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A NEW VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND ERTUGLIFLOZIN IN BULK AND TABLET DOSAGE FORM

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Abstract:

Objective: *A simple, sensitive and reproducible stability indicating RP-HPLC method for the simultaneous determination of Sitagliptin and Ertugliflozin in bulk and tablet dosage form has been developed and validated.*

Method: *Chromatographic separation was carried out on Agilent ODS C18 (4.6 x 150 mm, 5µ particle size) column using a mobile phase composed of acetonitrile: phosphate buffer (adjusted to pH 5.4 with 0.1 % OPA) in the ratio of 50:50 % v/v at a flow rate of 1.0 ml/min. The analyte was monitored using UV detector wavelength at 215 nm.*

Results: *The retention time was found to be 2.156 min and 3.067 min for Sitagliptin and Ertugliflozin respectively. The proposed method was found to be having linearity in the concentration range of 25-150 µg/ml for Sitagliptin (r2 0.9995) and 3.75-22.5 µg/ml for Ertugliflozin (r2 0.9998) respectively. The mean % recoveries obtained were found to be 99.10–99.93% for Sitagliptin and 98.91–101.17% for Ertugliflozin respectively. Stress testing which covered acid, alkali, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guide lines.*

Conclusion: *The proposed method can be successfully applied for the stability indicating RP-HPLC simultaneous determination of Sitagliptin and Ertugliflozin in bulk and tablet dosage form and in routine quality control analysis.*

Keywords: *Sitagliptin and Ertugliflozin, RP-HPLC, Forced degradation, Method validation.*

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INTRODUCTION:

Sitagliptin

Chemically(Fig.1),it is 3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one. It has a molecular formula of $C_{16}H_{15}F_6N_5O$ and molecular weight of 407.3136 g/mol. Sitagliptin and Ertugliflozin belongs to class of oral hypoglycemic and are anti Diabetic drugs used to treat type-2 Diabetes mellitus. Sitaglitpin selectively inhibits the dipeptidyl peptidase-4 (DPP-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.

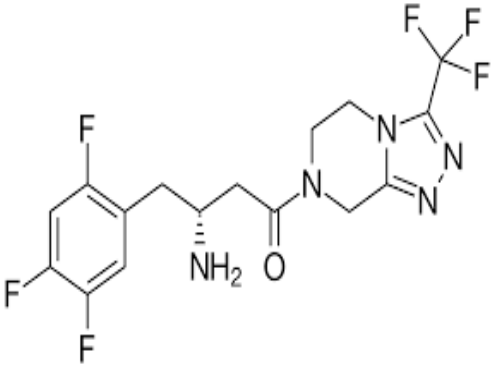

Fig.1: Chemical structure of Sitagliptin

Ertugliflozin

Chemically (Fig.2), it is (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 has a molecular formula of $C_{22}H_{25}ClO_7$ and molecular weight of 436.89 g/mol. Ertugliflozin is an anti Diabetic drug (selective inhibitors of the sodium-dependent glucose cotransporters (SGLT)), its activity increases glucose excretion, reducing hyperglycemia without the requirement of excessive insulin secretion.

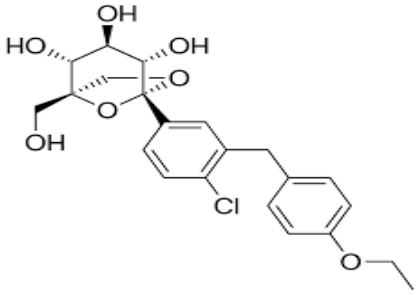

Fig.2: Chemical structure of Ertugliflozin

Literature survey revealed that few analytical methods were reported so far for both drugs in combination or in alone like Spectrophotometric [1-4], RP-HPLC[5-9], and HPTLC[10] methods. The aim of the present study was to develop a simple, precise, sensitive and selective stability indicating RP-HPLC method with UV detection for the analysis of Sitagliptin and Ertugliflozin in bulk and in combined tablet formulation.

MATERIALS AND METHODS:

Experimental:

Chemicals:

The pharmaceutical grade pure samples of Sitagliptin and Ertugliflozin were received as gift samples from Unichem Laboratories, Mumbai. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem.ltd. Mumbai. All the chemicals used were of analytical reagent grade (Qualigens Fine Chemicals Pvt. Ltd., Mumbai). Fixed dose combination tablet formulation (Steglujan) containing 15 mg of Sitagliptin and 100 mg of Ertugliflozin (Manufactured by Merck & Co) were procured from local market.

Instrumentation:

Quantitative HPLC was performed on Waters technologies 2695 series and UV detector module equipped with auto injector using empower software. An UV-2400PC Series UV/Visible double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements.

Chromatographic condition:

Mobile phase composition	Acetonitrile: phosphate buffer (adjusted to pH 5.4 with 0.1 % OPA) in the ratio of 50: 50 % v/v
Stationary phase	Agilent, C_{18} column (150 X 4.6mm, particle size 5µ)
UV detector wave length	215 nm
Run time	10 min
Flow rate	1.0 ml/min
Injection volume	20µl
Temperature	30^0C

The analytical Agilent C_{18} (150 mm x 4.6 mm, 5µ particle size) column was used at a flow rate of 1.0 ml/min and the UV detector wavelength was set at 215 nm. The injection volume was 10µL and temperature at 30ºc.

Preparation of Phosphate buffer:
Accurately weighed quantity of 1.36 g of potassium dihydrogen orthophosphate was dissolved in 1000 ml of water and then adjusted to pH 5.4 with 0.1% OPA. The buffer was filtered through 0.45 µ filter before use.

Preparation of Mobile Phase:
Potassium dihydrogen orthophosphate buffer and acetonitrile were filtered separately through 0.45 µ membrane filters. The filtered solvents were then mixed in the ratio of 50: 50 (% v/v) and degassed for subjecting mixture to sonication for 10 min and resultant solution used as mobile phase.

Preparation of diluent:
Potassium dihydrogen orthophosphate buffer (adjusted to pH 5.4 with 0.1% OPA) and acetonitrile and (50: 50 % v/v) used as diluent.

Preparation of standard solution:
Accurately Weighed and transferred 25mg of Sitagliptin, and 3.75mg of Ertugliflozin g working Standards into a 25 ml clean dry volumetric flasks, add 10ml of diluent, sonicated for 10 minutes and make up to the final volume with diluents

Sample solution preparation:
Accurately weighed equivalent weight of the combination powder sample transfeedr into a 100 ml volumetric flask, 75ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of Sitagliptin and & 150µg/ml of Ertugliflozin), 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Sitagliptin & 15µg/ml of Ertugliflozin)

Method validation:
Analytical validation parameters for this proposed method were determined according to ICH guidelines.

System suitability:
System suitability was carried out by injecting 20 µl of the standard solutions five times into the chromatographic system. The system suitability parameters were then evaluated for tailing factor, retention time and theoretical plates of standard chromatograms. % RSD for peak area of five replicate injections of standard solutions (% RSD NMT 2) were within the limits. The results for system suitability studies are presented in table 1.

Specificity:
The specificity of the method was performed by injecting standard and sample preparations. Chromatograms were recorded. The effect of wide range of excipients and other additives usually present in the formulations in the determination under optimum conditions was also investigated.

Linearity:
The linearity of an analytical method was determined on six concentration levels ranging from 25-125 µg/ml for Sitagliptin and 3.75-22.5µg/ml for Ertugliflozin**.** The calibration curve was constructed by plotting peak area against respective concentrations of Sitagliptin and Ertugliflozin respectively. The linearity of proposed method was then evaluated by linear regression analysis. The correlation coefficient, slope and intercept were calculated for both Sitagliptin and Ertugliflozin as shown in Fig.3 and Fig. 4.

Accuracy:
The accuracy of the test method was demonstrated by % recovery across its range by making three different concentrations at 50%, 100% and 150 % levels using standard addition method, where sample preparations were spiked with known amount of standard preparations and then each concentration was injected triplicate into the chromatographic system.

Precision
System precision
System precision was established by six replicate injections of the standard solution into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Method precision
The method precision study was performed by injecting six sample preparations of marketed formulations into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Intermediate precision
A study was carried out by injecting six standard preparations on different days into the chromatographic system. The corresponding peak areas were measured and % RSD was calculated.

Robustness
Robustness of the method was determined by small deliberate changes in flow rate, mobile organic phase temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust.

Forced degradation studies:
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results showed that for both the solutions, the retention time and peak area of Sitagliptin and Ertugliflozin are remained almost similar (%RSD less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr., which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented.

Acid degradation studies:
To 1 ml of stock solution Sitagliptin & Ertugliflozin 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. The resultant solution was neutralized by 2N NAOH before dilution and then diluted to obtain (100µg/ml of Sitagliptin & 15µg/ml of Ertugliflozin) solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Base degradation studies:
To 1 ml of stock solution Sitagliptin & Ertugliflozin 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 1°c. The resultant solution was neutralized by 2N HCL before dilution and then diluted to obtain (100µg/ml of Sitagliptin & 15µg/ml of Ertugliflozin) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Peroxide degradation studies:
To 1 ml of stock solution of Sitagliptin & Ertugliflozin 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^oc. For HPLC study, the resultant solution was diluted to obtain (100ppm & 15ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies:
The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (100µg/ml of Sitagliptin & 15µg/ml of Ertugliflozin) solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample

Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the (1000µg/ml of Sitagliptin & 150µg/ml of Ertugliflozin) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (100µg/ml of Sitagliptin & 15µg/ml of Ertugliflozin) solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION:

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for the estimation of Sitagliptin and Ertugliflozin in bulk and pharmaceutical dosage form. Separation was done by using mobile phase composed of acetonitrile: phosphate buffer (adjusted to pH 5.4 with 0.1% OPA) in the ratio of 50:50 % v/v on Agilent C18 (4.6 X 150mm, 5µ particle size) column at a flow rate 1.0 ml/min using UV detection at 215 nm. The retention times were found to be 2.156 min and 3.067 min for Sitagliptin and Ertugliflozin respectively. The Isobestic point of Sitagliptin and Ertugliflozin was found to be 215 nm (as shown in figure 3) after scanning 10µg/ml standard solutions of both Sitagliptin and Ertugliflozin in the UV region of 200-400 nm against reagent blank methanol and was utilized for HPLC method development.

Linearity was evaluated in the concentration range of 25-150 µg/ml for Sitagliptin and 3.75-22.5 µg/ml for Ertugliflozin. The calibration curves of Sitagliptin and Ertugliflozin were described by the equation y = 24363x + 20860 and y = 43385x + 2265 with correlation coefficient of 0.9999 as shown in figure 4 and figure 5 respectively. The standard and sample chromatograms in the specifity studies are shown in figure 6 and figure 7. System suitability results are shown in table 1. The %RSD in precision, accuracy and robustness studies were found to be less than 2.0%, indicating that the method was precise, accurate and robust. Accuracy data as shown in table 2. The validation summary parameters and assay results obtained from the marketed formulations are shown in table 3 and table 4. The results of robustness studies are shown in table 5 and table 6. The stress testing chromatograms for both Sitagliptin and Ertugliflozin are shown in figure 8 to figure 12 and results are shown in table 7 and table 8.

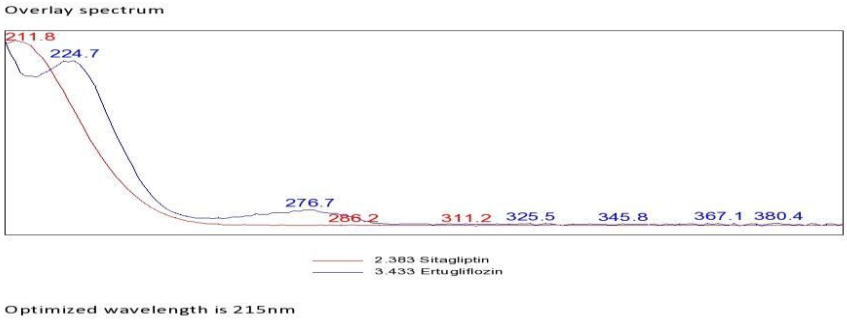

Fig. 3: Isobestic point of Sitagliptin and Ertugliflozin (1=215 nm)

Table 1: System Suitability Results

S.No	System suitability parameters	Sitagliptin	Ertugliflozin
1	Tailing factor (T_f)	1.40	1.30
2	Resolution (Rs)	5.4	
3	Retention time (Min)	2.154	3.054
4	Theoretical plates (N)	3493	4706

Table 2: Accuracy data

Sample	Level	Peak area*	Amount added (ml)	Amount recovered (mg)	Mean % Recovery *± SD
Sitagliptin	50%	3663649	0.5	49.20	99.16 ±0.17
	100%	4877404	1.0	99.77	99.43±0.55
	150%	6105326	1.5	149.74	99.93± 0.62
Ertugliflozin	50%	9724353	0.5	7.41	98.91±0.27
	100%	1303188	1.0	15.1	100.04±0.43
	150%	1646227	1.5	23.0	102.22±0.71

***Mean of three determinations**

Linearity:

The calibration curve was found to be linear over the concentration range of 25-150 µg/ml for Sitagliptin and 3.75-22.5 µg/ml for Ertugliflozin. The correlation coefficient was found to be 0.9999 for both Sitagliptin and Ertugliflozin respectively.

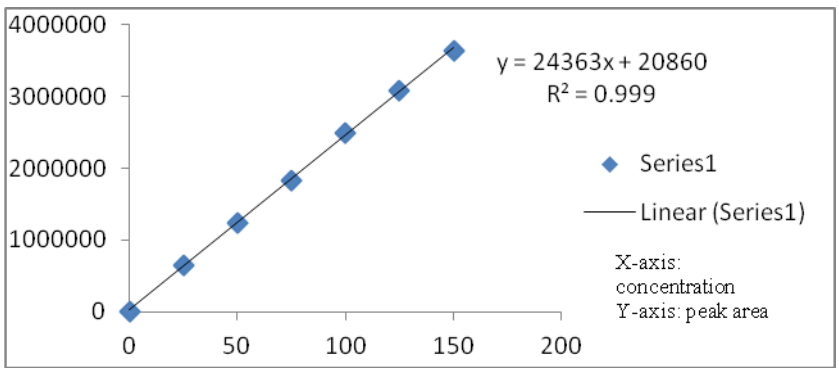

Fig.4: Linearity Graph of Sitagliptin (25-125 µg/ml)

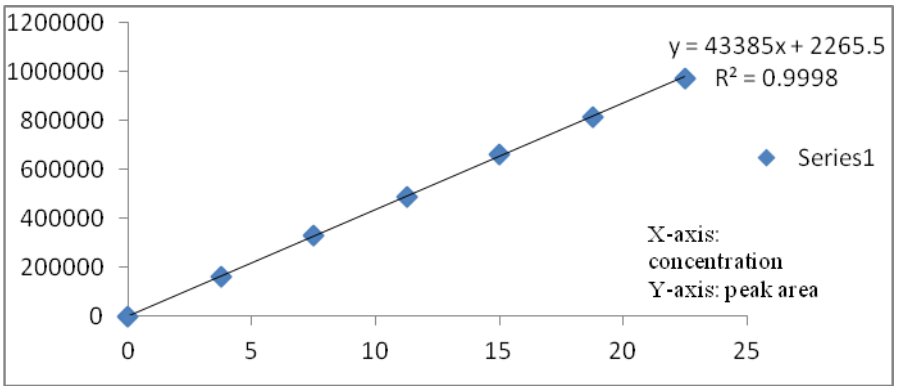

Fig.5: Linearity Graph of Ertugliflozin (3.75-22.5µg/ml)

Table 3: Validation Parameters of the proposed RP-HPLC Method

Parameter	Sitagliptin	Ertugliflozin
Regression equation	y =24363x + 20860	y = 43385x + 2265
Correlation coefficient	0.999	0.999
LOD (µg/ml)	0.68	0.08
LOQ (µg/ml)	2.04	0.244
System precision (% RSD)	0.5	1.0
Method precision (% RSD)	0.7	0.9
Intermediate precision (% RSD)	0.4	0.9

Table 4: Results of assay in Marketed formulation

Brand	Drug	Standard peak area	Sample peak area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay	%RSD*
Steglujan	Sitagliptin	2505419	2509253	100.0	99.96	100.05%	0.27
	Ertugliflozin	665177	667258	15.0	14.89	100.21%	0.34

***Mean of two determinations**

Specificity studies:

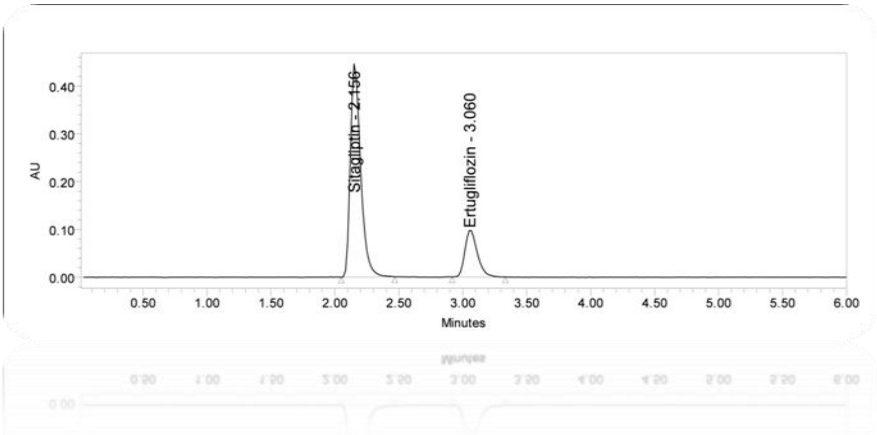

Fig.6: Typical chromatogram of standard

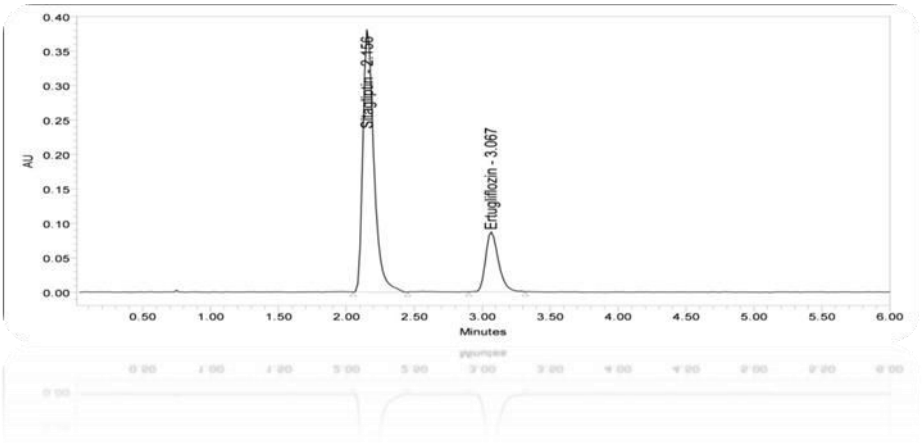

Fig.7: Typical chromatogram of sample

Robustness:

The developed method is robust with deliberate changes in variation of mobile organic phase composition, flow rate and temperature for both Sitaglitpin and Ertugliflozin respectively.

Table 5: Results of robustness study of Sitagliptin

S.No.	Parameter	Change Level	Sitagliptin			
			Retention time (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	0.8	2.023	2261375	1.38	3543
		1.2	2.504	2903109	1.39	3497
2.	Mobile organic phase composition (±10%v/v/v)	40:60	2.182	2603186	1.41	3729
		60:40	2.142	2571727	1.40	3588
3.	Temperature (±5°C)	25 ^{0}C	2.162	2537399	1.39	3615
		35 ^{0}C	2.140	24932348	1.40	3664

Table 6: Results of robustness study of Ertugliflozin

S.No.	Parameter	Change Level	Ertugliflozin			
			Retention time (min)	Peak area	USP Tailing	USP Plate count
1.	Flow rate (±0.2ml/min)	0.8	2.850	597699	1.31	4890
		1.2	3.525	753576	1.32	4853
2.	Mobile organic phase composition (±10%v/v/v)	40:60	2.850	686030	1.30	4926
		60:40	3.15	679380	1.29	4839
3.	Temperature (±5°C)	25 ^{0}C	3.525	753576	1.30	4962
		35 ^{0}C	2.850	597699	1.31	4962

Forced degradation studies:

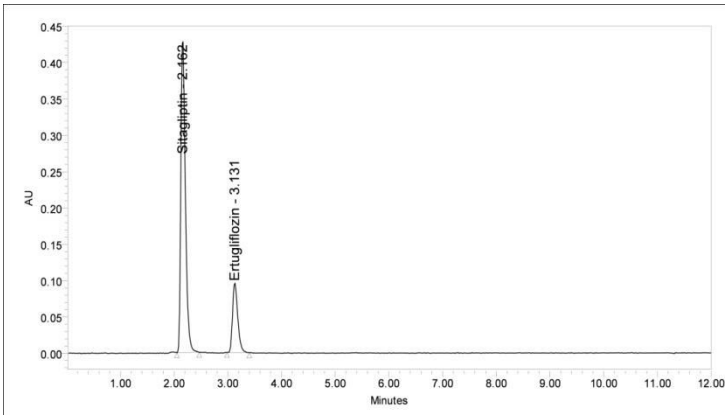

Fig.8: Chromatogram of Acid hydrolysis

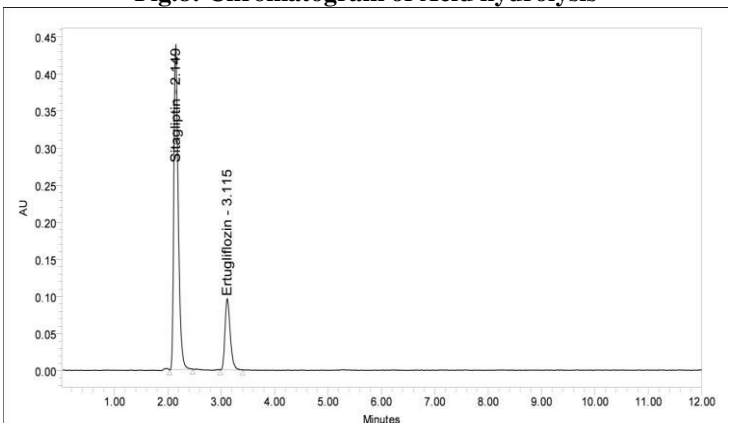

Fig.9: Chromatogram of Base hydrolysis

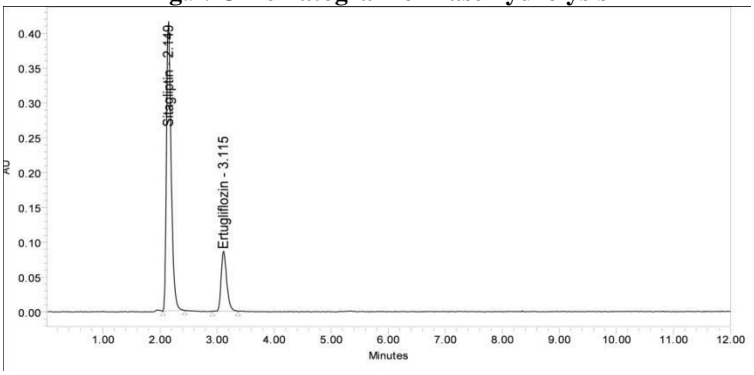

Fig.10: Chromatogram of Oxidation (peroxide)

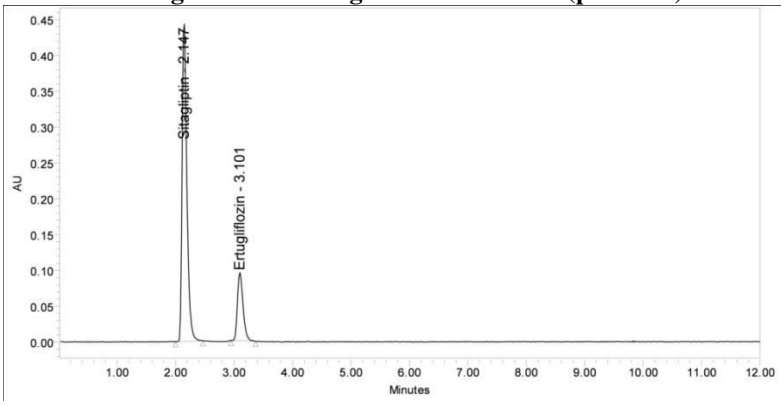

Fig.11: Chromatogram of Heat Exposure

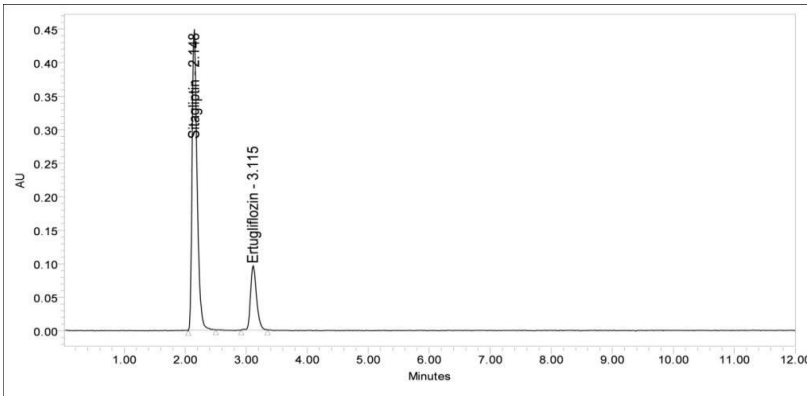

Fig.12: Chromatogram of UV Exposure

Table 7: Degradation Study of Sitagliptin

S.No.	Condition	Peak Area	Degradation % Assay	% Net Degradation
1	Acid degradation	2352972	93.82	6.18
2	Base Hydrolysis	2401720	95.77	4.23
3	Heat Exposure	2433718	97.27	2.96
4	Oxidation (peroxide)	2433718	96.25	3.58
5	UV Exposure	2445417	98.06	2.49

Table 8: Degradation Study of Ertugliflozin

S.No.	Condition	Peak Area	Degradation % Assay	% Net Degradation
1	Acid degradation	637384	95.73	4.27
2	Base Hydrolysis	639942	96.11	3.89
3	Heat Exposure	647666	97.27	2.73
4	Oxidation (peroxide)	647666	96.25	3.75
5	UV Exposure	652927	98.06	1.94

CONCLUSION:

From this study, it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Sitagliptin and Ertugliflozin in bulk & Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

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REFERENCES:

1. P. venkateswara rao. lakshmana rao and s.v.u.m Prasad. a new stability indicating Rp-hplc method for simultaneous estimation of Ertugliflozin and Sitagliptin in bulk and pharmaceutical dosage form and its validation as per Ich guidelines, indo american journal of pharmaceuitcal sciences ,2017 vol. 2(03),051-054.
2. G.Jeyabalan and Narendra Nyola. Analytical method development and validation of sitagliptin phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy, journal of applied sciences, 2013 Vol. 3 (01), pp. 095-098.
3. Karimulla S K. Vasanth P M .Ramesh T. Ramesh M. Method development and validation of Sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form, scholars research library,2013 vol 5(5) 168-174.

4. Kavitha D., S. K. Sahoo. Venkateswara Rao P., Nagamani M., Bhagyalaxmi development and validation of rp hplc method for determination of metformin and sitagliptin in bulk and pharmaceutical dosage form, journal of applied pharmaceutical research2013, pg:186-190.
5. Sai Sruthi Arige, Sai Datri Arige and Lakshmana Rao A. Development and validation of Sitagliptin and Simvastatin tablets by using rp-hplc method, International journal of applied pharmaceutical sciences, 2017 vol:4(1) pg:36-43.
6. Shailaja B. Jadhav*, Swati K. Kupkar, Deepali L. Dharam, Amol M. Jangam, Praveen D. Chaudhari., Development and validation of RP-HPLC and RP-HPTLC method for simultaneous estimation of Sitgaliptin phosphate and Metformin hydrochloride in bulk and dosage form, Indian journal of pharmaceutical education and research, 2013;47(1):13-16.
7. Sathish Kumar Konidala*, Pampana V V Sur esh Babu , Ranj, K S D Ranjitha., RP-HPLC Method Development and Validation for the Simultaneous Estimation of Sitagliptin and Simvastatin in Pharmaceutical Formulation, asian journal of pharmaceutical analysis,2016 6(2):pg-68-76.
8. Validation of Analytical Procedures: Text and Methodology, Proceedings of International Conference on Harmonization (ICH). Geneva, 2005.
9. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures, ICH-Q2A, Geneva (1995).
10. https://www.drugbank.ca/drugs/DB00245
11. https://pubchem.ncbi.nlm.nih.gov/compound/staglujan
12. https://www.ich.org/fileadmin/Public_Web_Site/ICH.../Q1A_R2__Guideline.pdf