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Research Article

Novel Simplified, New Analytical Method for Stress Degradation Study of Ertugliflozin an Oral Anti-diabetic Agent by RP-HPLC Method

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Abstract

Precise, simple and Stability indicating RP-HPLC method was developed and validated for Ertugliflozin in this study. Major objective of this proposed method for Ertugliflozin validated as per the guidelines of ICH and FDA. The following parameters were validated according to ICH guidelines in terms of System suitability, Solution stability, Linearity, Accuracy, Precision, Robustness and Stress degradation studies. In this proposed method, the separation was carried out using C18 Hypersil ODS Column (250 mm x 4.6 mm, 5 µm) with isocratic flow and the mobile phase utilized was acetate buffer and acetonitrile 60:40 (v/v). Ertugliflozin peaks were identified and separated at UV detection wavelength 240 nm. The pH of acetate buffer was adjusted to 4.0 with O-Phosphoric acid. The retention time for Ertugliflozin was found to be 2.30 ± 0.04 min. The results were found linear with concentration range 2.5 to 50 µg/ml (r^2 = 0.9998). The % recovery for the developed method found 98.48 to 99.43% for Accuracy and Precise for Intra and Interday method. No deliberate variation found for our developed method in flow rate, mobile phase and detection wavelength. The precision report for various concentrations (5 µg/ml, 7.5 µg/ml and 10µg/ml) found to be less than 2% for Intra and Interday precision. In forced degradation studies, Ertugliflozin was stable in Acidic, Alkaline, Thermal conditions whereas in Photolytic and oxidative conditions notable degradation was observed. The RP-HPLC method was validated according to ICH and FDA guidelines. The developed method was found to be Specific, precise, simple, sensitive, accurate, linear and thus to be considered stability indicating for Ertugliflozin pharmaceutical formulations and research tablets.

Keywords: Ertugliflozin; RP-HPLC; Stability Indicating; T2DM; SGLT2; ICH; USFDA

Introduction

As per 2017 report 6.28% largest number of the population suffered from Type 2 Diabetic mellitus which is considered to be an life threatening and cause remarkable changes in social and health care systems. Treating patients suffering with Type 2 diabetes mellitus need drug therapy with changes in lifestyle pattern like proper diet, exercise and prescribed lifestyle [1].

In T2DM drug therapy treating patients with SGLT found to be recurrent and positive. Gliflozin derivatives reduce the glucose reabsorption by kidney and improve urinary excretion of glucose thereby reducing the blood glucose level in patients suffering with Type 2 diabetic mellitus. In diabetic type 2 blood glucose level is found more and it is due to 90% absorption of glucose by SGLT, Inhibiting them by gliflozin reduces the level which was highly recommended therapy nowadays.

Ertugliflozin [$C_{22}H_{25}ClO_7$] chemically called as (1S,2S,3S,4R,5S) -5- (4-chloro- 3- (4-ethoxybenzyl) phenyl) -1-(hydroxymethyl)-6,8-dioxabicyclo [3.2.1] octane- 2,3,4- triol [3]. Ertugliflozin and fixed-

dose combinations of Ertugliflozin and Metformin, Ertugliflozin and sitagliptin have recently been approved by the US FDA as an adjunct to diet and exercise to improve glycemic control in adults with T2DM [4,5]. The structure of Ertugliflozin was shown in figure 1.

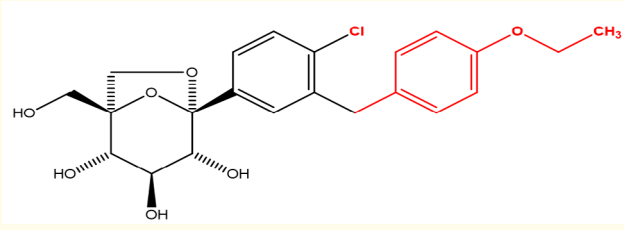

Figure 1: Chemical structure of Ertugliflozin.

Various analytical methods reported for Ertugliflozin for estimation and qualification but very limited number development methods reported for Ertugliflozin that too in its combination with Metformin and Sitagliptin. Ertugliflozin method development reported for it s combination with Metformin by RPHPLC [6-10], with Sitagliptin [11-17], in combination with both Metformin and Sitagliptin [18], bioanalytical methods using LC-MS, UPLC, Fluorescence spectroscopy [19-21]. As per our knowledge this is the first HPLC method developed for Ertugliflozin to know about its degradation products and drug stability in stress conditions.

Materials and Methods

Instrumentation

Chromatographic separation was carried out with the Shimadzu HPLC system consisting of a quaternary pump UV detector and auto-injector. This system consisted of a column compartment which supplies proper temperature to the column during the study. Data obtained by lab solution software and recorded into the HPLC system. For weighing 1mg sensitive Shimadzu electronic weighing balance and for stress degradation studies I-Therm thermostat oven were used.

Chemical and reagent

For this proposed method HPLC grade Acetonitrile, Triethylamine and HPLC grade distilled water procured from Loba Chemie Pvt Ltd, India. Working standards of Ertugliflozin and Research tablets were purchased from Supriya Life science Ltd, India.

Chromatographic parameters

- Equipment: SHIMADZU 2010CHT HPLC System
- Column: HYPERSIL C_{18} (250 x 4.6 mm, 5 µm particle size)
- Mobile phase: Acetate buffer: Acetonitrile (60:40 v/v)
- Wavelength: 240 nm
- Injection Volume: 20 µl
- Flow rate: 1 ml/min
- Run Time: 6 min
- Column Temperature: 28°C.

Preparation of standard stock solution

Accurately weighed 5 mg of Ertugliflozin and transferred into 50 ml volumetric flask added 10 ml mobile phase and sonicated the solution for 15 minutes. Final volume made with a mobile phase and filtered using a 0.45 µm filter. Degas the stock solution before filter and use this stock solution for further studies.

Preparation of sample solution

Weighed and transferred 5mg equivalent Ertugliflozin tablet powder. Crush the Powder using mortar and pestle. The fine powder was transferred into a 100 ml volumetric flask. Made the solution with mobile phase then sonicated for 20mins and finally degas it to remove the air bubbles. Filter the sample solution using 0.45 µm and utilize it for further studies.

Preparation of mobile phase

Mobile phase prepared by mixing Acetate buffer pH 4 with acetonitrile in the ratio of 60: 40.

Method validation

Ertugliflozin validating parameters studied as per the guidelines provided by ICH. The following validity parameters study for this drug as follows: - system suitability, linearity, accuracy, precision, solution stability, robustness and forced degradation study.

Forced degradation study

Forced degradation study conducted to know the stability of the drug under different stress conditions. Stress degradation studied, as per the guidelines by exposing the drug into acidic degradation, alkaline degradation, oxidative degradation, photolytic degradation and thermal degradation [23].

For Acidic degradation known concentration of sample solution mixed with 1 ml of HCl. After mixing neutralized the solution with 0.1N 1 ml sodium hydroxide and injected.

Alkaline degradation with known concentration of sample solution added 1 ml of sodium hydroxide diluted mixed and neutralized the solution 0.1N HCl before injecting the sample into the system.

For Oxidative degradation to 1 ml of sample solution 30% H_2O_2 added and refluxed the solution for 3 hours at room temperature. After 3 hours, inject the sample solution and read its peak area.

Thermal degradation, the fine powder of drug spreader in uniform surface and exposed to 105°C for 24 hours. After exposure, inject the sample into the system to study the degradation.

Photolytic degradation, the fine powder of drug sample spreader in uniform surface and kept under the UV light 254nm for 24 hours. After exposure read the peak area by injecting triplicate solution into the system.

Result and Discussion

Ertugliflozin drug used for treating the patient suffering with type 2 Diabetes mellitus T2DM, this drug involved in inhibiting the role of SGLT2 to identify a stable, sensitive and reliable chromatographic system. Different trials were conducted to identify the following right parameters for the study: column for separation, mobile phase and its composition, flow rate and column temperature as per ICH guidelines [24].

The weakly ionizable drug was found to be eluted using different columns with asymmetric and symmetric tailing factors during trial. In order to improve that separation column with the specified properties selected as below: C18 column 25 cm with 5 µm internal diameter. It was found that this column separates the analyte with good retention volume and peak shape as per the trail conducted.

Utilized different mobile phase with different compositions ratio selected for this study. Mobile phase with buffer pH 4 to 6 involved in eluting the analyte within 10 minutes and in few trails excellent RT value observed with mobile phase ratio 60:40 of Acetate buffer and acetonitrile.

Column temperature played an important role in eluting the analyte with a good amount of peak area which are reproducible in nature. For this study Column temperature 28°C considered as optimized one for further analytical experiments.

For flow rate identification 1 to 1.5 ml/min injection volume employed during the trail study and observed that high resulted separation with 1.0 ml/min whereas 0.5 ml/min separated the analyte with fronting effect and 1.5 ml/min failed to elute the analyte due to its over saturation effect.

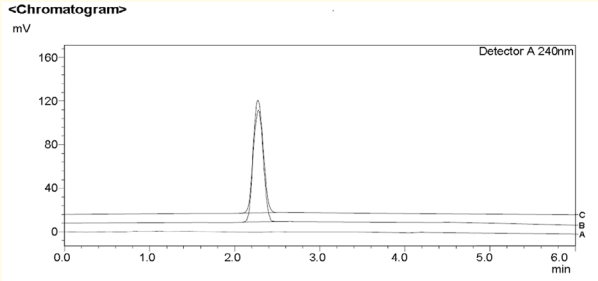

Figure 2: Chromatogram of Ertugliflozin in Optimized Condition A- Blank, B-Working Standard, C- Ertugliflozin Sample.

S. No	Injection no	RT	Area	USP Plate	USP Tailing Factor
1	Injection 1	2.3	248435	5745	1.01
2	Injection 2	2.29	248743	5482	1.01
3	Injection 3	2.3	249824	5675	1.03
4	Injection 4	2.31	248846	5841	1.05
5	Injection 5	2.29	249044	5501	1.02
6	Injection 6	2.31	248785	5493	1.04
Mean			248946.1667	5622.833333	1.03
S.D			473.0198375	152.82	0.016
% RSD			0.190008886	2.7	1.59

Table 1: System suitability report of Ertugliflozin.

The reliable separation found with flow rate 1 ml/min, the linearity value found to be linear, the concentration range 2.5 to 50 microgram with the calibration value Y = 46645x + 6180 (r^2 = 0.9997).

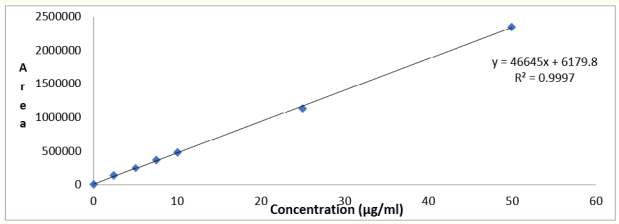

Figure 3: Calibration Curve of Ertugliflozin at 240 nm Wavelength.

S. No	Conc (µg/ml)	*Mean peak area ± SD (% RSD)
1	0	0
2	2.5	123866
3	5	248581
4	7.5	360357
5	10	482703
6	25	1142597
7	50	2349631
8	80	3784552
9	100	4766870
10	120	5682572
Slope		47424
Intercept		-3215
Coefficient Correlation value		0.99996

Table 2: Linearity Results.

*Mean of three replicates.

Accuracy

Recovery study conducted for a system proposed method by elevating the concentration into 50% to 150%. Recovery report was found more reliable with the number of samples studied.

Precision

Intermediate and Method precision conducted for the developed method by injecting 6 samples of triplicate solution prepared for this experiment. The Precision value found less than 2% with accuracy which clarified that our method remains simple, precise and accurate.

Robustness

There is no deliberate variation experienced for the method employed.

Drug	Spiked Conc (µg/ml)	Total Conc (µg/ml)	*mean peak area ± SD (%RSD)	Drug found (µg/ml)	% Recovery
Ertugliflozin	2.5 (50%)	7.5	367674 ± 1568 (0.43)	7.39	98.48
	5 (100%)	10	494932 ± 953 (0.19)	9.94	99.43
	7.5 (150%)	12.5	617829 ± 1658 (0.27)	12.41	99.29

Table 3: Result of % Recovery Method.

*Mean of three replicates.

S. No	Parameters	Condition	Research Sample	Working Standard
			*Mean peak area ± SD (%RSD)	*Mean peak area ± SD (%RSD)
1	Flow rate	(0.8 ml/min)	232748 ± 3657 (1.6)	227903 ± 1348 (0.6)
2	Flow rate	(1.2 ml/min)		
3	Mobile phase	(43: 57)	224775 ± 2504 (1.10)	231989 ± 2011 (0.9)
4	Mobile phase	(47: 53)		

Table 4: Result of Robustness Method.

*Mean of three replicates.

Forced degradation studies

The drug withstand the stress condition introduced into the sample which are in solid and liquid form stability state. Good amount of Degradation happens with acidic degradation compared with Photolytic, alkaline, oxidative, thermal conditions without disturbing the retention time of the drug. Data for various analytical parameters summarized below for the above developed method proved that peak purity not altered with the stress conditions implied during the forced degradation studies.

Stress Condition 0.1N	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)
Standard drug (untreated)	248893	100	-
Acidic degradation	244275	98.14	1.86
Alkaline degradation	245281	98.55	1.45
Oxidative degradation	237285	95.34	4.66
Thermal degradation	239165	96.09	3.91
Photolytic degradation	231297	92.93	7.07
Stress Condition 1N			
Standard drug (untreated)	248893	100	-
Acidic degradation	214041	86.01	13.99
Alkaline degradation	225645	90.67	9.33
Oxidative degradation (30%)	233750	93.93	6.07
Thermal degradation (30%)	229589	92.26	7.74
Photolytic degradation (30%)	236605	95.07	4.93

Table 5: Data of Stress degradation studies Ertugliflozin.

*Mean of three replicates.

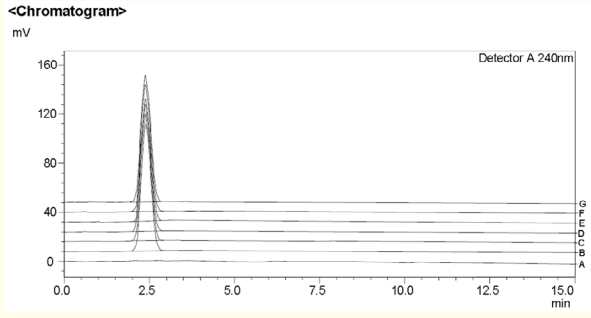

Figure 4: Stress conditions Chromatogram of Ertugliflozin A-Blank, B-Standard, C-Acidic, D-Alkaline, E-Oxidative, F-Thermal, G-Photolytic degradation.

Conclusion

Various methods reported for estimation of Ertugliflozin in combination with Sitagliptin and Metformin. Whereas as per our knowledge this is the first method reported for Ertugliflozin alone. Estimation of Ertugliflozin alone is needed one for current conditions to study the metabolite products of Ertugliflozin and its biochemical purity. The proposed method validated for Ertugliflozin as per the guidelines of FDA and ICH. Validation parameters report such as Specificity, Accuracy, Precision, Linearity and Robustness were found within the acceptable level. The chromatographic condition optimized for this method as follows C18 Hypersil ODS Column (250 mm x 4.6 mm, 5 µm) with isocratic flow and the mobile phase utilized was acetate buffer and acetonitrile 60:40 (v/v) with injection volume 20 µl. The drug detected at UV detection wavelength 240 nm. Forced degradation reports summarized that the drug remains stable in Acidic, Alkaline, Thermal conditions whereas slightly unstable Photolytic and in oxidative conditions with altering or interfering with the retention time of Ertugliflozin. Hence, our proposed method can be employed for estimating and evaluating the stress degradation of Ertugliflozin API and Pharmaceutical Formulations.

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Conflict of Interest

Author declares No conflict of interest for this research work.

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