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Analytical Method Development by HPLC to Evaluate the Stress Degradation Stability Profile of Ertugliflozin

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ABSTRACT: Diabetes is a chronic disease in which the body does not make or properly utilize the insulin, a hormone that is required to convert blood sugar, starches, and other food into energy by circulate the glucose from blood into the body cells". People are going on making new drugs and new formulations of the existing drugs within very short period of time. To get the regulatory permission for marketing, company has to submit required data including the analysis reports as to prove that their drug product is of required quality for its intended use. T2DM, the predominant type of diabetes accounting for >90% of all diabetes cases, is a progressive disease involving parallel defects of glucose metabolism in multiple tissues. Common risk factors for T2DM include increasing age, smoking, being overweight or obese, physical inactivity and poor nutrition. Ertugliflozin is an oral, selective inhibitor of sodium glucose co-transporter-2 (SGLT2) which inhibits renal glucose reabsorption and results in urinary glucose excretion (UGE) and reductions in plasma glucose and haemoglobin A1c (A1C) in patients with type 2 diabetes mellitus (T2DM).The present developed analytical method was developed using 0.1% Formic acid in water: 0.1% Formic acid in ACN as the mobile phase. The developed method was found to be sensitive and time and cost effective with the reduced the use of organic chemicals in the mobile phase. Improved stability-indicating RP-HPLC method was developed for the estimation of ertugliflozin. Retention times compared to the best method reported. The developed method could be suitable for routine analysis of the drugs in bulk and tablet formulation after complete validation as per ICH guideline

Keywords: HPLC, Ertugliflozin, diabetes, Analytical method, (SGLT2), Stability

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1. INTRODUCTION

Drugs are inevitable part of our life. We use the drugs throughout our life. The quality of the medicines are an essential feature as it directly affects the life of consumers. The quality of any pharmaceutical product or material can be best evaluated by analyzing it. "Analysis is a division of science that deals with the qualitative and quantitative measurement of any substance". Analysis can give us the answer what (Qualitative analysis) is present and how much (Quantitative analysis) is present in the matter. [1-2]

Analysis is found in almost every branch whether it is data analysis, market analysis or pharmaceutical Analysis. Among these the pharmaceutical analysis deals with the quality of pharmaceutical products and ultimately life of the consumer. [3] Another requirement for quality drug is that the regulatory and government agencies became stringent in case of poor-quality drug products. Quality of any drug product can be known by a series of tests starting from the testing of raw material, intermediates and finished products etc. Drug analysis deals with the identification, characterization and quantification of drugs in singular or in combination as in dosage forms, biological fluids and bulk drugs. [4] As a pharmaceutical analyst we are interested in qualitative analysis of given drug(s) whether it is present in the given material and in quantitative analysis we are interested in the amount of drug(s) present in the given sample.

2. MATERIAL AND METHODS:

MATERIALS

Ertugliflozin reference standards were purchased from Manus Aktteva Biopharm LLP 303, 3rd Floor, Royale Manor, Law Garden, Besides Rangwala Towers, Behind N.C.C. Ground, Dhulia Kot Road, Ellisbridge, Ahmedabad - 380006, Gujarat, India.. Acetonitrile and water of HPLC grade were used and formic acid was used of analytical grade were purchased from Chempura Enterprises Indore M.P.India.

Method Development

Selection of wavelength: Ertugliflozin, 10 µg/mL was scanned between 200-400 nm using a

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UV-visible spectrophotometer. Wavelength was selected from the spectra of above solutions. Ertugliflozin showed absorbance response at a wavelength of 224 nm, thus it was selected as a wavelength of detection. Fig. 4.1 represents the overlay UV spectrum.

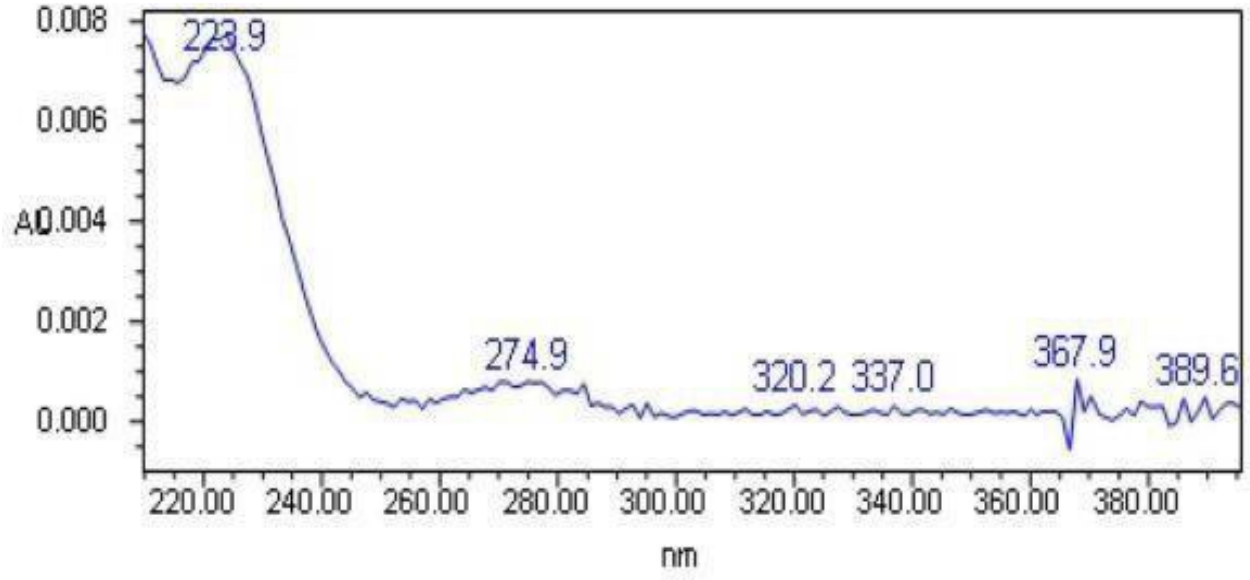

FIGURE 1- UV SPECTRUM OF ERTUGLIFLOZIN SHOWING SELECTION OFWAVELENGTH DETECTION

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Table-1 - Preliminary trial runs for Method Development

Sr.No.	Trial Column	Mobile phase	Observation
1	KROMASIL - C18 (250 mm × 4.6 mm, 5 µm)	0.1% formic acid in water : Acetonitrile(30:70)	No peak eluted till 40 min. run time
2	KROMASIL - C18 (250 mm × 4.6 mm, 5 µm)	0.1% formic acid in water : Acetonitrile (50:50)	No peak eluted till 40 min run time
3	KROMASIL - C18 (250 mm × 4.6 mm, 5 µm)	0.1% formic acid in water :0.1% formic acid in Acetonitrile (20:80)	Peak symmetry of the peaks was not good (improper peak shape) with increased retention time 42.15 min
4	KROMASIL - C18 (250 mm × 4.6 mm, 5 µm)	0.1% formic acid in water :0.1% formic acid in Acetonitrile (40:60)	Peak symmetry of the peaks was good but with increased retention time 46.25 min.
5	KROMASIL - C18 (250 mm × 4.6 mm, 5 µm)	0.1% formic acid in water :0.1% formic acid in Acetonitrile(80:20)	Peak symmetry was good but peak eluted at 1.6 min
6	KROMASIL - C18 (250 mm × 4.6 mm, 5 µm)	0.1% formic acid in water :0.1% formic acid in Acetonitrile(70:30)	Peak symmetry was good with system suitability parameters in limits Peak eluted at 7.24 min

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FIGURE 2- HPLC Chromatogram of Analytical Method Development Trial-1 - Blank

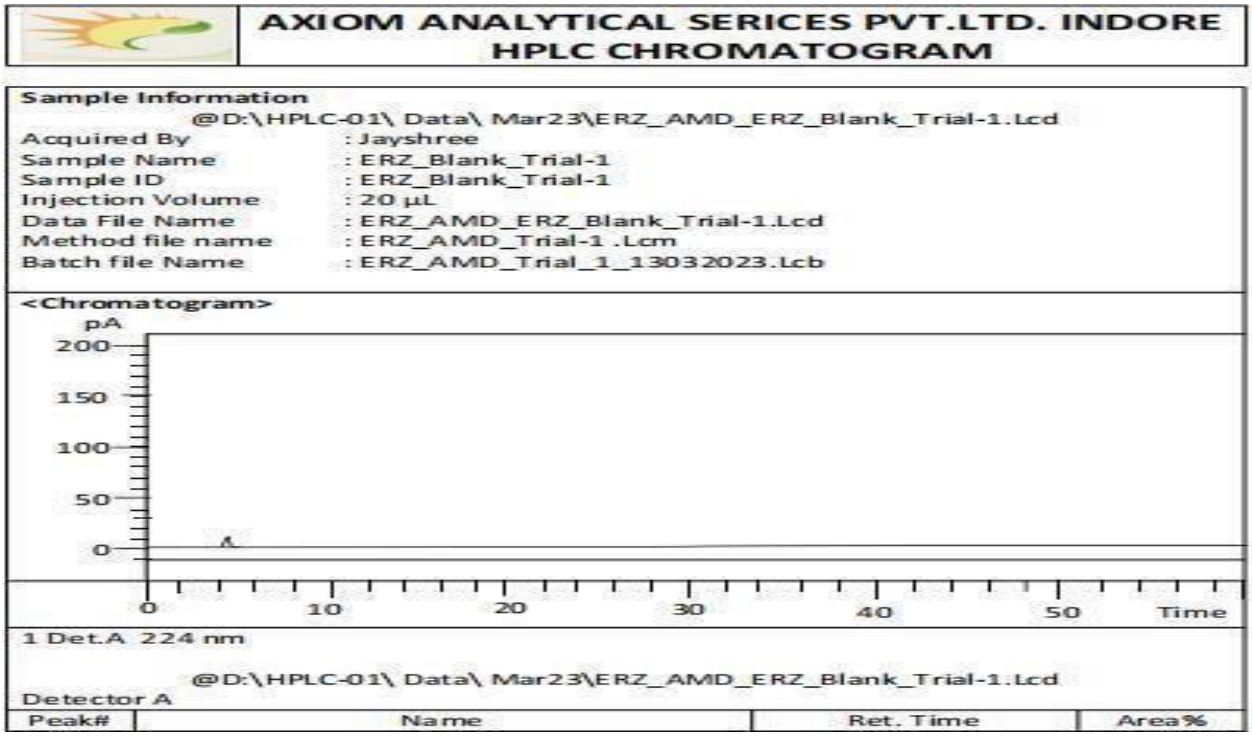

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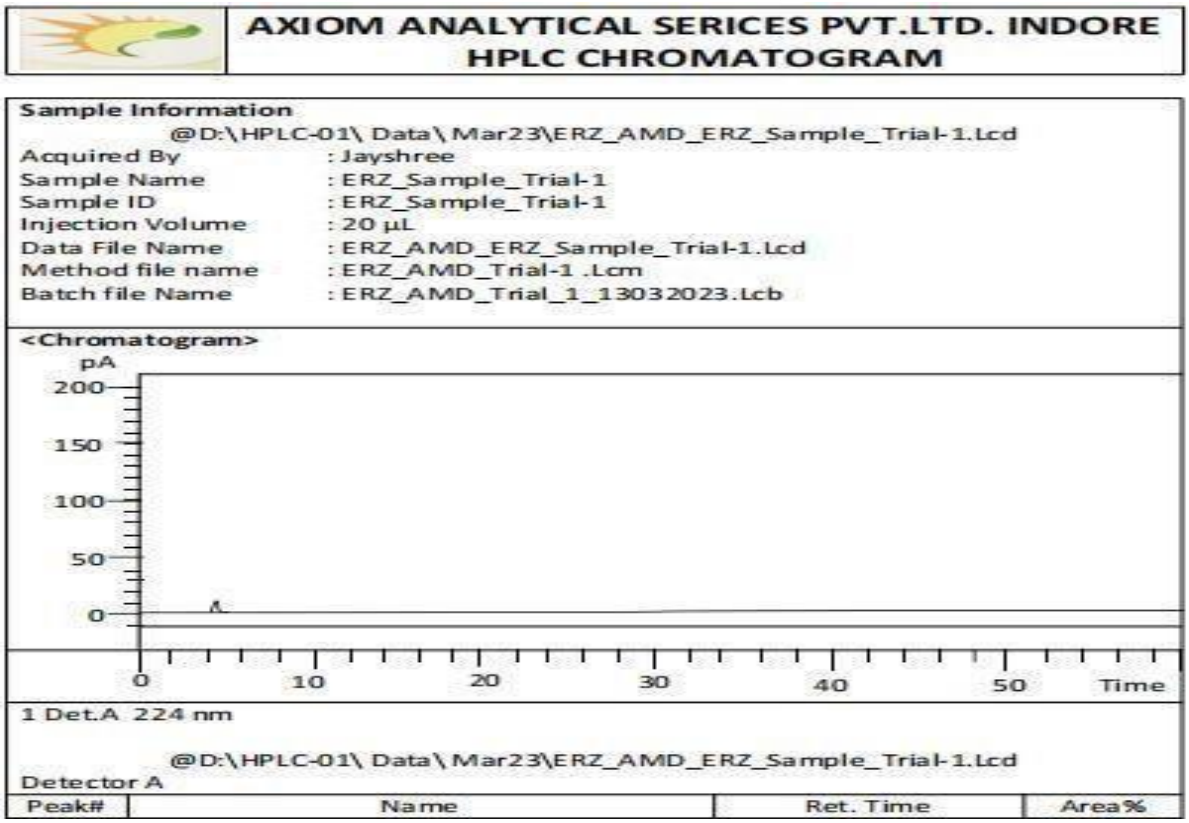
FIGURE 3-HPLC Chromatogram of Analytical Method Development Trial-1 - Sample



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Analysis of Sample on Developed Method

- **Chromatographic Condition:**

HPLC-system: HPLC Waters 2695 with Empower Software
Analytical column: Kromasil-C18, 5.0 µm, 250 x 4.6mm,
Mobile phase: 0.1% formic acid in water /0.1% formic acid in Acetonitrile

Flow: 1 ml/min for analytical column

Auto-sampler temperature:15±2°C **column temperature:**35°C **Injection volume:** 20 µl

Run time: 20.0 min.
Chromatographic data acquisition software: Empower-3
Preparation of mobile phase A : 0.1% formic acid in water: Spike 1000 ml of water with 1 ml of formic acid.

Preparation of mobile phase b: 0.1% formic acid in acetonitrile: Spike 1000 ml of acetonitrile with 1 ml of formic acid.

Preparation of column/needle wash solution: (0.1 % formic acid in Acetonitrile: water (50:50, v/v)): Mix 500 mL of Acetonitrile and 500 mL of water in are agent bottle, add 1 mL of formic acid and sonicate using ultra-sonicator.
Preparation of diluent: (Acetonitrile: Water = 40: 60, v/v): Mix 400 mL of acetonitrile and 600 mL of water in a reagent bottle.

Standard solution:- Dissolve 50.25 mg of the Ertugliflozin Standard in the Diluent and dilute to 100.1 ml with the Diluent.

Sample solution:- Dissolve 50.63, 50.65 mg of the substance under examination in theDiluent and dilute to 100.0 ml with the Diluent.

- **Forced Degradation Studies**

Regulatory guidelines: stress degradation study
The Different international guidelines represent stress degradation studies. The ICH

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guidelines that are applicable to stress degradation studies are as follow;

1. ICH Q1A:- New Drug Substances and Products Stability Testing,
2. ICH Q1B:- Photo stability Testing of New Drug Substances and Products,
3. ICH Q2B:- Validation of Analytical Procedures: Methodology. [2, 4]

The sample was also treated with described following **Acid Hydrolysis, Alkaline Hydrolysis, Oxidation Degradation, Thermal Degradation** and **Photolytic** D**egradation** conditions. 20 µL of the resulting solutions were injected into the HPLC system and the chromatograms were recorded.

Acid hydrolysis: Forced degradation in acidic condition was performed by adding 1 mL of standard solutions of ertugliflozin (20 µg/mL) to 6 ml methanol: water (1:1). To start the reaction, pH

3.0 was adjusted using 0.1 M hydrochloric acid. The mixture was incubated at 45°C for 2 hours. The solution was then allowed to reach at room temperature, neutralized to pH 7.0 by the addition of 0.1 M sodium hydroxide, and diluted to 10 mL with the mobile phase to get a final concentration of ertugliflozin (2 µg/mL).

3. Results and Discussion

Method Development and optimization

The optimization of method by trial and error method to obtain a chromatogram with good better number of theoretical plates, and tailing factor for the component peak. To optimization of the analytical RP-HPLC method, preliminary trial injections were performed by changing mobile phase (Table 1). Buffer selected for the present analysis was 0.1% Formic acid in water and 0.1 % in acetonitrile to avoid peak splitting. . When the drug samples were scanned on UV Spectrophotometer between 200 and 400 nm, the λ max was found 224 nm. UV spectrum is shown in Fig. 3. Kromasil Octa Decile Silen (C-18) columns are rugged, highly retentive, and widely available. Optimal separation and peak shapes were obtained on Kromasil Octadecylsilane (C-18) column with

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dimensions of 250 mm × 4.6 mm, 5 µm indicating that it was suitable for the estimation of ERTU . Theoretical plates are an important characteristic of the column which indicates the ability of the column to produce sharp, narrow peaks for achieving good resolution. Under optimized test conditions, a Kromasil Make column with 250 mm length 4.5 Internal Diameter and a particle diameter of 5 µm produces theoretical plates of 10,000–12,000 . In the optimized trial, the observed system suitability parameters were theoretical plates 11,261, tailing factor 1.1 reflecting that theselected column was ideal for the estimation of the drug. In the trial runs, for the ideal separation of the selected drugs, mixtures of solvents like 0.1 % Formic acid in water and acetonitrile in different proportions were tried on C18 column. In the optimized method, the mobile phase selected was 0.1 % Formic acid in water and acetonitrile (70:30% v/v), as the peak shape of ERTU was good with optimum system suitability parameters. The flow rate was optimized on the basis of analyte peak quality and minimum consumption of the mobile phase, and the flow rate selected for the analysis was 1.0 ml/min. ERTU was eluted at 7.24 min. Analytes were eluted on Kromasil-C18, 150 x 4.6 mm, 5µm column using an isocratic elution mode having mobile phase composition of 0.1% formic acid in water :0.1% formic acid in Acetonitrile (70:30 %v/v). Analyte were detected at 224 nm. 20µL fixed-

loop injector was used for the injection of the sample with the flow rate of 1.0 ml min−1. Retention times were 7.24 min for ertugliflozin as shown in Fig 14. The Non degraded sample were analyzed on that devolved method to check the quantitative assay results of sample and found % Assay is 99.89%.

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Stress or forced degradation studies:

The important part of method development of a stability indicating method is to assess the presence of impurities under the main analyte peak. The analyte peak was checked for its purity/homogeneity, which is usually evaluated by determining the purity angle and purity threshold. According to the ICH Q2 (R1) guidelines, in forced degradation studies, purity threshold should be greater than purity angle and % degradation should be less than 20 to consider the method as stable. The developed method was specific and stable for the estimation of the drug. The % degradation of ERTU was 1.02–6.52 The co-elution of degradants with the drugs was absent. The purity threshold for the drugs was found to be greater than the purity angle see table below. In forced degradation studies, purity threshold was found to be greater than purity angle for the drugs inferring the absenceof co-elution of degradants with the drugs, and the analyte peaks were pure. Percent degradation ofless than 10 for the drugs demonstrates that the developed method was specific and stable.

Table : 2 Drug Forced Degradation Profile

Sr.No	Degradation Condition	% Assay	% Degradation in Assay Observed
1.	Without Degradation	99.89 %	-
2.	Acid Degradation	76.63 %	23.26 %
3.	Alkali Degradation	62.73 %	37.16 %
4.	Oxidation Degradation	38.16 %	61.82 %
5.	Thermal Degradation	99.91 %	No Degradation Observed
5.	Photolytic Degradation	99.97 %	No Degradation Observed

The drug shows no degradation in Thermal and photo degradation when exposed to high temperature and UV light. The assay results are found 99.91 and 99.97 for both the

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degradation condition, The drug shows maximum degradation in oxidation degradation condition and assay results found is 38.16 %. the drug also shows degradation in acid hydrolysis and Alkali degradation and assay results are found during these degradation are 76.63 % 62.73% respectively and the developed method is capable to quantify the amount of drug degradation accurate.

4. CONCLUSION

The RP-HPLC method which used as a stability-indicating method of analysis is an analytical procedure that is capable to differentiate between the major active pharmaceutical ingredients from any degradation/decomposition products formed during storage under defined storage conditions. This stability- indicating assay method development studies the effect of stressors on a drug by after Forced Degradation of drug substance, which helps in understanding the stability of the drug during storage under different Stressed conditions and analysis. Few methods were reported for the estimation of ERTU by RP-HPLC. In the present method, ERTU was eluted at 7.52 min . The present method was developed using 0.1% Formic acid in water : 0.1% Formic acid in ACN as the mobile phase using Kromasil Make C-18 analytical column with the flow rate of 1.0 ml/min on 224 nm wave length . The developed analytical method was found to be specific and cost effective with reduction in the ratio of organic solvent in the moving phase. Improved stability-indicating RP-HPLC method was developed for the estimation of Ertugliflozin. retention times compared to the best method reported. The drug shows no degradation in Thermal and photo degradation when exposed to high temperature and UV ligh and shows maximum degradation in oxidation degradation condition and assay results found is 38.16 % . and shows less degradation in acid hydrolysis and Alkali degradation than oxidation degradation all the samples were analyzed on new developed method and the developed method is capable to quantify the amount of drug degradation accurate. The developed method could be suitable for routine analysis of the drugs in bulk and tablet formulation after complete validation as per ICH guideline.

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