugliflozin and25µg/ml to 150µg/ml for Sitagliptin. Correlation coefficients were found to be 0.9994&0.9995for Ertugliflozin& Sitagliptin respectively (Fig No:5&6).

Accuracy

The accuracy of the method was established by performing recovery studies according to the ICH guidelines. Spiked samples were prepared by spiking pre-analyzed sample solutions with the pure drug at three different concentration levels each in triplicate. Mean percentage recovery values at three different concentrations of the two drugs were calculated. The % mean recovery of Ertugliflozin (99.94%) &sitagliptin (100.08%) (Table No.4).

Limit of detection and limit of quantitation

LOD and LOQ were calculated using the formulae based on the standard deviation of the y-intercept of regression lines and the slope of the calibration curve. LOD and LOQ were calculated using the following formula.

$$LOD=3.3\sigma/SLOD=3.3\sigma/S \quad LOQ=10\sigma/SLOQ=10\sigma/S$$

S is the mean of the slope, and σ is the standard deviation of the intercept.

The LOD and LOQ of Ertugliflozin and Sitagliptin were experimentally calculated by injecting six injections of each drug and the results were given in Table No.5. The results of LOD and LOQ confirmed that the developed method has good sensitivity.

Robustness

To ensure the inerrancy of the method by altering the chromatographic conditions like column temperature, mobile phase composition, flow rate, etc can be reported. Minute changes in the operational conditions were allowed and the extent to which the method was robust was determined. The results were reported in TableNo.6.

Forced Degradation Studies

Stress degradation studies were performed as per the ICH guidelines (R2) Stability Testing of New Drug Substances and Products, using the proposed validated analytical method.

Acid Degradation:

Take 1ml of the standard stock solution and add 1ml of 2N Hcl refluxed for 30min 60^0C. From the above solution10 µl was injected into the system and the chromatograms were recorded to evaluate the stability of the sample. (FigNo.7))

Alkali Degradation Studies:

Take one ml of standard stock solution of Ertugliflozin and Sitagliptin, add one ml of 2N sodium hydroxide, and reflux for 30mins at 60^0C. The solution was diluted to the required concentration and fed 10µl into the HPLC system, record chromatograms to report stability (FigNo.8).

Oxidative Degradation

Take 1ml of standard stock solution of Ertugliflozin and Sitagliptin, was subjected to oxidative degradation by refluxing with 20% v/v H_2O_2 for 30 min at 60°C. After that, cool it down and dilute to optimized concentration then inject 10 µl into HPLC and calculate the degradation percentage(Fig No.9).

Dry Heat Degradation Studies:

Take the standard drug solution and hold it in the oven for 6 hours at 105 ° C to test dry heat degradation, the solution was diluted to the required concentrationand10µl was introduced into the device and the chromatograms were reported to verify the stability (Fig No.10).

Photo Stability Studies:

Photolytic degradation was carried out by exposing the standard solution of Ertugliflozin and Sitagliptin to UV light in the UV chamber for 1 day and the resultant solution was diluted to the required concentration, 10µl were introduced into the HPLC system, and chromatograms were recorded to assess the stability (Fig No.11).

Neutral Degradation Studies:

In neutral degradation studies refluxing the medication with water at a temperature of 60ºC for 6hrs. The resulting solution was diluted to a suitable concentration, about 10 µl of the solutions were injected into the column, and the chromatograms were recorded to assess the stability of the drugs (Fig No.12).

RESULTS AND DISCUSSION

In this study, separation of ERT and SIT was performed on the C18 column. Optimization of mobile phase composition was performed based on resolution among drugs and degradation products, asymmetric factor, and theoretical plates. The mobile phase consisted of 0.01N KH2PO4 and acetonitrile in a ratio of 50:50 % v/v was selected to give sharp and well-resolved peaks for ERT and SIT. The retention times for ERT and SIT were 0.883±0.3 and 1.465±0.2 min, respectively. UV overlaid spectra of ERT and SIT showed that both the drugs absorbed appreciably at 240 nm, so the same wavelength was selected as the detection wavelength during degradation studies. The optimized method was validated as per ICH Q2 guidelines. The method was specific in presence of degradation products as shown in figs. 7 to 12. The linearity was proven by calibration curves and it was found to be linear over the range of 3.75-22.5 µg/ml for ERT and 25-150 µg/ml for SIT. The data of correlation coefficient (R^2 value) for ERT and SIT were 0.9994 and 0.9995, respectively. The intermediate precision was tested by interday precision and was found to be less than 1.5% against the acceptance limit of 2%. The low RSD (%) value indicated that the method is more precise. The recovery studies for accuracy were tested at three different levels (50, 100, and 150%) and their results were between 98-102%, thus the accuracy of the method was proven. The detection limits (LOD) for ERT and SIT were 0.139 µg/ml and 0.18µg/ml, respectively, while quantitation limits (LOQ) were 0.421 and 0.56µg/ml respectively. The lowest LOD value indicated the method is more sensitive. The robustness of the method was studied by changing the flow rate of the mobile phase from 1 ml/min to 0.9 ml/min and 1.1 ml/min and pH change of ±0.1 units. The mobile phase composition was changed to 65:35, by increasing the percentage of buffer, the retention time for ERT and SIT were observed to be 0.892 min and 1.528 min, respectively. The corresponding assay value for all robust conditions was between 98-102% against the Assay value of optimized conditions. Solution stability study was done as a part neutral degradation study and the results revealed that ERT and SIT were stable in solution up to 24 h. The method has fulfilled all validation parameters as per ICH

guidelines. A forced degradation study was carried out by subjecting both drugs to acid and alkali hydrolysis, chemical oxidation, and photolytic and thermal degradations.

CONCLUSION

A simple, accurate, and precise method was developed for the simultaneous estimation of the Ertugliflozin and Sitagliptin in the Tablet dosage form. The results of stress testing undertaken according to the ICH guidelines reveal that the method is specific and stability indicating. **CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGMENTS

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ABBREVIATIONS

RP-HPLC: Reverse phase high-performance liquid chromatography; ICH: International council for harmonization; LOD: Limit of detection; LOQ: Limit of quantification; T2DM: Type 2 diabetes mellitus; SIT: Sitagliptin; ERT: Ertugliflozin; DPP: Dipeptidyl peptidase; FDA: Food and Drug Administration; GLP: Glucagon-like peptide; SGLT: Sodium-glucose cotransporter; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

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Table No.1: System suitability parameters for Ertugliflozin and Sitagliptin

S no	Ertugliflozin			Sitagliptin			
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 No.2: Linearity of Ertugliflozin and Sitagliptin.

Ertugliflozin		Sitagliptin	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
3.75	16526	25	179882
7.5	31857	50	352998
11.25	50107	75	526199
15	64415	100	688920
18.75	80398	125	881276
22.5	96760	150	1028107

Table No.3: System precision table of Ertugliflozin and Sitagliptin

S. No	Area of Ertugliflozin	Area of Sitagliptin
1.	64450	684926
2.	64263	690546
3.	64432	697041
4.	64128	693133
5.	63679	699376
6.	63500	703947
Mean	64075	694828
S.D	398.4	6752.7
%RSD	0.6	1.0

Table No.4: Accuracy data of Ertigliflozin and Sitagliptin

% level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% recovery	Mean % Recovery	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% recovery	Mean % Recovery
50%	7.5	7.53	100.40	99.94%	50	49.77	99.54	100.08%
	7.5	7.49	99.86		50	50.07	100.14	
	7.5	7.47	99.60		50	49.61	99.22	
100%	15	15.12	100.80		100	101.6	101.6	
	15	14.77	98.46		100	99.06	99.06	
	15	14.92	99.46		100	99.57	99.57	
150%	22.5	22.63	100.58		150	151.28	100.85	
	22.5	22.18	98.58		150	149.57	99.71	
	22.5	22.66	100.71		150	151.56	101.04	

Table No.5: Sensitivity table of Ertugliflozin and Sitagliptin

Molecule	LOD	LOQ
Ertugliflozin	0.139	0.421
Sitagliptin	0.18	0.56

Table No.6: Robustness data for Ertugliflozin and Sitagliptin

S.no	Condition	%RSD of Ertugliflozin	%RSD of Sitagliptin
1	Flow rate (-) 1.1ml/min	0.45	0.4
2	Flow rate (+) 1.3ml/min	0.4	0.8
3	Mobile phase (-) 75B:25A	0.94	0.4
4	Mobile phase (+) 65B:35A	0.47	0.4
5	Temperature (-) 25°C	0.47	0.9
6	Temperature (+) 35°C	0.80	0.3

Table No.7: Assay Data of Ertugliflozin

S.no	Standard Area	Sample area	% Assay
1	64450	63900	99.63
2	64263	64016	99.81
3	64432	63803	99.48
4	64128	64109	99.95
5	63679	64176	100.06
6	63500	63782	99.44
Avg	64075	63964	99.73
Stdev	398.4	162.4	0.25
%RSD	0.6	0.3	0.25

Table No.8: Assay Data of Sitagliptin

S.no	Standard Area	Sample area	% Assay
1	684926	692670	99.59
2	690546	694710	99.88
3	697041	696705	100.17
4	693133	690323	99.25
5	699376	699040	100.51
6	703947	705746	101.47
Avg	694828	696532	100.15
Stdev	6752.7	5440.7	0.7822
%RSD	1.0	0.8	0.8

Table No.9: Degradation data of Ertuglifazolin and Sitagliptin

Drug Name	Parameters	% Drug degraded
Ertuglifazolin	Acid	8.24
	Alkai	5.76
	Oxidation	7.03
	Thermal	3.92
	UV	1.63
	Water	0.61
Sitagliptin	Acid	7.73
	Alkali	4.45
	Oxidation	4.53
	Thermal	5.55
	UV	1.89
	Water	0.73

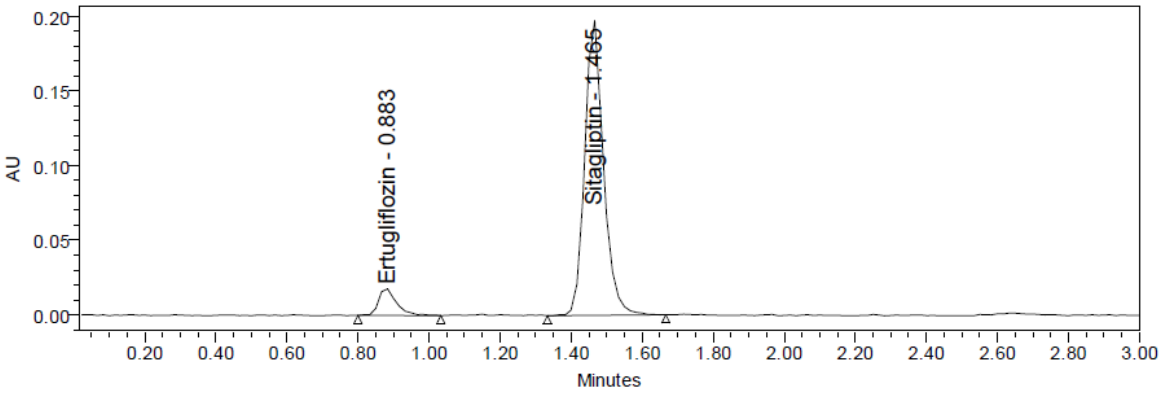

Figure No.3: Chromatogram of standard

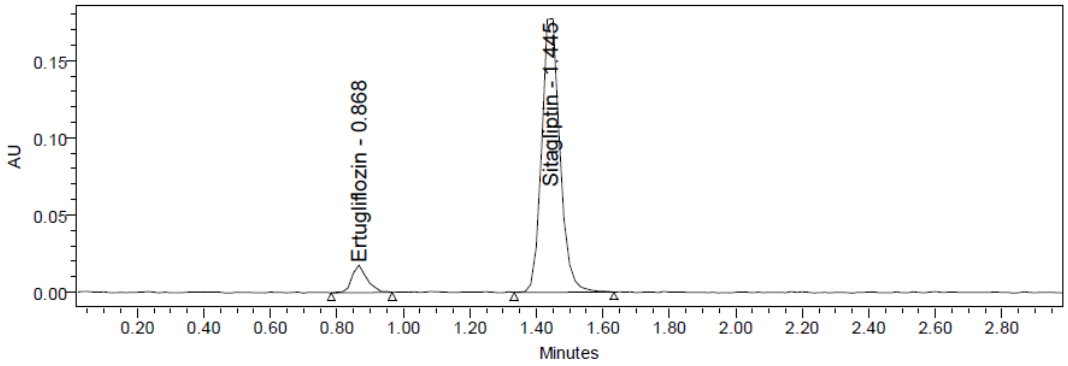

Figure No.4: Chromatogram of sample

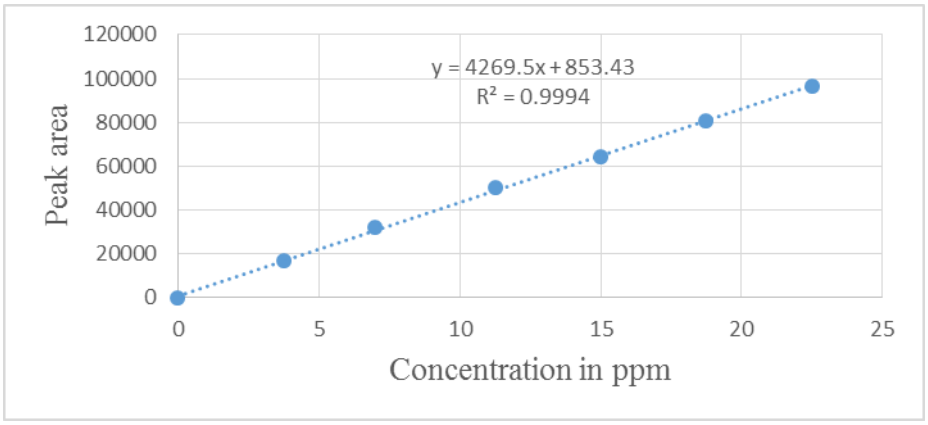

Figure No.5: Calibration curve of Ertigliflozin

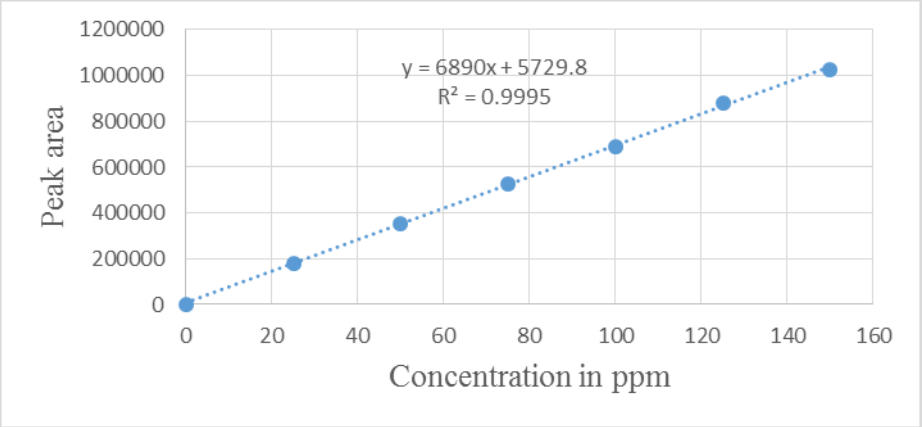

Figure No.6: Calibration curve of Sitagliptin

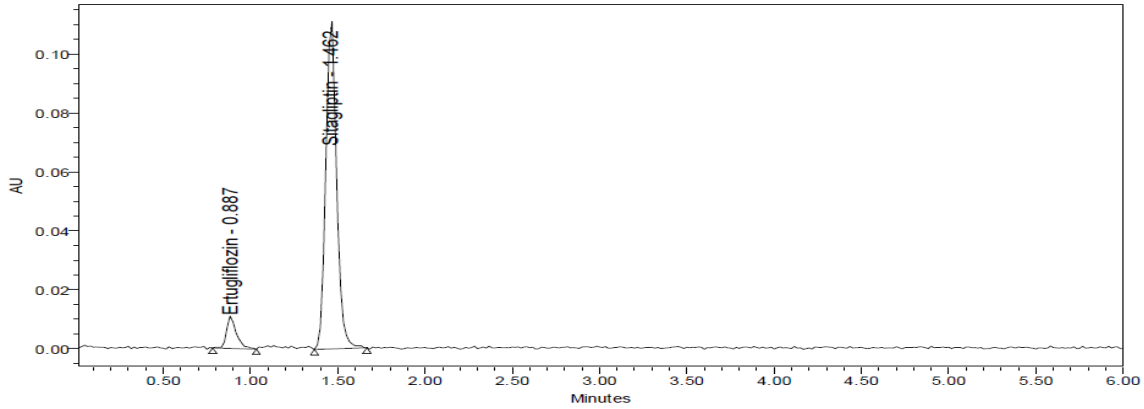

Figure No.7: Acid chromatogram of Ertugliflozin and Sitagliptin

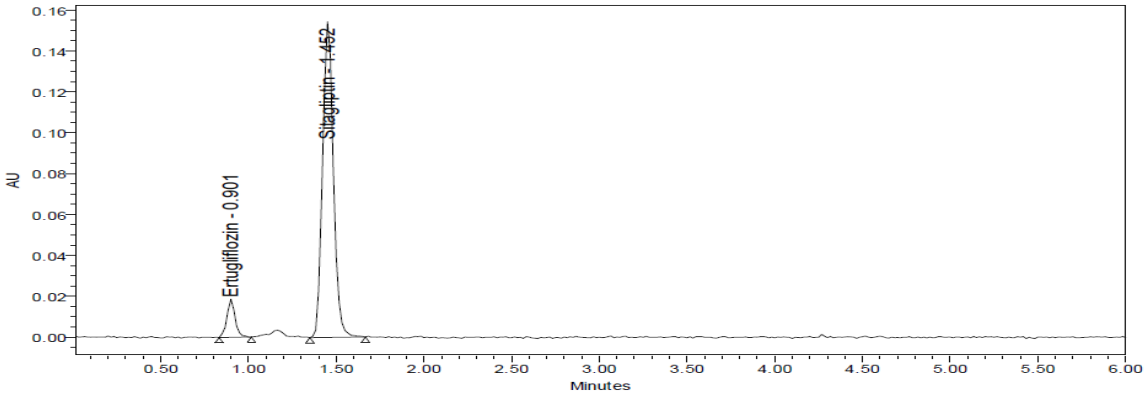

Figure No.8: Base chromatogram of Ertugliflozin and Sitagliptin

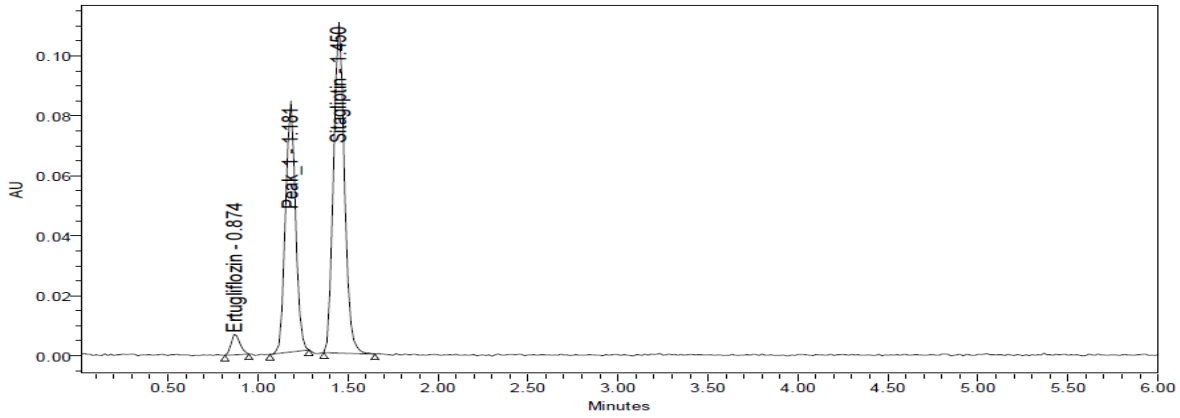

Figure No.9: Peroxide chromatogram of Ertugliflozin and Sitagliptin

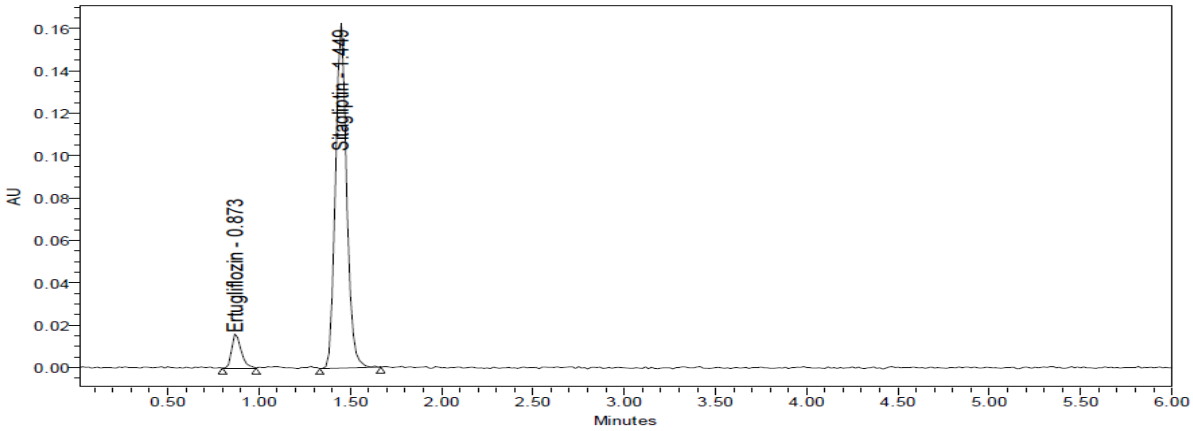

Figure No.10: Thermal chromatogram of Ertugliflozin and Sitagliptin

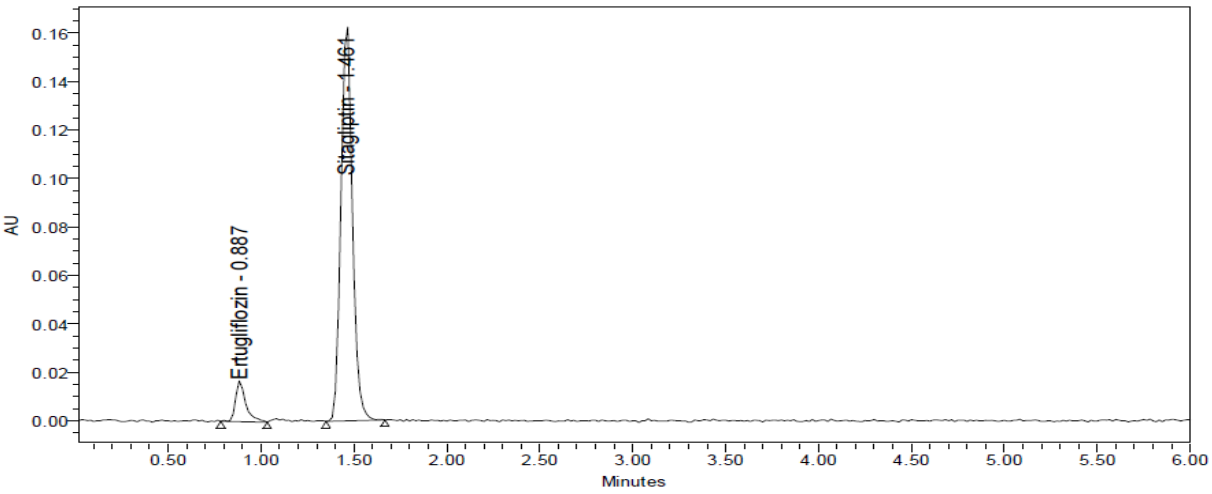

Figure No.11: UV chromatogram of Ertugliflozin and Sitagliptin

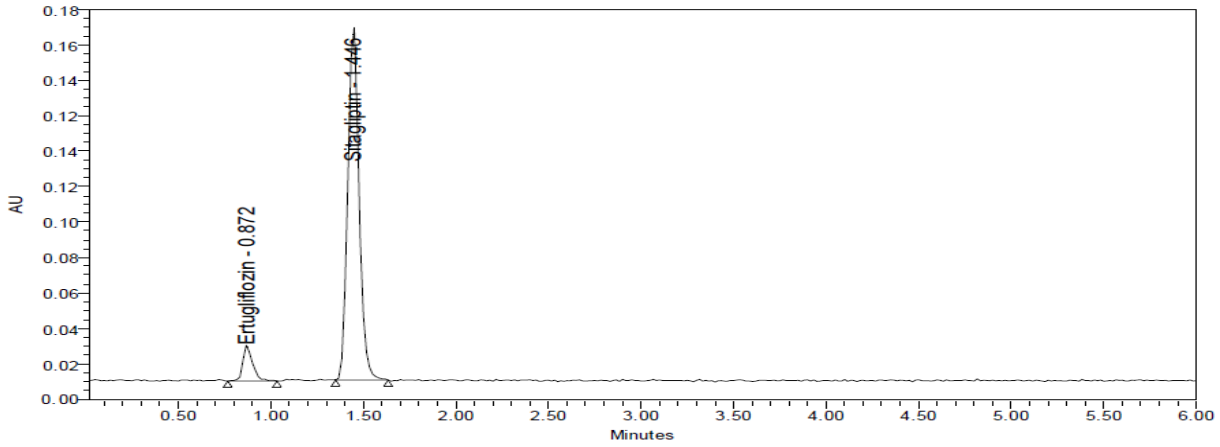

Figure No.12: Water chromatogram of Ertugliflozin and Sitagliptin