A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ERTUGLIFLOZIN IN DOSAGE FORM

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ABSTRACT

To develop precise, accurate, reproducible and validate stability indicating HPLC method for determination of Ertugliflozin in API and pharmaceutical formulation as per ICH Q2 R1 Guideline. The adequate separation was carried using Kromasil C18 column (25 cm x 0.46 cm) 5µ as a stationary phase and a mixture of 0.01M pH 5.5 KH_2PO_4 Buffer: ACN (70:30 v/v) as a mobile phase with the flow rate of 1.0 ml/min and the effluent was monitored at 220nm using single wavelength UV detector. A stability indicating HPLC method has been developed for analysis of the drug in the presence of the degradation products and is validated with different parameters such as linearity, Precision, Accuracy, Robustness and Specificity. It involved various time interval study in which methanol and distilled water were used as solvents. Beer's law was obeyed in the concentration range of 5-25µg/ml. A retention time of Ertuagliflozin was5.52 min. A recovery of Ertugliflozin in tablet formulation was observed in the range of 99.83-100.13%.Degradation of Ertugliflozin was found to occur in acid, alkaline, hydrogen peroxide and thermal conditions whereas it was found to be photo stable. The proposed method was found to be specific, accurate, precise and robust can be used for estimation of Ertugliflozin in API and pharmaceutical formulation.

KEYWORD: Ertugliflozin, Recovery, Validation, RP-HPLC

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INTRODUCTION

Ertugliflozin is used for the treatment of diabetes mellitus type 2 and functions to improve glycemic control in adults when combined with the diet and exercise [01,02]. Ertugliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2) which are responsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transporter mechanism causes blood glucose to be eliminated through the urine. Using Ertugliflozin leads to heavy glycosuria (glucose elimination in the urine), which can lead to weight loss and the tiredness. Ertugliflozin is also associated with hypotensive reactions [03,04].

Ertugliflozin was approved by the FDA on 22th December 2017.

The chemical name of Ertugliflozin 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. The molecular formula is $C_{22}H_{25}ClO_7$. The molecular weight of the Ertugliflozin is 436.89 g/mol.

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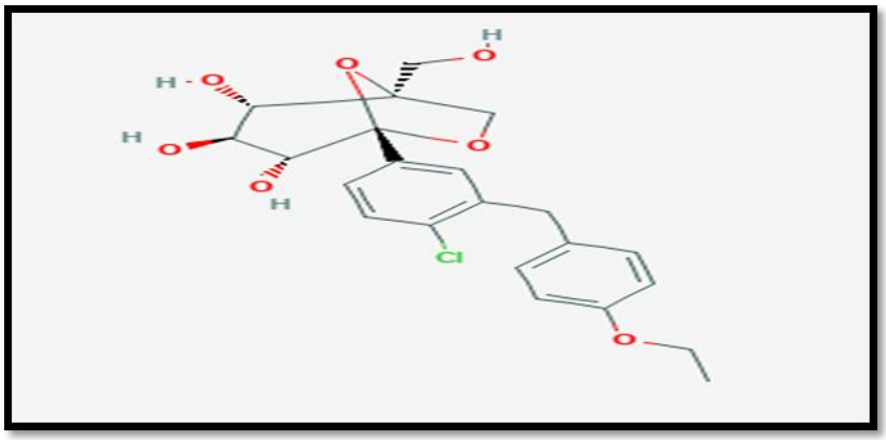

Figure 1: Structure of Ertugliflozin

As per the literature review, Ertugliflozin was estimated with other drug combination by different methods such as reversed-phase HPLC (RP-HPLC) [05,06,07], liquid chromatography-mass spectroscopy (LC-MS) [08] and stability study by HPLC [09,10,11].

Butno HPLC method has been reported for stability indicating analytical method and validation for the estimation of Ertugliflozin in its bulk or single pharmaceutical dosage form. Therefore, the aim of the present work was to develop stability indicating HPLC method for the estimation of Ertugliflozin in its dosage forms. Because analytical methods must be validate before use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference in Harmonization (ICH)[12] guidelines, by assessing its selectivity, linearity, accuracy, and precision, limit of detection and limit of quantification.

MATERIALS AND METHODS

Chemicals and reagents

Ertugliflozin was procured as a gift sample from Pfizer Limited, Mumbai, India. Steglatro tablets were purchased from local market. Acetonitrile, Methanol of HPLC grade, Potassium hydrogen phthalate, Hydrochloric acid, Sodium hydroxide and Hydrogen peroxide (50%) of AR grade were purchased from E. Merck (India) Ltd., Mumbai.

Apparatus

The HPLC instrument used was 1260-Infinity II (Agilent technologies) with U.V detector and N2000 software. Other instruments used are UV-Visible Spectrophotometer (Shimadzu 1800 Double beam UV Probe 2.33), electronic analytical weighing balance (AUX 200), Digital Ultra Sonicator (Labman) and Melting point apparatus (Veegomatic 130S).

Preparation of standard stock solution(1000µg/ml)

Standard stock solution of Ertugliflozin was prepared by dissolving 50 mg of Ertugliflozin in 50 ml of methanol in a 50 ml clean dry volumetric flask and the standard solutions was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 1000 µg/ml of Ertugliflozin. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Ertugliflozin.

Preparation of working standard solution (100µg/ml)

Working standard solution of Ertugliflozin was prepared by taking 1 ml of stock solutions of Ertugliflozin in to clean dry 10 ml volumetric flask and make up volume with diluent to get a concentration of 100 µg/ml of Ertugliflozin.

Preparation of test solution

Twenty Tablets (Steglatro 10 mg) were accurately weighed and a powder equivalent

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to 25 mg was weighed and transferred into 25 ml clean dry volumetric flask. About 10 ml of diluent was added and sonicated for 5 minute to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluent (stock solution). The above sample solution suitably diluted to get a concentration of 10 µg/ml of Ertugliflozin.

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures.[12]

System suitability

System suitability tests were carried out on freshly prepared standard solution of Ertugliflozin under optimized chromatographic condition and parameters were studied to evaluate the suitability of the system. Results are shown Table.1

Table 1: System suitability testing

Parameter	Result
Retention Time	5.52
Theoretical plate	4125
Asymmetry	1.24
Resolution	-

Linearity

The linearity for Ertugliflozin was assessed by analysis of combined standard solution in range of 5-25µg/ml, 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were pipette out from the Working standard solution of Ertugliflozin (100µg/ml) and transfer to 10 ml volumetric flask and make up with methanol to obtain 5, 10, 15, 20 and 25µg/ml.

In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted.

Precision

Repeatability was determined by applying six replicates of standard solution (10µg/ml Ertugliflozin). The intraday and interday precisions were determined by responses of three replicates on same and different days for the standard concentration (10, 15, 20 µg/ml). The results were reported in terms of % RSD.

Accuracy

Recovery study was carried out by standard addition method where known amount of standard concentration at 80%, 100% and 120% of the test solution were spiked in the test solution in triplicate. The area of each solution peak was measured at 220nm. The amount of Ertugliflozin was calculated at each level and % recoveries were computed.

LOD and LOQ

The LOD and LOQ were estimated from the set of 3 calibration curves used to determination method linearity.

The LOD may be calculated as, LOD = 3.3 × SD / Slope and LOQ may be calculated as, LOQ = 10 × SD / Slope

Where, SD = Standard deviation of Y intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

Robustness

Following parameter were changes one by their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (±0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of mobile phase was changed (±0.2) 5.3 and 5.7.

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3. Ratio of mobile phase was changed (±2) 0.01M pH 5.5 KH_2PO_4 Buffer: ACN (68:32 v/v) and 0.01M pH 5.5 KH_2PO_4 Buffer: ACN (72:28 v/v).

Forced degradation studies

Acid Hydrolysis

Acid decomposition studies were performed by transferring 1 ml of working standard solution in to 10 ml of volumetric flask. 2 ml of 1 N HCl solution was added and solution was heated for 90mins at 75°C.After time period 2 ml of 1 N NaOH was added to neutralize the solution and make up the volume with diluent to get 10µg/mlErtugliflozin.

Alkaline Hydrolysis

Base decomposition studies were performed by transferring 1 ml of working standard solution in to 10 ml of volumetric flask. 2 ml of 1 N NaOH solution was added and solution was heated for 60mins at 75°C. After time period 2 ml of 1 N HCl added to neutralize the solution and make up the volume with diluent to get 10µg/mlErtugliflozin.

Oxidative Hydrolysis

Oxidative degradation studies were performed by transpiring 1 ml of working standard solution in 10 ml of volumetric flask. 2 ml of 3% H_2O_2 solutions was added and mixed well and keep for 180mins at room temperature. After time period the volume was adjusted to get 10µg/ml for Ertugliflozin.

Thermal Degradation

Thermal Degradation studies were performed 1 ml of working standard solution was transferred in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 95ºC for 180mins. Then the volume was adjusted with diluents to get 10µg/mlErtugliflozin.

Photolytic degradation

Photo degradation studies were performed 1 ml of working standard solution was transferred in to 10 ml of volumetric flask. The volumetric flask was kept under UV chamber for 72 hrs. Then the volume was adjusted with diluent to get 10µg/ml Ertugliflozin.

RESULT AND DISCUSSION

Optimized chromatographic condition

A chromatographic separation of drug was achieved using Kromasil C18 column (25cm x 0.46 cm) 5µ at an ambient column temperature. The samples were eluted using Mobile phase of 0.01M pH 5.5 KH_2PO_4 Buffer: ACN (70:30 v/v). Drug and degradants were monitored at detection wavelength of 220 nm **(Figure 2)**, the flow rate was 1 ml/min, injection volume was 20 µl. Mobile phase and samples were degassed by ultra-sonication for 20 min and filtered through 0.45µm Nylon (N66) 47 mm membrane filter. The chromatograms of the prepared standard solution of Ertugliflozin were recorded under optimized chromatographic conditions **(Figure 3).**

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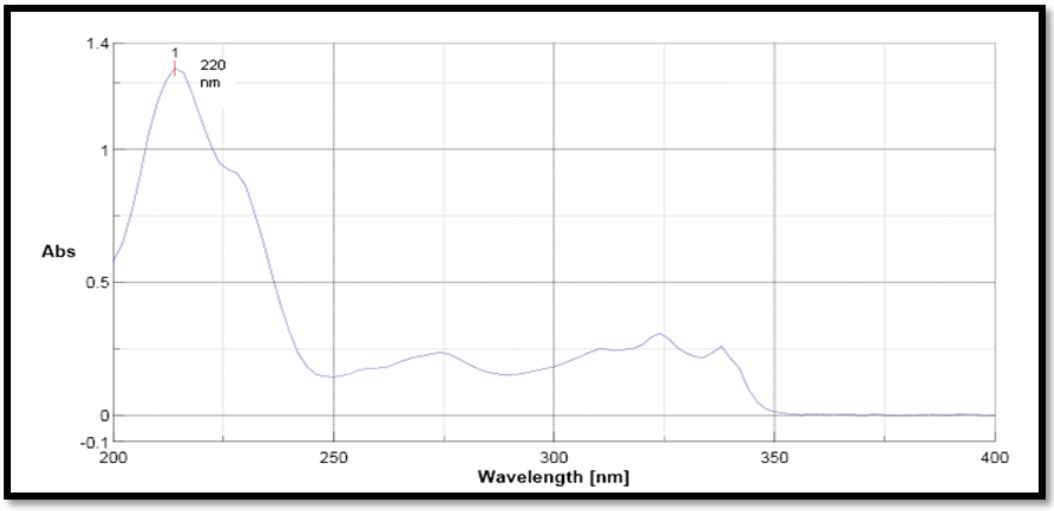

Figure 2: UV Spectrum of Ertugliflozin showing selection of wavelength

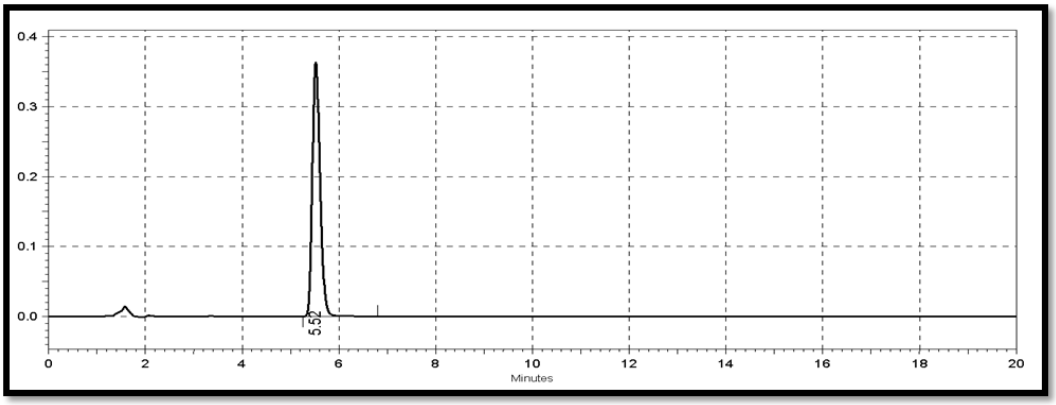

Figure 3: Optimized chromatogram of Ertugliflozin

Method Validation

Linearity

The method was found linear over concentration range of 5-25 µg/ml. Correlation co-efficient for calibration curve Ertugliflozin was found to be 0.9914.

The regression line equation for Ertugliflozin is as following:

$y = 63116x – 193239$, $R^2 = 0.9914$.**(Table 2, Figure 4, Figure 5)**

Table 2: Linearity range of Ertugliflozin

Sr. No	Concentration (µg/ml)	Area
1	5	158614.407
2	10	412624.866
3	15	691996.174
4	20	1122937.360
5	25	1381370.089

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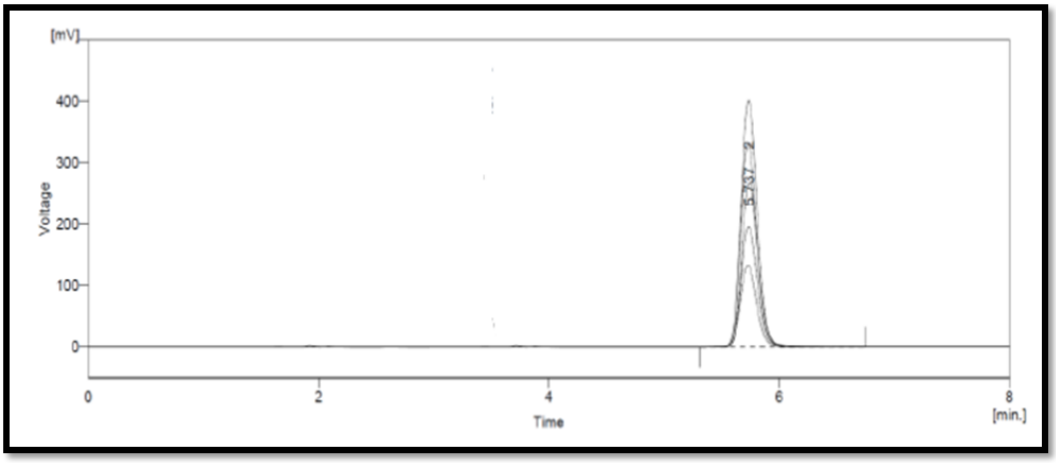

Figure 4: Overlay Chromatogram of Ertugliflozin (5-25 µg/ml)

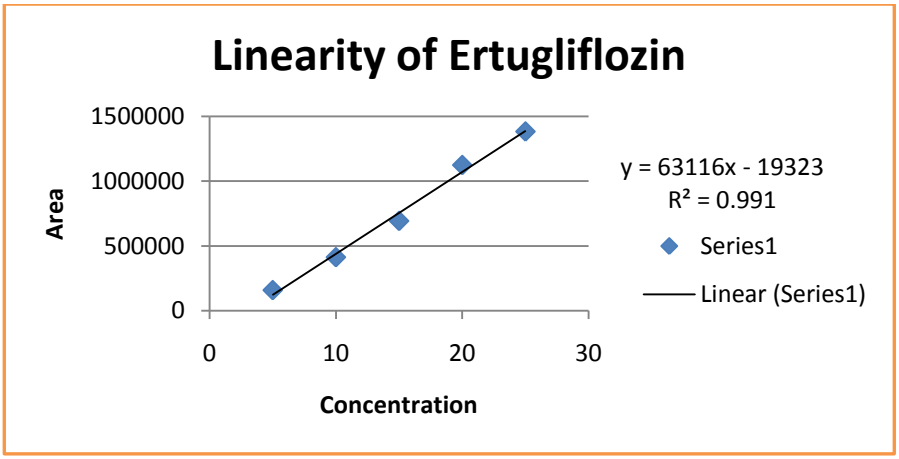

Figure 5: Calibration Curve of Linearity of Ertugliflozin

Precision

The % RSD for repeatability was found to be 1.832 for Ertugliflozin. The % RSD of Intraday Precision was found to be 0.908-1.064 for Ertugliflozin. The % RSD of Interday precision was found to be 1.283-1.884 for Ertugliflozin. Hence, the method is precise. **(Table 3)**

Table 3: Repeatability, Intraday and Interday Precision of Ertugliflozin

Parameter	Conc. (µg/ml)	Peak Area* ± SD	%RSD
Repeatability	10	407150.721 ± 7458.554	1.832
Intraday Precision	10	407696.611 ± 3866.067	0.948
	15	688335.197 ± 7326.926	1.064
	20	1152686.381 ± 10466.071	0.908
Interday Precision	10	398067.623 ± 6224.883	1.564
	15	691187.030 ± 8867.121	1.283
	20	1098150.138 ± 20689.366	1.884

*Average of six determinations

Accuracy

Accuracy was determined by recovery studies of Ertugliflozin, known amount of standard was added to the pre analyzed sample. The study was done at three different concentration levels. Recoveries werein between 99.83-100.13 % which is in accordance with ICH guidelines which proves method to be accurate. **(Table 4)**

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Table 4: Accuracy data of Ertugliflozin

Sr. No.	Conc. Level	Amount of Test Solution (µg/ ml)	Amount of Stand. added (µg/ ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± SD
1	**80%**	10	8	8.08	101.06	99.83±1.5
2		10	8	8.02	100.27	
3		10	8	7.85	98.16	
4	**100%**	10	10	9.99	99.86	99.91±0.79
5		10	10	10.07	100.72	
6		10	10	9.91	99.14	
7	**120%**	10	12	12.16	101.34	100.13±1.84
8		10	12	12.12	101.03	
9		10	12	11.76	98.01	

LOD and LOQ

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected. It may be expressed as a concentration that gives a signal to noise ratio of approximately 3:1. The LOD calculated from formulae was found to be 0.162µg/ml. The LOQ was found to be 0.491µg/ml.

Robustness

The Robustness was evaluated by mobile phase composition, Flow rate and pH value of mobile phase. During studies of Robustness there was not much change retention time and symmetry of peak. The effect of changes was found to be within the acceptance criteria which are shown in below table. The %RSD should be less than 2%.**(Table 5)**

Table 5: Robustness data of Ertugliflozin

Sr. No.	Condition	Variation	Area	%RSD
1	Flow rate (± 0.1 mL/min)	0.8 ml/min	401467.883 ± 5673.241	1.413
2		1.2 ml/min	411582.995 ± 6584.657	1.6
3	pH (±2)	5.3	406758.843 ± 5991.047	1.473
4		5.7	390427.033 ± 5391.279	1.381
5	Mobile phase (±2)	72:28	394129.197 ± 4882.392	1.239
6		68:32	410350.120 ± 7859.520	1.915

Analysis of marketed formulation

Applicability of the proposed method was tested by analysing the commercially available formulation. The assay result was 99.70 ± 0.5957%, respectively of the labelled amount.

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Table 6: Analysis of marketed formulation

Formulation	Label claim	% Label claimed (% Assay* ± SD)	%RSD
Ertugliflozin (Steglatro)	10mg	99.70± 0.5957	0.597

*Average of three determinations

Forced degradation studies

The degradation nature of Ertugliflozin was investigated under different stress degradation (Hydrolysis, Oxidative, Photolytic and Thermal) conditions recommended by International Conference on Harmonization (ICH) using HPLC.

The degradation studies indicate thatErtugliflozin is more susceptible to Hydrolytic degradation. It was stable on exposure to light and dry heat in the solid state.The degradation products were well resolved from the pure drug with significant differences in their retention time values.

The method was therefore being considered to be stability indicating for tablet solid dosage form. **(Figure 6, Figure 7, Figure 8, Figure 9 and Figure 10)**

The result of forced degradation studies is summarized in **(Table 7).**

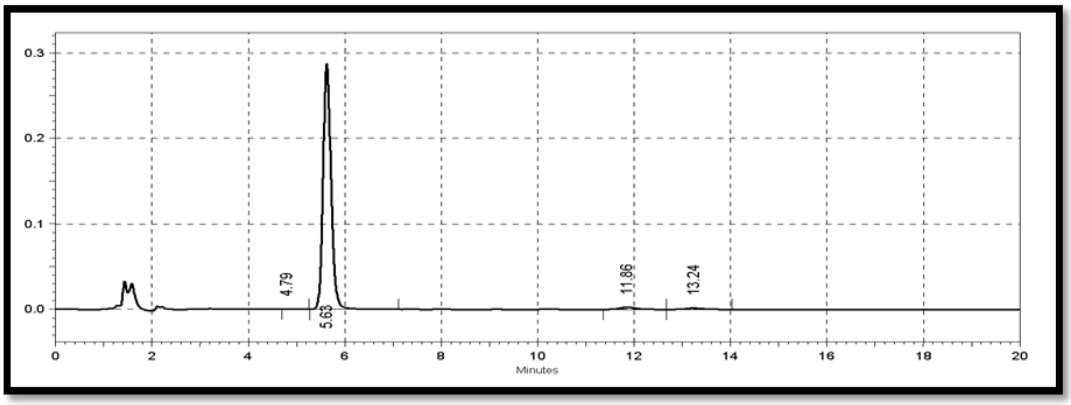

Figure 6: Chromatogram of Acid hydrolysis of Ertugliflozinat 220 nm

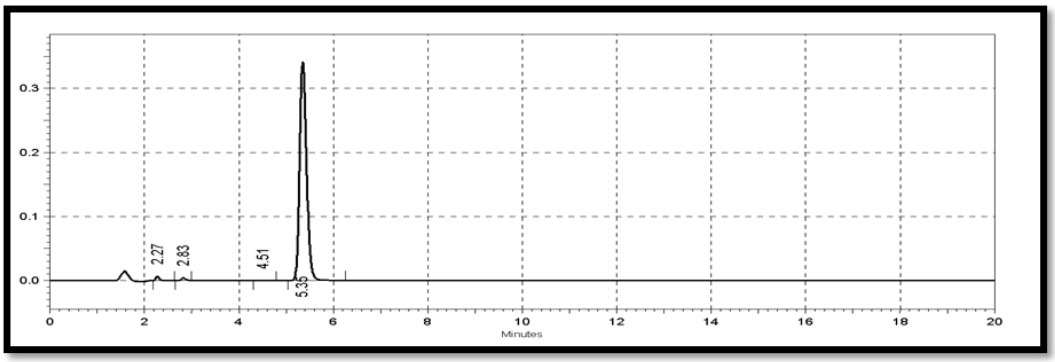

Figure 7: Chromatogram of Base hydrolysis of Ertugliflozin at 220 nm

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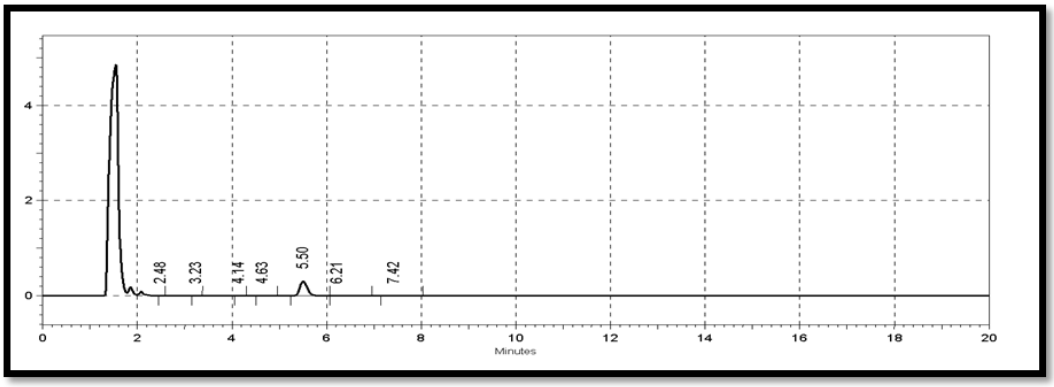

Figure 8: Chromatogram of Oxidative degradation of Ertugliflozin at 220 nm

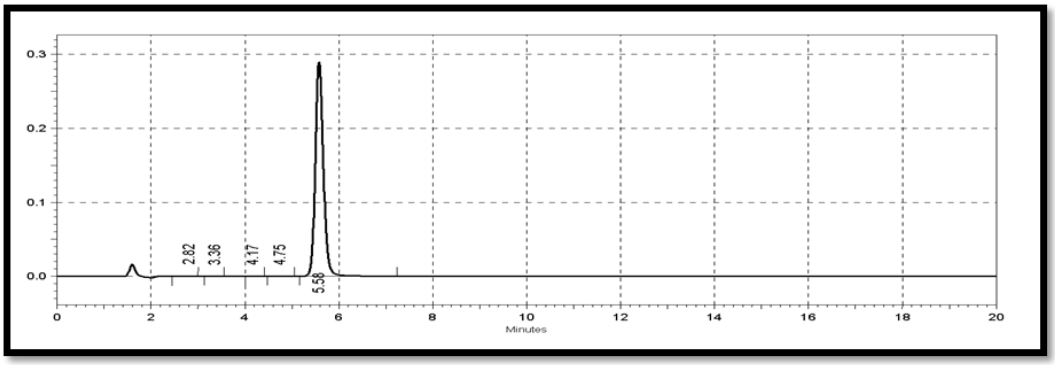

Figure 9: Chromatogram of Thermal degradation of Ertugliflozin at 220 nm

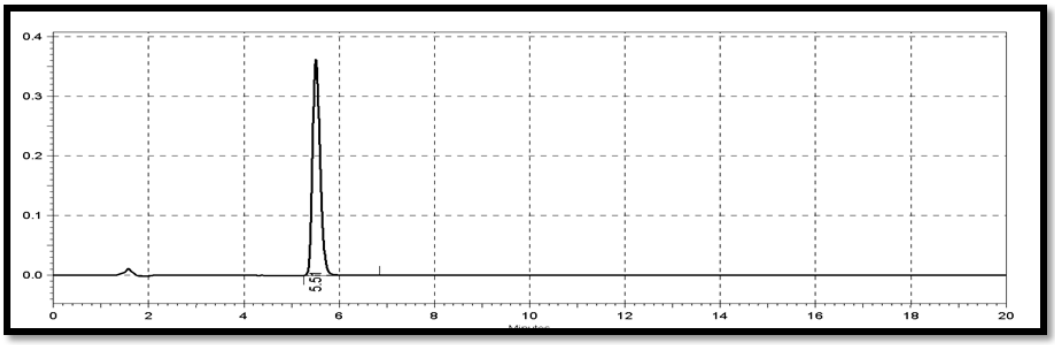

Figure 10: Chromatogram of Photolytic degradation of Ertugliflozin at 220 nm

Table 7: Summary of Forced Degradation study of Ertugliflozin

Sr. No.	Degradation Condition	RT of Analyte	RT of Degradation peak	%Degradation
1	1 N HCl at 75°C, 90mins	5.63	4.79, 11.86, 13.24	17.95
2	1 N NaOHat 75°C, 60mins	5.35	2.27, 2.83, 4.51	14.48
3	3% H_2O_2at RT, 180mins	5.50	2.48, 3.23, 4.14, 4.63, 6.21, 7.42	26.23
4	Thermal at95ºC, 180mins	5.58	2.82, 3.36, 4.17, 4.75	15.32
5	UV light, 254nm, 72 hrs	5.51	-	1.52

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Table 8: Summary of Validation parameter

Sr. No.	Parameter		Limit	Value
1	**Specificity**		No interference	Specific
2	**Linearity**	**Correlation Coefficient**	r^2> 0.999	0.9993
		Regression Line equation	-	y=149.82x+736.9
3	**Range**		-	5-15µg/ml
4	**Precision**	**Repeatability**	RSD < 2	1.50
		Intraday	RSD < 2	1.48
		Interday	RSD < 2	1.73
5	**Accuracy**		98-102%	99.63-10013%
6	**Assay**		98-102%	100.13
7	**LOD**		-	0.717 µg/ml
8	**LOQ**		-	2.210 µg/ml
9	**Robustness**		RSD < 2	Robust

CONCLUSION

The proposed stability-indicating HPLC method was validated as per ICH guidelines and can be applied for the determination of Ertugliflozin in Tablet dosage forms. The method was found to be accurate, precise, robust and specific as the drug peak did not interfere with the extra peaks aroused during the forced degradation studies. No interference from any components of pharmaceutical dosage form can be successfully applied to perform the routine analysis of the drug Ertugliflozin in pharmaceutical formulations.

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