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Novel Stress Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ertugliflozin and Sitagliptin in Bulk and its Formulation

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ABSTRACT

A selective, sensitive RP-HPLC method was developed for the simultaneous estimation of the Ertugliflozin (ETR) and Sitagliptin (SGT) in bulk and its dosage form. The separation and determination was carried on water's C18 column capacitate (250X4.6 mm, 5 µm particle size), retention times of Ertugliflozin and Sitagliptin were found to be 2.39 and 4.60 min. respectively. The wavelength was fixed at 215 nm with PDA detection. The mobile phase was consisted mixture of 0.5 mM potassium dihydrogen ortho phosphate buffer: Methanol in the ratio of 55:45 v/v, pH 5.3 was adjusted with HCl and flow of mobile phase was maintained 1mL/min. The calibration curve was linear and regression co-efficient (R^2) value found to be 0.999 and concentration ranging from 37.5-112.5 and 250-750 µg/mL for Ertugliflozin and Sitagliptin respectively. The quantization limit and detection limit of the method were found 0.1 & 0.3 µg/ml and 0.4 and 1µg/ml for Ertugliflozin and Sitagliptin.

Keywords: Ertugliflozin, Sitagliptin, Reversed Phase High Performance Liquid Chromatography, Methanol.

INTRODUCTION

A novel class of anti-diabetic drugs, which are inhibitors of dipeptidyl-peptidase IV (DPP4),which included sitagliptin,vildagliptin and saxagliptin[1,2,3,4,5]. Type 2 *diabetes mellitus* (T2DM) is a progressive disease, for the treatment of many patients they require combination therapy to maintain over time glycemic levels[6,7]. Efficacy and safety of the addition of Ertugliflozin in patients with Type 2 diabetes mellitus inadequately controlled with metformin and Sitagliptin[8,9,10]. Ertugliflozin is an oral

sodium–glucose transporter 2 inhibitor. The study assessed the efficacy and safety of co initiation of Ertugliflozin and sitagliptin compared with placebo in patients with T2DM in adequately controlled on diet and exercise[11,12]. Ertugliflozin (1S,2S,3S,4R,5S) 5[4 chloro 3[4ethoxyphenyl]methyl]phenyl]1 (hydroxymethyl)6,7 dioxabicyclo[3.2.1]2,3,4 triol.[13] Sitagliptin chemically7[(3R)3amino1oxo 4(2,4,5 trifluorophenyl)butyl] 5,6,7,8 tetrahydro 3(trifluoromethyl) 1,2,4 triazolo[4,3a]pyrazine phosphate (1:1) monohydrate[14,15,16]. The placebo-adjusted differences in changes from baseline in systolic blood pressure were not statistically significant. Ertugliflozin is used for the treatment a higher prevalence of genital mycotic infections occurred in men and women with Ertugliflozin compared with placebo[17]. The most of the methods were reported for the separation and estimation of Sitagliptin, metformin and few are only on estimation of Sitagliptin.[18,19,20,21,22,23,24,25] The structures of Ertugliflozin and Sitagliptin showed in Figures 1 and 2.

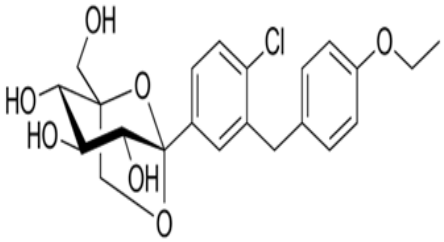

Fig. 1. Structure of Ertugliflozin

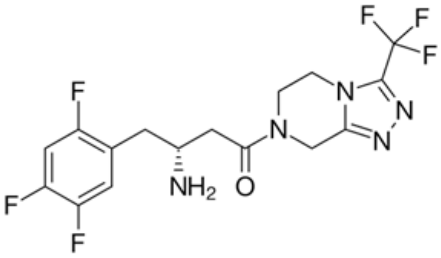

Fig. 2. Structure of Sitagliptin

EXPERIMENTAL

Apparatus

The HPLC was LC Waters (Waters, Milford, MA, USA), Electronic Weighing Balance (LC-GC India), pH Meter (Elico, Model LI 612), Ultrasonic bath (Enertech), Thermostatic oven (Thermolab), Micropipettes (Genie), Data Processing software (Empower 2), Photodiode array detector (Waters, model 2998), Autosampler (Waters, model 717 plus).

MATERIALS AND METHODS

Reagents & chemicals

All the chemicals and reagents in this experiment were of analytical grade. Water was double distilled and filtered with a membrane filter. Methanol – HPLC grade (Merck, India), hydrochloric acid and potassium di hydrogen ortho phosphate (SD fine chem, India) were used to prepare mobile phase. Pharmaceutical grade standard drugs viz., Ertugliflozin and Sitagliptin were kindly gifted by Ajanta Pharma Ltd, Mumbai, India. The combined tablet formulation contains 15 mg of Ertugliflozin and 100mg of Sitagliptin (Steglujan, Natco) purchased from local market of Kurnool.

Preparation of standard solution

Weigh accurately 10 mg of Ertugliflozin & Sitagliptin and transferred in to individual 10 ml volumetric flasks with small quantity of mobile phase. The solution was sonicated for 10 min. and volume made with mobile phase and concentration 1000 µg/ml. This solution further diluted for the preparation of working standard solutions to get final concentrations of 75 µg/mL of Ertugliflozin and 500 µg/mL of Sitagliptin working standard solutions.

Preparation of sample solution

Twenty tablets were weighed and finely powdered. The average weight of tablets was determined. The powder equivalent to 10 mg of ETR was weighed and transferred to a 10 mL volumetric flask. 10 mL of diluent was added to disintegrate tablets completely by using ultra sonicated for 10 minute. The aliquot portion of the filtrate was further diluted to get final concentrations 75 µg/mL of ETR and 500 µg/mL of SGT. The solution was filtered through membrane filter. The 20 µL of this solution was injected in to HPLC system.

Chromatographic Settings

The mobile phase used for the development of method was 0.5 mM potassium dihydrogen ortho phosphate buffer: Methanol in the ratio of 55:45 v/v, pH 5.3 was attuned with HCl and flow of mobile phase was filtered through membrane filter and flow rate was kept 1mL/min. The effluents were supervised at 215 nm with PDA detector and injected 20 µl of solution through chromatographic column.

RESULTS AND DISCUSION

Method development

The method was developed with different buffers and organic solvents but the composition of potassium dihydrogen ortho phosphate and methanol was showed good resolution, symmetrical peaks, high theoretical plates, and low retention times of both Ertugliflozin and Sitagliptin. The optimized parameters were showed in Table 1.

Table 1: Optimized conditions for separation and estimation of Ertugliflozin, Sitagliptin

S. No	Parameter	Description/Value
1	Stationary Phase	Waters C18 (250X4.6X5)
2	Mobile Phase	0.5 mM Potassium dihydrogen ortho phosphate buffer (pH 5.3) and Methanol in the ratio of 55:45 v/v.
3	Flow rate	1 ml/min.
4	Detection Wavelength (Isosbestic Point)	215 nm
5	Detector	Photo diode array
6	Injection	Autosampler -Waters, model 717 plus
7	Injection volume	20 µl
8	Column Temperature	35
9	Run time	6 min.
10	Diluent	Mobile phase
11	Retention Times	Ertugliflozin : 2.3 min. Sitagliptin : 4.6 min.

Method validation

The different method validation parameters were performed as per ICH norms. The all parameters showed good results and they met ICH guidelines of acceptance.[26]

System suitability constraints

The system suitability parameters were showed good theoretical plates 3985 and 6425 for ETR and SGT. The tailing factor was less than 2 for both drugs. They showed good resolution between peaks 11.27 and showed fine peak areas. The chromatograms were showed in Fig. 3,4,5 and results were tabulated in Table 2.

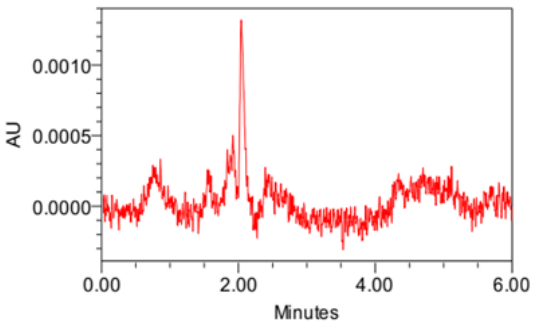

Fig. 3. Blank chromatogram

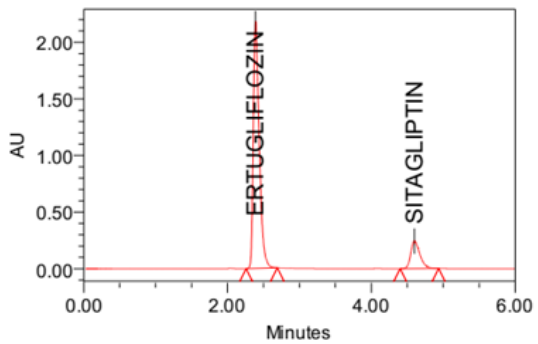

Fig. 4. Standard chromatogram

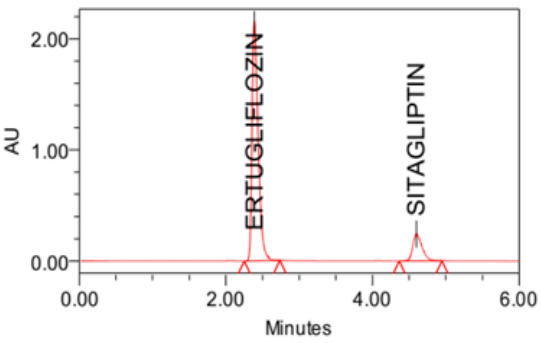

Fig. 3. Sample chromatogram

Table 2: System suitability results of ETF & SGT

S. No	Parameter	ETF	SGT
1	Theoritical Plate Count	3985	6425
2	Peak Area	12553232	6608681
3	Peak Height	2151554	240210
4	RT	2.39	4.603
5	Tailing	1.58	1.35
6	Resolution	-	11.27
7	S/N	6.014	670

Specificity

The stress degradation studies were implies the specificity of the method. Different parameters were evaluated depend upon separation between degradants and active moiety, as well as method showed ability to analyze analyte in the presence of other products.

Common Suggested procedure for Linearity

The calibration curve linear over concentration range and R^2 values were found to be 0.999 for both Ertugliflozin and Sitagliptin. The standard solution was showed linearity concentration range from 37.5-112.5µg/mL for Ertugliflozin & 250-750 µg/mL for Sitagliptin. The data of graphs were showed in Figures 6 & 7.

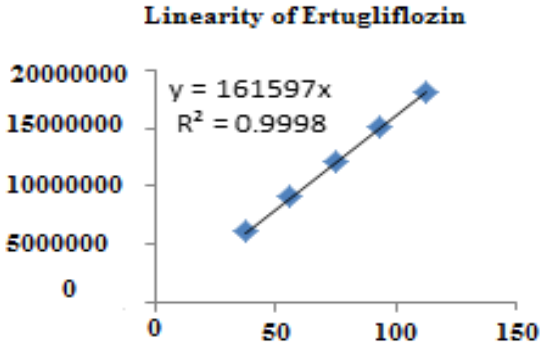

Fig. 6. Linearity graph of Ertugliflozin

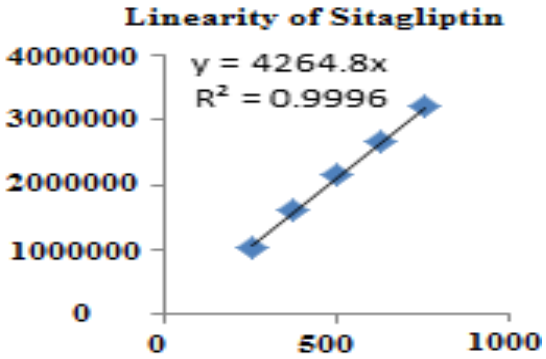

Fig. 7. Linearity graph of Sitagliptin

Precision

The precision was assessed through system precision and method precision. The method precision was estimated through assay. The optimized concentrations of standard and sample solutions were injected in to chromatographic system for the system precision and method precision. The %RSD values varied from 0.55-0.66%. The results of the method showed good precision of the values. The results were tabulated in Table 3 and 4.

Table 3: System precision

S. No	Peak Area	
	ETF	SGT
1	12508045	2132304
2	12521643	2140220
3	12372333	2115333
4	12372949	2117149
5	12381516	2136308
6	12424701	2149651
Average	12430197.83	2131827.00
STDEV	68481.10	12219.79
% RSD	0.55	0.57

Table 4: Precision results of ETF & SGT

S. No	Peak Area		% Assay	
	ETF	SGT	ETF	SGT
1	12381516	2117149	98.64	98.71
2	12372949	2132304	98.57	99.42
3	12508045	2115333	99.64	98.63
4	12521643	2140220	99.75	99.79
5	12372333	2136308	98.56	99.61
6	12424701	2149651	98.98	100.23
Average	12430197.83	2131827.50	99.02	99.40
STDEV	68481.10	13386.11	0.55	0.62
% RSD	0.55	0.62	0.55	0.62

Accuracy

The accuracy of the method was planned by standard addition process. The concentration of 50% solution showed % mean recovery 99.90 & 100.91 for Ertugliflozin & Sitagliptin respectively. The concentration of 100% solution showed % mean recovery 100.18 & 100.29 for Ertugliflozin & Sitagliptin respectively. The concentration of 150% solution showed % mean recovery 100.84 and 99.86 for Ertugliflozin & Sitagliptin respectively. The results were tabulated in Table 5.

Table 5: Accuracy results of ETZ & SGT

Parameters	Peak Area	Amount added(µg)	Amount recovered (µg)	% of recovery	% mean recovery
Ertugliflozin					
50%	6209103	37.13	37.09	99.90	99.90
100%	12465890	74.26	74.48	100.29	100.29
150%	18802423	111.39	112.33	100.84	100.84
Sitagliptin					
50%	1071497	37.13	37.46	100.91	100.91
100%	2127492	74.26	74.39	100.18	100.18
150%	3181111	111.39	111.23	99.86	99.86

Limit of detection & Limit of quantification

The LOD and LOQ were estimated 12.71µg/ml-42.37µg/ml for Ertugliflozin and 8.59µg/ml-28.65µg/ml for Sitagliptin. The limit of detection and quantitation limits performed based on the slope and standard deviation.The method showed ability to detect Ertugliflozin & Sitagliptin at low level of concentrations. The chromatograms were showed in Figures 8, 9.

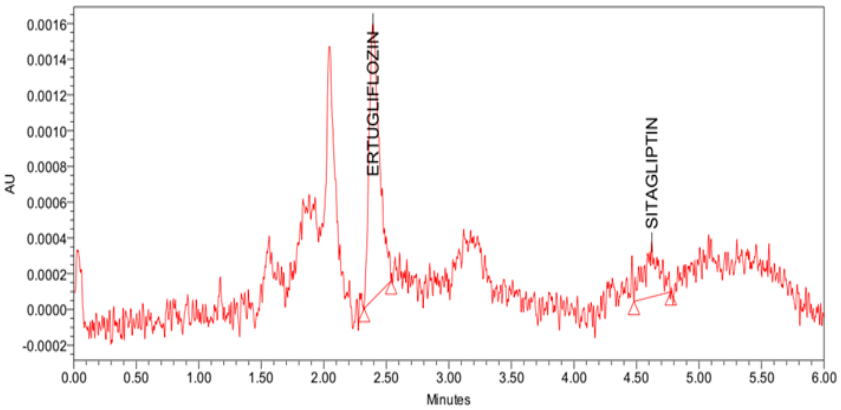

Fig. 8. Chromatogram of LOD

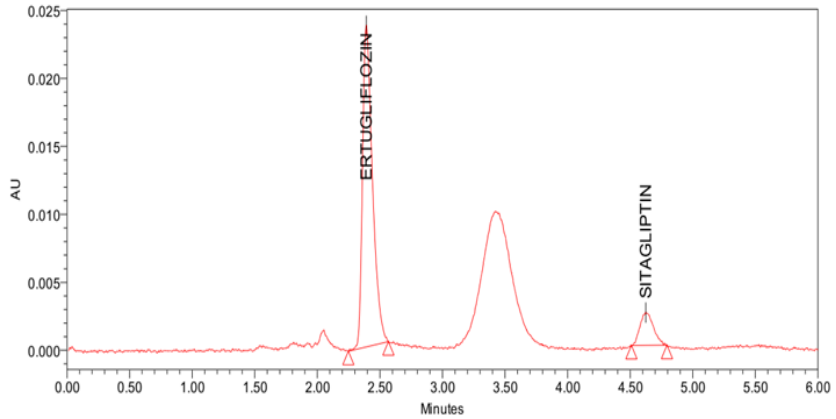

Fig. 9. Chromatogram of LOQ

Robustness

The robustness of the method was performed with deliberate change of flow rate, temperature and mobile phase composition. The changed parameters were showed good percentage assay values. The percentage assay values were in between 99.24% -101.47% for Ertugliflozin and 99.51-101.08 for Sitagliptin respectively. They met acceptance criteria according to ICH guidelines. The results were tabulated in Table 6.

Table 6: Robustness of ETZ & SGT

S. No	Parameter	Condition	Ertugliflozin			Sitagliptin		
			RT	Peak Area	% Assay	RT	Peak Area	% Assay
1	Flow	0.8 ml/min.	1.92	12357028	99.24	3.71	2134138	99.51
2		1 ml/min.	2.39	12553232	100.00	4.60	2144839	100.00
3		1.2 ml/min.	3.82	13837314	101.47	6.01	2177984	101.08
4	Temp	30 °C	2.39	12553345	100.01	4.64	2141427	99.85
5		35 °C	2.39	12553232	100.00	4.60	2144839	100.00
6		40 °C	2.40	12581162	100.23	4.66	2154377	100.36
7	Mobile Phase	B:M 55: 42 v/v	2.81	12532136	99.84	4.55	2139894	99.77
8		B:M 55:45 v/v	2.39	12553232	100.00	4.60	2144839	100.00
9		B:M 55:48 v/v	2.68	12574123	100.17	4.66	2152468	100.36

Assay of Ertugliflozin and Sitagliptin in commercial dosage form

The assay of the method was performed for tablet formulation. Powdered 20 tablets from that accurately weighed powder equivalent to 161.56 mg of Ertugliflozin. The final concentration was prepared as 75 µg/mL of Ertugliflozin and 500 µg/mL of Sitagliptin. The % assay values were 99.02% & 99.40% for Ertugliflozin and Sitagliptin. The method was used for routine analysis of Ertugliflozin and Sitagliptin estimation in combined dosage form. The results were showed in Table 7.

Table 7: Assay table for ETR and SGT

S.NO	ETR		SGT	
	Peak Area	% Assay	Peak Aea	% Assay
1	12381516	98.64	2117149	98.71
2	12372949	98.57	2132304	99.42
3	12508045	99.64	2115333	98.63
4	12521643	99.75	2140220	99.79
5	12372333	98.56	2136308	99.61
6	12424701	98.98	2149651	100.23
Mean	12430197.83	99.02	2131827.50	99.40
STDEV	68481.10	0.55	13386.11	0.62
% RSD	0.55	0.55	0.62	0.62

Force degradation studies

The stability studies were implemented on the Ertugliflozin and Sitagliptin. The method showed, there was no interference of degradants and blank. The developed RP-HPLC method verifies the proficiency of stability indicating method for the analysis of Ertugliflozin and Sitagliptin. Different stress indicating studies were conducted with 0.1 N HCl, refluxed for 3 H at 70^{O}C, Base (0.1 N NaOH refluxed for 4H at 70^{O}C), H_2O_2 (3% H_2O_2 Stored at room temperature for 2 H), hydrolytic for 6H at 70^{O}C and UV light (near UV 250 nm for 5 days). The % degradation in all the stress conditions were observed up to 9%. Proposed method was found to be resolved the degraded products from the analytes peak. The average assay results in all the conditions were approximately 90%. The results were tabulated in Table 8 and chromatograms of degradation studies were showed from Figure 10 to 14.

Table 8: Degradation studies of ETR & SGT

Stress conditions	% Assay of active moiety			
	ETZ	% degradation	SGT	% degradation
Acid (0.1 N HCl, refluxed for 2 H at 70^{O}C)	91.01	-8.99	90.47	-9.53
Base (0.1 N NaOH refluxed for 3H at 70ºC)	91.12	-8.88	90.36	-9.64
H_2O_2 (3% H_2O_2 Stored at room temperature for 2 H)	90.22	-9.78	90.10	-9.90
Hydrolytic for 4H at 70^{o}C	91.29	-8.71	91.08	-8.92
UV light (near UV 250 for 5 days)	91.21	-8.79	90.58	-9.42

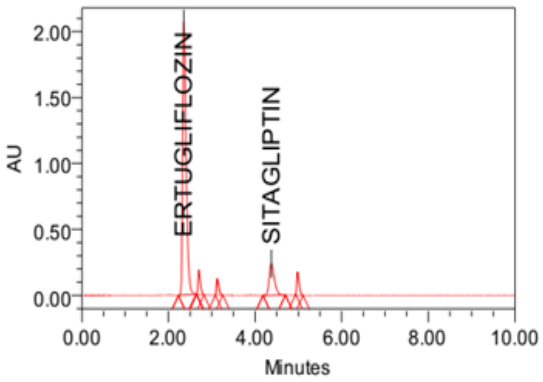

Fig. 10. Acid degradation

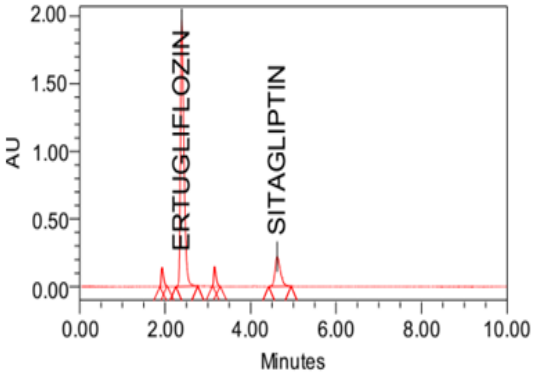

Fig. 11. Alkaline degradation

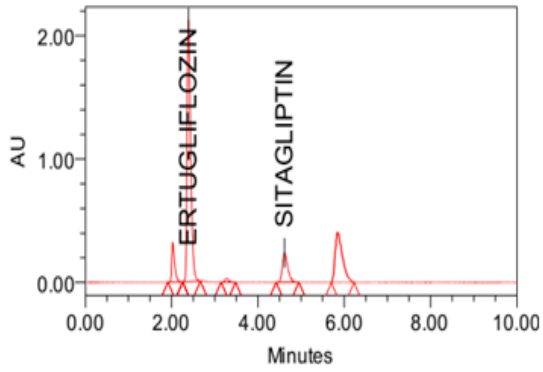

Fig. 10. Peroxide degradation

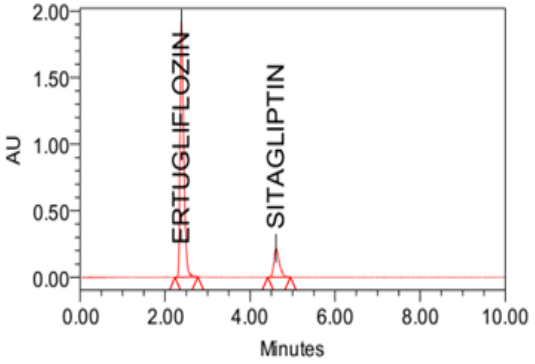

Fig. 11. Hydrolytic degradation

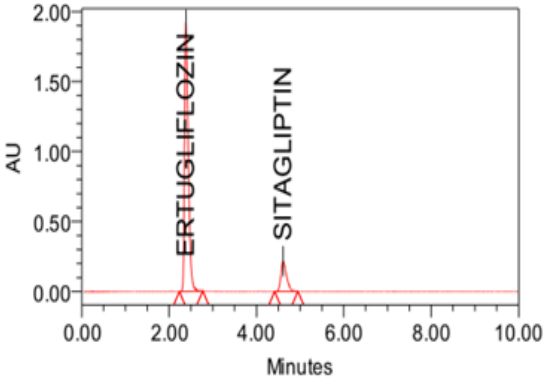

Fig. 12. UV degradation

CONCLUSION

The developed and validated simultaneous estimation of Ertugliflozin and Sitagliptin by RP-HPLC method was showed low tailing factor and high theoretical plates. The method was exposed good precision, accuracy and robustness, met the all values with in the limit according to ICH guidelines. The linearity graphs showed good linearity between different concentrations solutions of ETR and SGT, the R^2 value were found to be 0.999 for both ETR and SGT. The LOD and LOQ values were found to be 0.1 and 0.4 µg/ml for ETR and 0.3 and 1 µg/ml for SGT. The results of LOD and LOQ specified sensitivity of the method and detected ETR and SGT at low concentration. The forced degradation studies were accomplished with acid, alkaline, peroxide, hydrolytic, UV-light conditions. The results of the method were showed high stability and method was used for the routine analysis bulk and its pharmaceutical dosage forms.

Table for LOD& LOQ

Parameter	ETF	SGT
Slope	16159	4264
STDEV	68481.00	12219.00
LOD	12.71µg/ml	8.59 µg/ml
LOQ	42.37 µg/ml	28.65 µg/ml

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