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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ERTUGLIFLOZIN AND SITAGLIPTIN BY RP- HPLC

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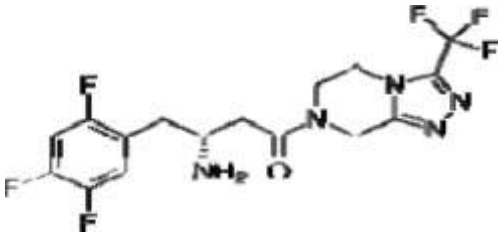
ABSTRACT

A new sample, sensitive, accurate, precise and reproducible RP-HPLC method has been developed for the simultaneous estimation of sitagliptin and ertugliflozin in bulk and pharamaceutical dosage form using ODS column in isocracticmode.the mobile phase consisted 0.1% TFA methanol: Acetonitraile the detection was carried out at 250nm.The method was linear over the concentration range for sitagliptin 40-200µg/ml and for ertugliflozin 6-30µg/ml the recoveries of sitagliptin and ertugliflozin was found to be 100.26 and 100.18 respectively. The LOD and LOQ for sitaglptin were found to be 1.140 and 3.450 respectively. The LOD and LOQ for ertugliflozin were found to be 0.324 and 0.981 respectively. The validation method were carried out utilizing ICH gulidelines . the described HPLC method for the analysis of pharmaceutical formulation containing combined dosage forms.

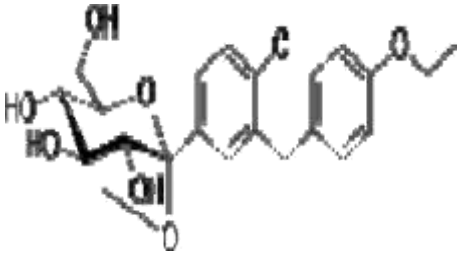
INTRODUCTION

Sitagliptin is an oral dipeptidyl peptidiase (DPP-4) inhibitor used in conjuction with diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus. The effect of this medication leads to glucose dependent increases in insulin and decreases in glucagon to improve control of blood sugar. Sitagliptin was granted FDA approval on oct-16-2006.

STRUCTURE OF SITAGLIPTIN



Ertugliflozin is a hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase(DPP-4) inhibitor class of drugs.Thisezyme is inhibiting drug is to be used alone or incombination with metformin or a thiazolinedione.



STRUCTURE OF ERTUGLIFLOZIN

HPLC METHOD DEVELOPMENT INSTRUMENTATION

The chromatographic system consisted of a waters H-Class UPLC chromatogram equipped with BEH C18 100X2.1mm, 1.8µ, LC-20AD pumps and an SPD-20A photo diode array(PDA) detector. Samples were injected into the system through a Rheodyne 7725 injector valve via and integrated by empower-2 software.Soluble of the compound was enhanced by sonication on an

ultrasonicator (PCI Analytics PC181). PVDF membrane filters used for filtration were purchased from Merck Millipore.

DRUGS AND CHEMICALS

The reference standard samples of sitagliptin and Ertugliflozin were obtained from suven life sciences Ltd. Acetonitrile and Orthophosphoric acid and used were of HPLC grade , milli-Q water was used throughout the analysis.

PREPARATION OF 0.1% ORTHOPHOSPHORIC ACID:

Transferred 1ml of orthophosphoric acid in 1000ml flask, made up the volume with water and mixed well.

PREPARATION OF THE MOBILE PHASE :

0.1% OPA and acetonitraille was mixed in the ratio of 55.45% v/v and sonicated to degas.

PREPARATION OF DILUENT :

Water and acetonirtaile was mixed in the ratio of 50:50% v/v and used as diluent for preparing drug solutions.

PREPARATION OF THE MIXED WORKING STANDERED AND SOLUTION OF SITAGLIPTIN AND ERTUGLIFLOZIN:

Accurately weighed and transfeered 25 mg of the sitagliptin and 10mg of ertugliflozin working standards into a 25ml clean dry volumetric flask ,add ¾ volume of diluent sonicated for 5min and make upto volume with diluents (100ppm of sitagliptin and 15ppm of ertugliflozin).

METHOD OF VALIDATION

ACCURACY

To determine the accuracy of the proposed method, different amounts of bulk sample of Sitagliptin and Ertugliflozin within linearity limits were taken and analyzed by the proposed method.Accuracy for Sitagliptin and Ertugliflozin was conducted by spiking therug to the placebo powder at three different levels of the test concentration (i.e. 50%, 100%, and 150%) and each level three times. The mean % Recovery and % RSD values were calculated. The %Recovery value was found to be in between 98.0 % to 102.0%.

`PRECISION

To ascertain theneffectiveness of method system suitability tests were carried out on freshly prepared standard stock solution containing 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin. 2 µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the peak response of sample were calculated.

LINEARITY

To establish linearity, a stock solution containing 1000 µg/mL of Sitagliptin and 150 µg/mL of Ertugliflozin were prepared using diluent and further diluted to yield solutions in the concentration range of 25-150 µg/mL of Sitagliptin and 3.75-22.5 µg/mL Ertugliflozin. The solutions were prepared and analyzed in triplicate. The experiment was repeated thrice by preparing different solution and analyzed by injecting 2 µL in UPLC.

SYSTEM SUITABILITY

System suitability was assessed by analyzing the mixed standard drug solution (100 ppm of Sitagliptin and 15 ppm of Ertugliflozin) and calculating the chromatographic parameters such as resolution, theoretical plates, and tailing factor.

SPECIFICITY:

Specificity is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and the excipients) showed almost no interfering peaks within retention time ranges. Fig. 6.5 and 6.6 show the representative chromatograms for standard and the formulation. The figures show that the selected drugs were clearly separated. Thus the proposed UPLC method is selective.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

LOD and LOQ values were calculated from the average standard deviation and slope from the calibration curve as per ICH guideline.

ROBUSTNESS:

Robustness study was done by applying small deliberate changes in the

chromatographic conditions and studying the system suitability parameters of both the drugs. The conditions selected for testing were the flow rate, column oven temperature and composition of the mobile phase. The study was conducted on a mixed standard solution containing 100 µg/ml of Sitagliptin and 15 µg/ml of Ertugliflozin. The results remained unaffected by small variations in these conditions.

FORCED DEGRATION STUDIES

PREPARATION OF STANDARD STOCK SOLUTION OF SITAGLIPTIN AND ERTUGLIFLOZIN

Accurately Weighed and transferred 25 mg of Sitagliptin and 10 mg of Ertugliflozin working Standards into a 25 mL clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 min and make up to the final volume with diluents. (1000 ppm of Sitagliptin and 150 ppm of Ertugliflozin).

Acid induced degradation study

In acid hydrolytic degradation, to 5 mL of standard stock solution of Sitagliptin and Ertugliflozin 5 mL of 1 N Hydrochloric acid was added and refluxed for 60 min at 60^0C and then neutralized with 5 mL of 1N sodium hydroxide solution. The resultant solution was diluted to obtain 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin, 2 µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

Base induced degradation study

In base hydrolytic degradation, to 5 ml of standard stock solution of Sitagliptin and Ertugliflozin 5 ml of 1N sodium hydroxide solution was added and refluxed for 60 min at 60^0C and then neutralized with 5 ml of 1N Hydrochloric acid. The resultant solution was diluted to obtain 100 µg/ml of Sitagliptin and 15 µg/ml of Ertugliflozin, 2µl solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

Peroxide induced degradation study

In peroxide degradation, 5 mL of 20% hydrogen peroxide (H2O2) was added to 5 mL of standard stock solution of Sitagliptin and Ertugliflozin. The solutions were kept on Bench top for 60 min. For UPLC study, the resultant solution was diluted to obtain 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin, 2 µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

Thermal induced degradation study

In thermal degradation, 5 mL of standard stock solution of Sitagliptin and Ertugliflozin was placed in oven at 105°C for 12 h. For UPLC study, the resultant solution was diluted to obtain 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin, 2 µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

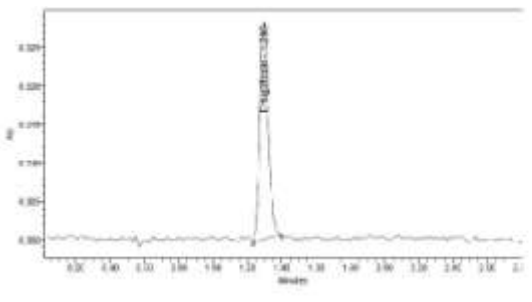
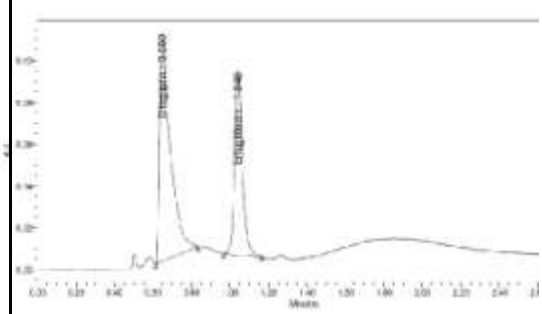
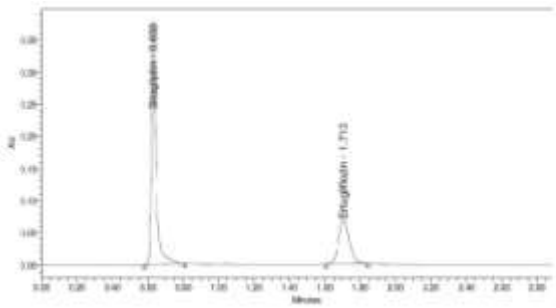
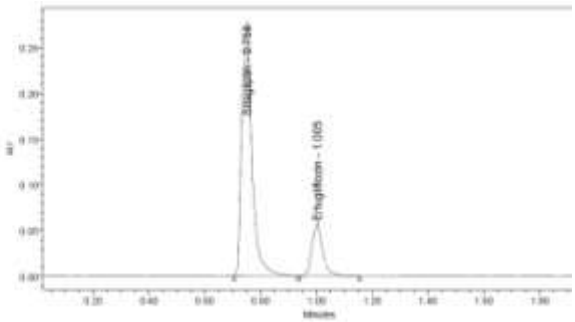
Neutral degradation study

In Neutral degradation, To 5 mL of standard stock solution of Sitagliptin and Ertugliflozin 5 mL of water was added and refluxed for 6 h at 60^0C. The resultant solution was diluted to obtain 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin, 2µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

Photolytic degradation study

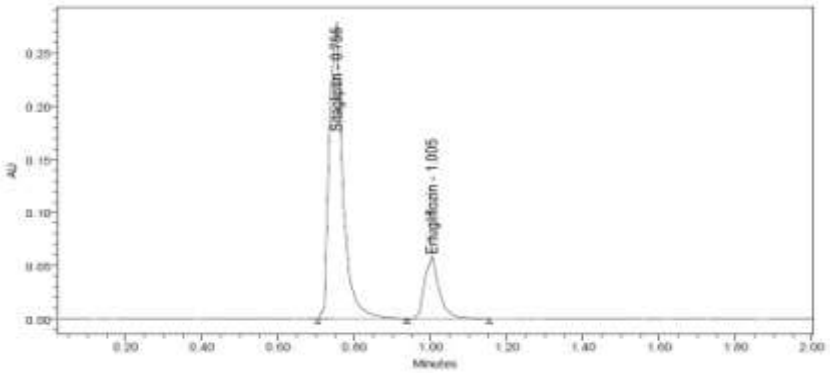
The photochemical stability of the drug was also studied by exposing the 5 mL of standard stock solution of Sitagliptin and Ertugliflozin to UV Light by keeping the beaker in UVChamber for 1hours or 200-Watt hours/m^2 in photo stability chamber. For UPLC study, the resultant solution was diluted to obtain 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin, 2 µL solutions were injected into the UPLC system and the chromatograms were recorded to assess the stability of sample.

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS AND METHOD DEVELOPMENT

	
Trial-1 **CHS C18 100 X 2.1mm 1.7µ** **Methanol: Water (50: 50%v/v)**	**Trail-2** **SB C18 100 X 2.1mm 1.7µ column,** **Acetonitrile : water (50:50%v/v)**
	
Trial-3 **CHS C18 100 x 2.1mm 1.7µ column,** **Acetonitrile : OPA (50:50 %v/v)**	**Optimized Conditions** **BEH C18 100 X 2.1mm 1.7µ column,** **Acetonitrile : OPA (50:50%v/v)**

OPTIMIZED CHROMATOGRAPHIC CONDITIONS OF THE PROPOSED METHOD

S. No.	Parameter	Value
1	Stationary phase	BEH C18 100 X 2.1mm 1.8□
2	Mobile phase	0.1% OPA: Acetonitrile
3	Flow rate	0.3 mL/min
4	Column temperature	30ºC
5	Volume of injection	2 µL
6	Detection wavelength	220 nm
7	Run time (min)	2 min



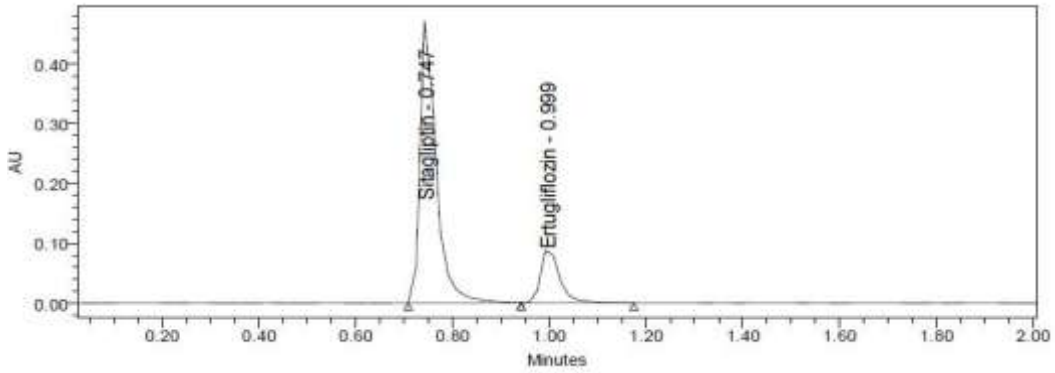
TYPICAL CHROMATOGRAM OF THE MIXED STANDARD SOLUTION SITAGLIPTIN AND ERGITLIFLOZIN

RECOVERY OF ERTUGLIFLOZIN

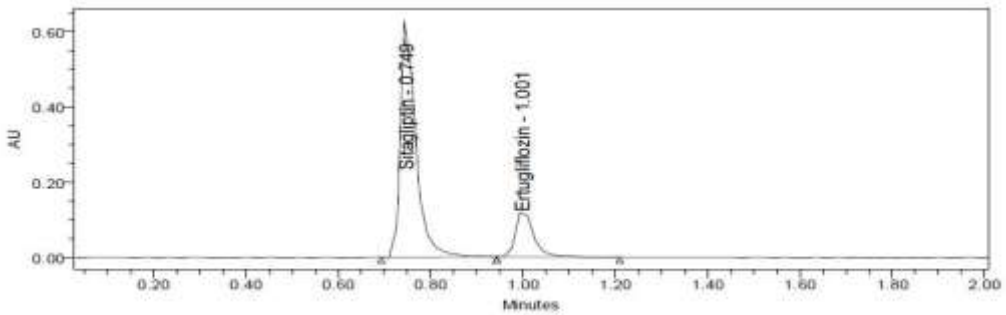
Accuracy Level	Peak area	Amount added(µg/mL)	Amount Found(µg/mL)	Recovery %	Mean % Assay
50%	76841	7.5	7.5122	100.16	
	77021	7.5	7.5294	100.39	100.3
	77003	7.5	7.5277	100.37	
100%	154650	15	14.965	99.77	
	154120	15	14.914	99.43	99.49
	153894	15	14.893	99.28	
150%	232920	22.5	22.462	99.83	
	232914	22.5	22.462	99.83	99.92
	233560	22.5	22.524	100.10	

RECOVERY OF SITAGLIPTIN

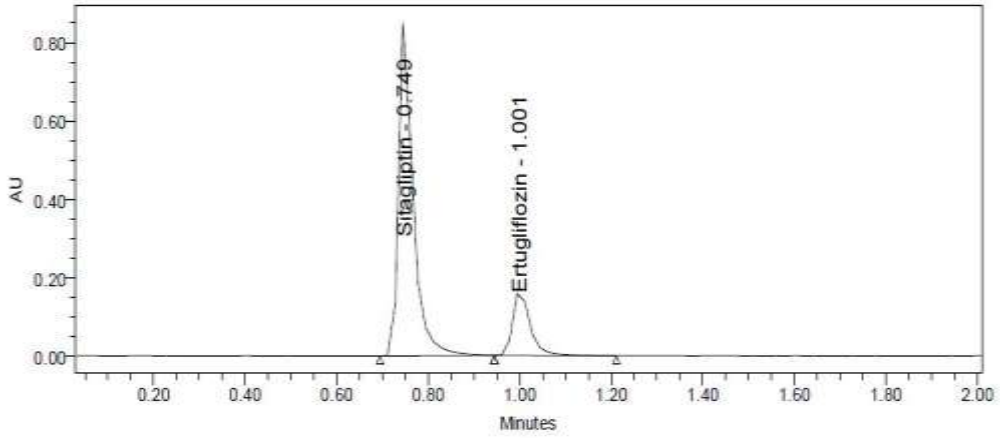
Accuracy Level	Peak area difference	Amountadded (µg/mL)	Amount Found(µg/mL)	% Recovery	Mean % Assay
	346850	50	50.479	100.96	
50%	337108	50	49.093	98.19	99.90
	345437	50	50.278	100.56	
	690644	100	99.362	99.36	
100%	698072	100	100.420	100.42	99.97
	695968	100	100.120	100.12	
	1037264	150	148.65	99.10	
150%	1040630	150	149.12	99.42	99.34
	1041414	150	149.24	99.49	



TYPICAL CHROMATOGRAM OF ACCURACY AT 50% LEVEL



TYPICAL CHROMATOGRAM OF ACCURACY AT 100% LEVEL
TYPICAL CHROMATOGRAM OF ACCURACY AT 150% LEVEL

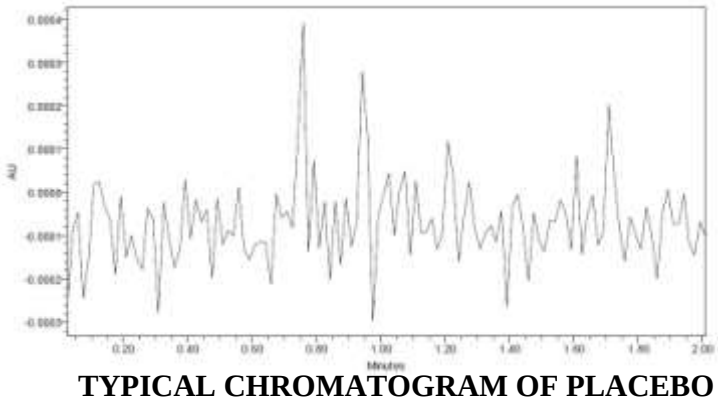


INTRA-DAY PRECISION DATA

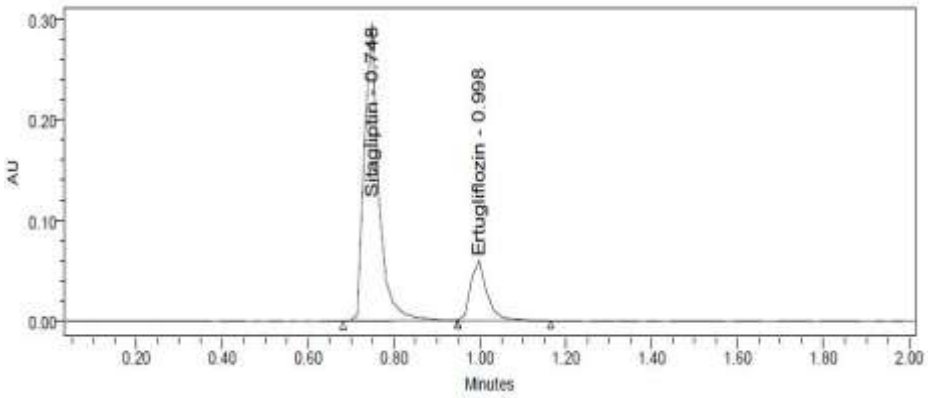
S.NO	**Injection**	**Sitagliptin**	**Ertugliflozin**
1.	Injection-1	524622	135420
2.	Injection-2	522093	135128
3.	Injection-3	522996	134883
4.	Injection-4	526012	134106
5.	Injection-5	522371	134545
6.	Injection-6	529315	132149
	Mean	524568	134372
	SD	2758.5	1181.0
	% RSD	0.5	0.9

INTERMEDIATE PRECISION DATA

S.NO	**Injection**	**Sitagliptin**		**Ertugliflozin**	
		Day-1	**Day-2**	**Day-1**	**Day-2**
1.	Injection-1	524622	514622	155420	151420
2.	Injection-2	522093	512093	155128	153710
3.	Injection-3	522996	522996	154883	150240
4.	Injection-4	526012	521012	154106	151720
5.	Injection-5	522371	521371	154545	152470
6.	Injection-6	529315	520315	152149	151179
	Mean	521652		154372	
	SD	4611.72		1756.72	
	% RSD	0.8		1.15	



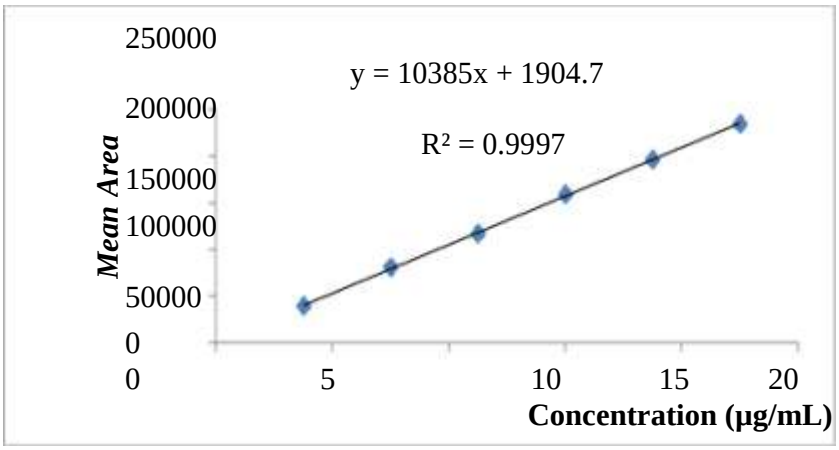
TYPICAL CHROMATOGRAM OF PLACEBO



TYPICAL CHROMATOGRAM OF SITAGLIPTIN AND ERTUGLIFLOZIN

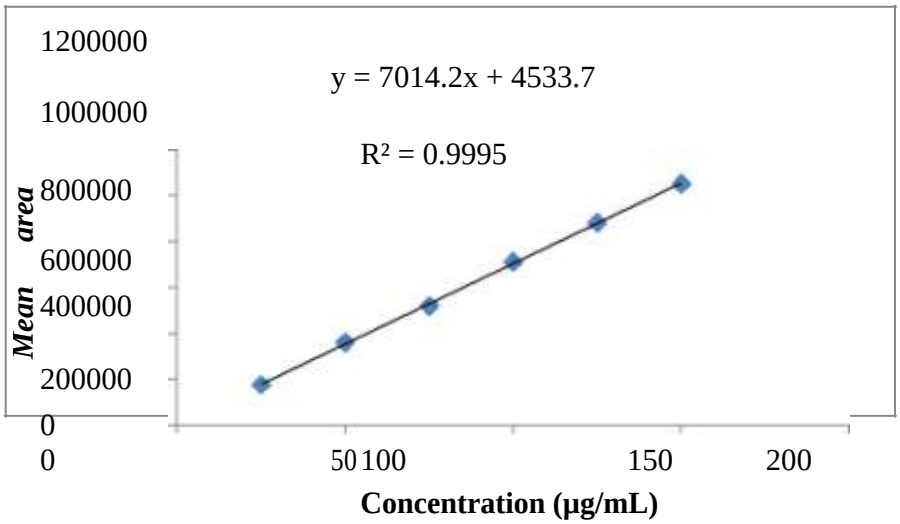
LINEARITY OF ERTUGLIFLOZIN

Concentrationof ertugliflozin(µg/mL)	Peak area	Mean area	RSD
3.75	38947	39830	2.31
	40780		
	39764		
7.5	80434	81399	2.95
	79633		
	84131		
11.25	113285	117314	2.97
	119319		
	119339		
15	155435	159206	2.05
	161287		
	160895		
18.75	190430	196545	3.62
	204356		
	194850		
22.5	234982	234980	0.01
	234949		
	235009		



LINEARITY OF SITAGLIPTIN

Concentration of Sitagliptin (µg/mL)	Peak Area	Mean Area	RSD
25	183485	177139	3.85
	178016		
	169917		
50	362655	362110	1.27
	366395		
	357280		
75	527131	520121	1.85
	509152		
	524081		
100	731128	713894	3.89
	681833		
	728721		
125	894455	883144	1.14
	879776		
	875200		
150	1063368	1053250	0.83
	1048390		
	1047992		

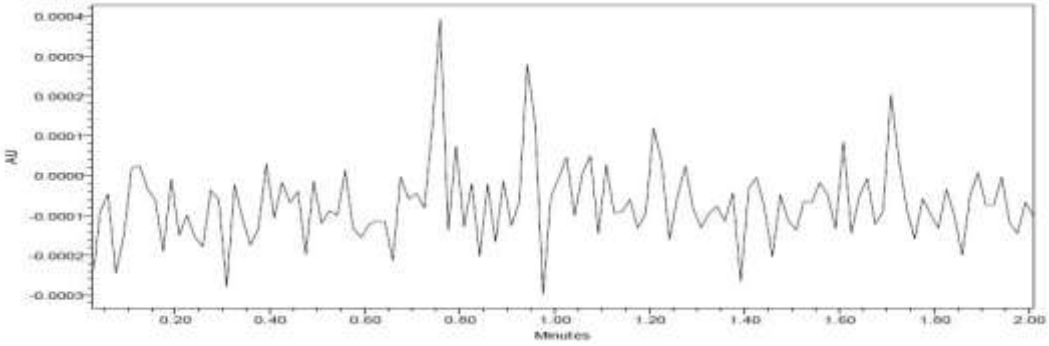


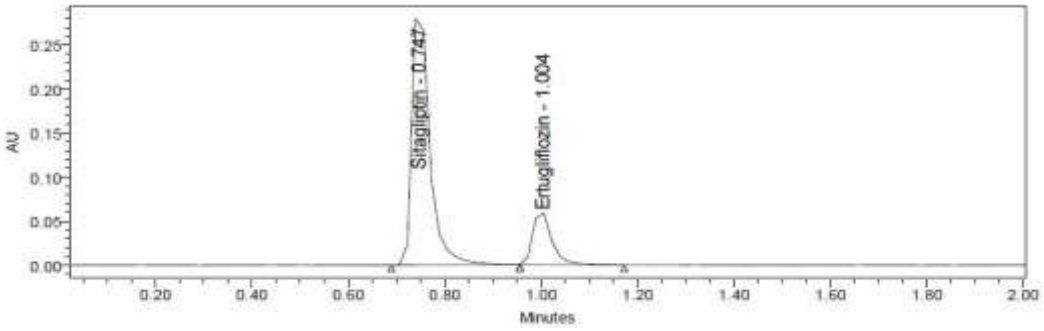
LINEARITY PLOT OF SITAGLIPTIN

SYSTEM SUITABILITY

	Parameter	Sitagliptin	Ertugliflozin
1.	Retention time (min)	0.747	0.998
2.	Peak area	660704	141262
3.	Resolution	-	3.7
4.	Theoretical Plates	2395	3437
5.	Tailing Factor	1.52	1.26

SPECIFICITY TYPICAL CHROMOTOGRAM OF PLACEBO





SPECIFICITY TYPICAL CHROMOTOGRAM OF ERTUGLIFLOZIN AND SITAGLIPTIN

LOD AND LOQ VALUES OF THE METHOD

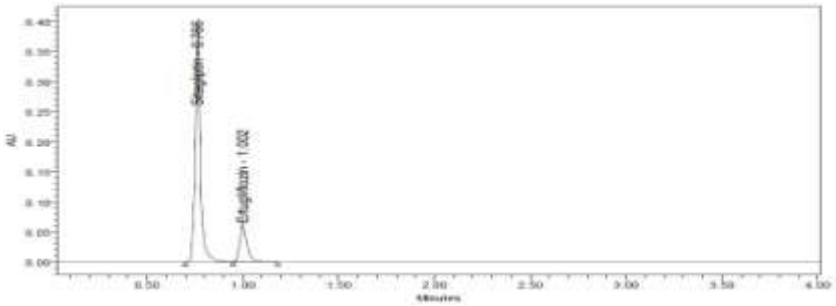
S. No	Parameter	Sitagliptin		Ertugliflozin	
1	LOD	1.140		0.324	
2	LOQ	3.450		0.981	

ROBUSTNESS DATA OF ERTUGLIFLOZIN

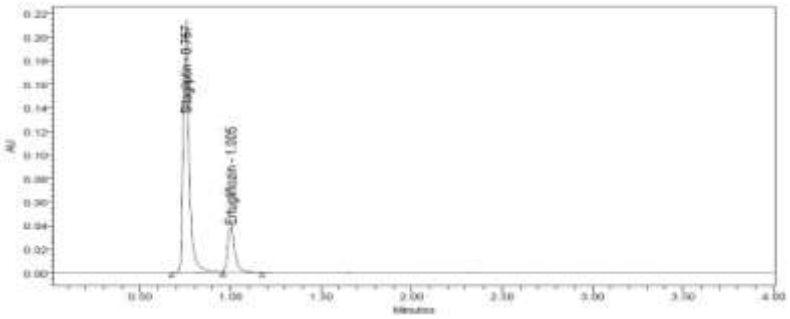
Chromatographic conditions	% assay	Theoretical Plates	Asymmetry	Resolution	Retention time
Water: Methanol(60:40% v/v)	99.07	3435	1.29	3.9	0.985
Water: Methanol (40:60% v/v)	98.99	2982	1.35	3.7	3.8
0.2 mL/min	101.0	3284	1.37	4.0	1.036
0.4 mL/min	99.7	2583	1.29	3.8	0.0971
28°C	99.2	3160	1.31	3.9	1.010
32°C	100.1	3292	1.26	3.9	0.997

ROBUSTNESS DATA OF SITAGLIPTIN

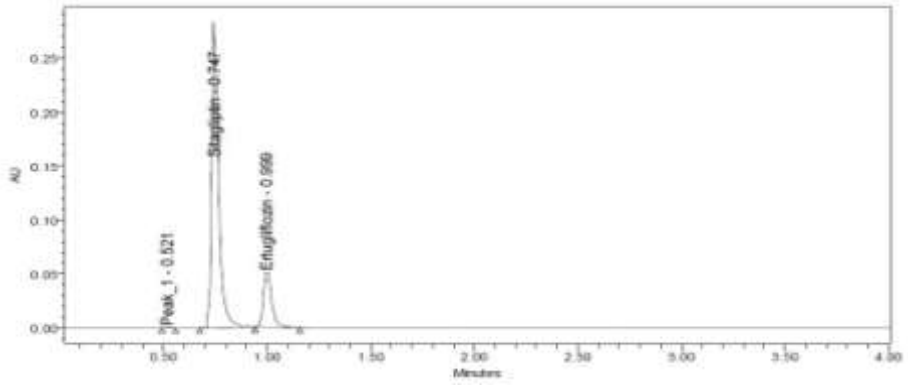
Chromatographic conditions	% assay	Sitagliptin		
		Theoretical Plates	Asymmetry	Retention time
Water: Acetonitrile (60:40% v/v)	98.81	3104	1.69	0.742
Water: Acetonitrile (40:60% v/v)	99.12	2378	1.57	0.768
0.2 mL/min	99.02	2359	1.59	0.780
0.4 mL/min	99.18	2114	1.58	0.733
28°C	98.02	2014	1.47	0.761
32°C	98.87	2039	1.51	0.747



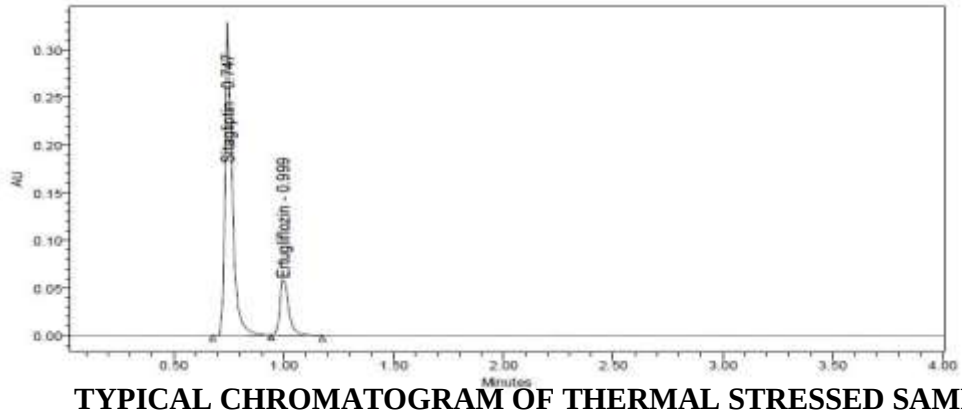
TYPICAL CHROMATOGRAM OF ACID STRESSED SAMPLE



TYPICAL CHROMATOGRAM OF BASE STRESSED SAMPLE

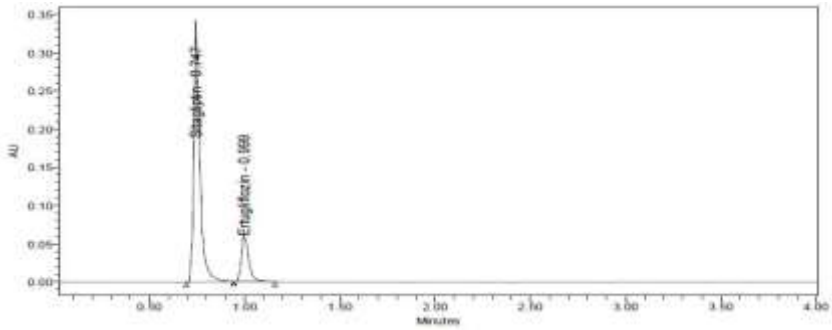


TYPICAL CHROMATOGRAM OF PEROXIDE STRESSED SAMPLE

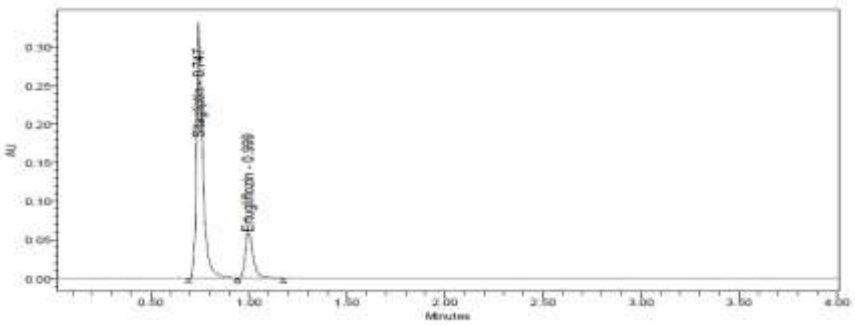


TYPICAL CHROMATOGRAM OF THERMAL STRESSED SAMPLE

Nature of degradation	**Stress conditions**	**Sitagliptin**		**Ertugliflozin**	
		Purity of angle	**Purity of Threshold**	**Purity of angle**	**Purity of Threshold**
Acid sample	1N HCl refluxed for 60 min at 60°C	0.311	0.814	0.102	0. 789
Base sample	1N NaOH refluxed for 60 min at 60°C	0.107	1.301	0. 038	0.417
Peroxide sample	10% peroxide for 60 min Bench top	0. 471	0.810	0. 734	2.841
Thermal sample	oven at 105°C for 12 h	0. 879	3.992	0.425	0.614
	in UV Chamber for	0.920	1.501	0.471	7.507
UV sample	1hours or 200-Watt hours/m^2				
Water sample	water refluxed for 6 h at 80^0C	0.510	0.711	0.117	1.770



TYPICAL CHROMATOGRAM OF WATER STRESSED SAMPLE



TYPICAL CHROMATOGRAM OF PHOTOLYTIC DEGRADATION SAMPLE

PEAK PURITY DATA OF SITAGLIPTIN AND ERTUGLIFLOZIN

FORCED DEGRADATION DATA OF SITAGLIPTIN AND ERTUGLIFLOZIN

Stress sample	Stress conditions	% Assay	%
Control sample		100.30	-
Acid sample	1N HCl refluxed for 60 min at 60°C	95.51	4.79
Base sample	1N NaOH refluxed for 60 min at 60°C	95.74	4.56
Peroxide sample	20% peroxide for 60 min Bench top	93.09	7.21
Thermal sample	oven at 105°C for 12 h	98.44	1.86
	in UV Chamber for		
UV sample	1hours or 200-Watt	98.37	1.93
	hours/m^2		
Water sample	water refluxed for 6 h at 60^0C	99.25	1.05

SUMMARY AND CONCLUSION:

The present analytical method was developed by studying different parameters. The column used for the study was BEH C18 100 X 2.1 mm 1.8µ column because it gave good separation and peakshapes. Ideal λ max for be at 220 nm as the peak purity was good. Injection volume was selected to be 2µL which gave a good peak area. The flow rate was fixed at 0.3 mL/min for giving satisfactory retention times. A mixture of 0.1% OPA and Acetonitrile (50:50% v/v) was found to be ideal for the proposed study as it resulted in good resolution of the drugs. Run time was selected to be 3 min because the analysis gave peaks around 1.009 and 1.284 ±0.02 min of Sitagliptin and Ertugliflozin respectively. The percent recovery was found to be in between 98.0 to 102.0%. The analytical method was found to be linear over the range 25-150 µg/mL of Sitagliptin and 3.75-22.5 µg/mL Ertugliflozin of the target concentration. The analytical method passed both the robustness and ruggedness tests. In both the cases, relative standard deviation was below 2.0. The developed method is simple, sensitive, precise and accurate for simultaneous quantitative estimation of Sitagliptin and Ertugliflozin in tablet dosage forms and has certain advantages over the methods reported in literature. This method is simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. The solvent system used in this method is economical due to the use of water and Acetonitrile. The % RSD values were within 2.0 and the method was found to be precise. The results of the validation parameters of the method lie within the prescribed limits. The method did not show any interference from excipients. These results confirm the suitability of the proposed RP-HPLC method for accurate and precise analysis of Sitagliptin and Ertugliflozin in combined tablet dosage forms.

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