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(RESEARCH ARTICLE)

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Determination of ertugliflozin in pharmaceutical formulations by RP-HPLC method

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Abstract

A novel, specific, accurate, rugged, precise reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Ertugliflozin in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using Symmetry ODS C18 (4.6×250mm, 5µm) column with a mobile phase containing a mixture of Methanol: Phosphate Buffer pH-3.6 in the ratio of 35:65%v/v. The flow rate was 1.0 ml/min and effluent were monitored at 235 nm and a peak eluted at 2.552 min and column oven temperature was maintained ambient. Calibration curve was plotted with a range from 6-14 µg/ml. The LOD and LOQ values of Ertugliflozin were found to be 1.2µg/ml and 3.6µg/ml respectively. The percentage recovery of the Ertugliflozin was found to be within the limits. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ertugliflozin in bulk drug and in its pharmaceutical dosage form. The proposed method was applied for the analysis of tablet formulations, to improve QC and assure therapeutic efficacy.

Keywords: Ertugliflozin; RP-HPLC; Accuracy; Validation; ICH Guidelines**.**

1. Introduction

Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters (SGLT), more specifically the type 2 which is responsible for about 90% of the glucose reabsorption from glomerulus. (Figure.No.1). Ertugliflozin is an oral, selective inhibitor of sodium glucose co-transporter-2 (SGLT2) that results in urine glucose excretion (UGE) and decreases in plasma glucose and haemoglobin A1c (A1C) in individuals with type 2 diabetes mellitus (T2DM). Ertugliflozin is a newly discovered chemical entity with the chemical name of (1S,2S,3S,4R,5S[4-Chloro-3-(4ethoxybenzyl)phenyl]-5-[4-Chloro-3-(4ethoxybenzyl) phenyl] -1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol.[1-4] Ertugliflozin is used for the treatment a higher prevalence of genital mycotic infections occurred in men and women with ertugliflozin compared with placebo.[5-7]

Literature review revealed that only few HPLC method [8-11] have been reported for the determination of ertugliflozin and a pharmacokinetic study which used LC-MS method for the determination of ertugliflozin. HPLC has become a widely used tool for the routine determination and separation of drugs either alone in pure form or in admixture with other drugs or degradation products and in pharmaceutical formulations.[11-14]Existing literature reveals that there are only few methods for the assay of ertugliflozin in bulk and dosage forms. Hence an attempt has been made to develop a new simple, reliable, and reproducible, isocratic RP-HPLC methods to estimate the ertugliflozin in bulk and

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pharmaceutical formulation with good precision, accuracy, linearity and reproducibility respectively. The proposed method was validated as per ICH guidelines [15-17].

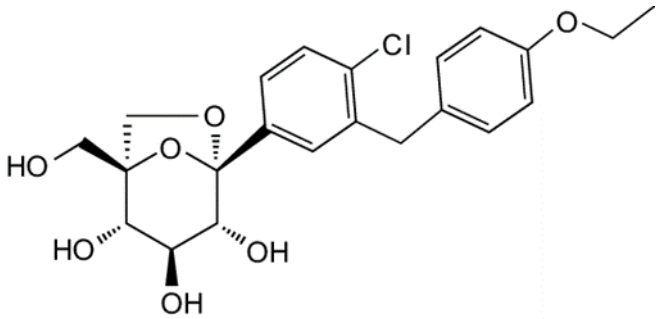

Figure 1 Structure of Ertugliflozin

2. Experimentation

2.1. Equipment

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

2.2. Reagents and chemicals

Acetonitrile, Methanol and water of HPLC grade were procured from Rankem lab ltd. ertugliflozin was received as gift samples from Reddys Labs Ltd., Hyderabad, India, respectively. Steglatro ® and tablets were purchased from local market. Nicolan Healthcare Pvt Ltd

2.3. HPLC conditions

Symmetry ODS C18 (4.6×250mm, 5µm) column was used as the stationary phase. A mixture of Methanol: Phosphate buffer adjusted to pH-3.6 in the ratio of 35:65%v/v was used as the mobile phase. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 235 nm. The injection volumes of samples and standard were 20µl.

2.4. Standard solutions

A stock solution containing 1000µg/ml of Ertugliflozin was prepared by dissolving Ertugliflozin in mobile phase. A working standard solution contain in 10 -30µg/ml of EGZ was prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

2.5. Assay of tablet formulation

Table 1 Table for Assay

Tablet formulation	Drug	Amount present (mg/tab)	Amount found* (mg/tab)	% label claim*
T1	EGZ	5	5.19	100.99%
T2	EGZ	15	14.91	99.98%

T1 and T2 are two different brands of tablet formulations. EGZ denotes Ertugliflozin respectively.*Each value is average of six determinations.

20 tablets (each tablet contains 5 mg of Steglatro ® tablets) were accurately weighed and calculated their average weight. Then it was taken into a mortar and crushed to fine powder and uniformly mixed. A quantity of powder equivalent to10 mg of EGZ was weighed and transferred to a 10 ml standard flask. The drug was initially dissolved in diluent and sonicated for 10 minutes. The volume was made up to 10 ml with mobile phase. Then the solution was filtered using 0.45-micron syringe filter. After that 0.1ml of the above filtrate was diluted to 10 ml with the diluent so as to give a concentration of 10µg/ml of Ertugliflozin. Then 20µl of this solution was injected in to the column and chromatogram was recorded and shown in Fig.2.Each concentrations of EGZ in the tablet formulation were calculated

by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in table1.

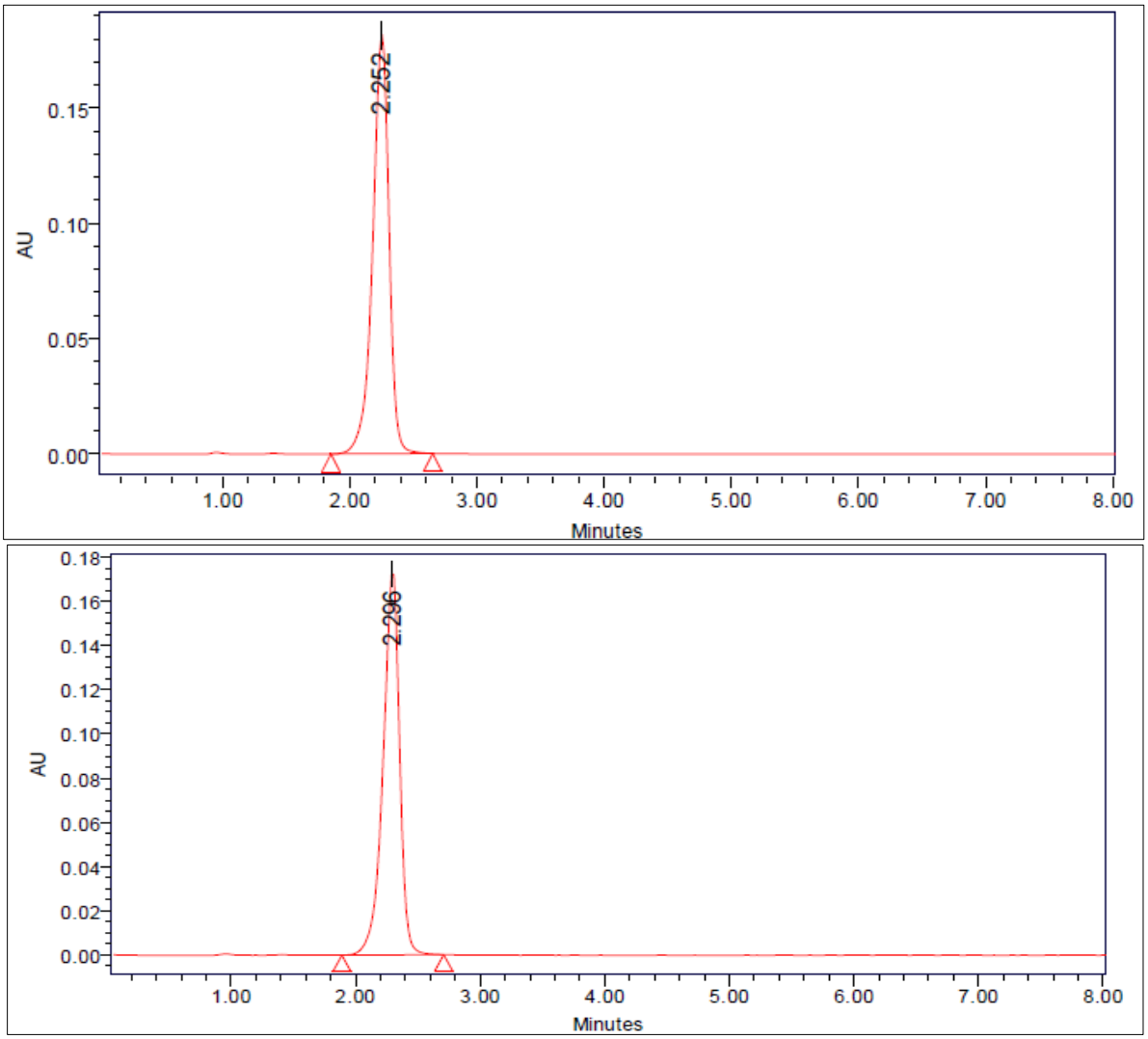

Figure 2 Assay Chromatogram of Ertugliflozin.

3. HPLC method development

3.1. Optimized Chromatographic condition (Fig.3)

Column: Symmetry ODS C18 (4.6×250mm, 5µm) column

Temperature: Ambient

Wavelength: 235 nm

Mobile phase ratio:Methanol: Phosphate buffer adjusted to pH-3.6, 35:65%v/v Flow rate 1.0mL/min

Injection volume: 10 µl

Run time: 8 minutes

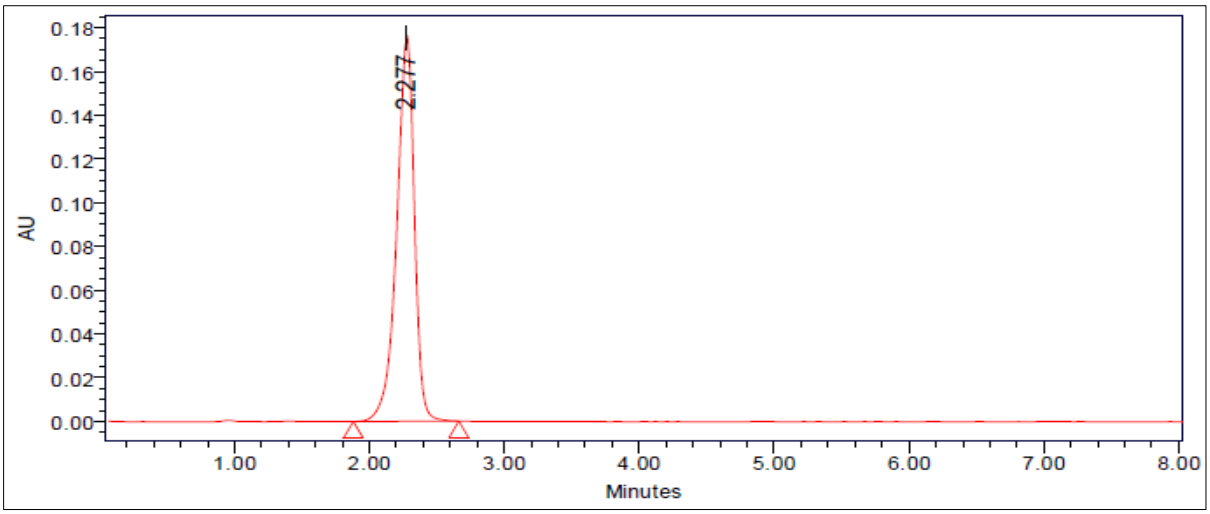

Figure 3 Optimized chromatogram of ertugliflozin

3.2. Validation of the method

3.2.1. Preparation of mobile phase

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.6):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

3.2.2. Preparation of mobile phase

Accurately measured 350 ml (35%) of Methanol, 650 ml of Phosphate buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Table 2 System suitability results of Ertugliflozin

Sr. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ertugliflozin	2.277	1652847	185647	6589	1.24
2	Ertugliflozin	2.277	1653658	186254	6587	1.26
3	Ertugliflozin	2.267	1654521	185475	6584	1.28
4	Ertugliflozin	2.265	1653564	186594	6582	1.29
5	Ertugliflozin	2.277	1658745	185684	6895	1.24
Mean			1654667			
Std. Dev.			2355.764			
% RSD			0.142371			

3.2.3. Diluent Preparation

The Mobile phase was used as the diluent.

System suitability studies

The system suitability test was carried out on freshly prepared stock solution of EGZ to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in Table 2. The values obtained were

demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within ± 2% standard deviation range during routine performance of the method.

Linearity and Range

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 6-14 µg/ml. 20µl of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas (Y axis) against the amount of drug in µg/ml (X axis). Peak area of linearity range and the parameters were calculated and presented in table 3 respectively. The linearity curve of Ertugliflozin was shown in Figure.4.

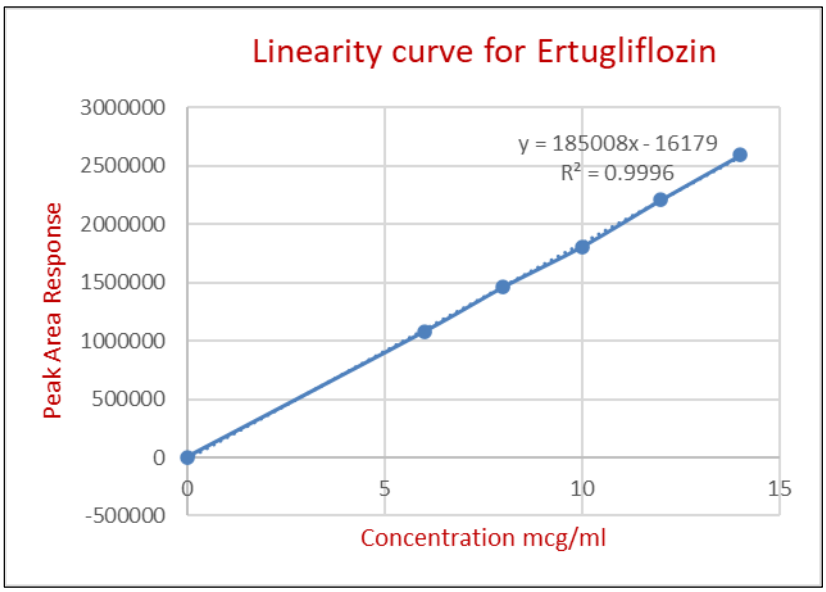

Figure 4 Linearity curve Ertugliflozin

Table 3 Analytical performance parameters of linearity curve

S.No	parameters	Ertugliflozin
1	Linear dynamic range(µg/ml)	6-14
2	Correlation coefficient	0.9996
3	Slope (m)	185008
4	Intercept	16179
5	Curve fitting	99.96
6	LOD(µg/ml)	1.2
7	LOQ(µg/ml)	3.6

3.2.4. Limit of detection and Limit of quantification

The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD = 3.3X {Residual Standard deviation/Slope}.

The LOD for Ertugliflozin was confirmed to be 1.2g/ml.

The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ = 10 X {Residual Standard deviation/Slope}.

The LOQ for Ertugliflozin was confirmed to be3.6µg/ml

3.2.5. Accuracy

The accuracy of the method was determined by recovery experiments. Placebo was spiked with known quantities of standard drugs at levels of 50 to 150% of label claim. The recovery studies were carried out 3 times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in table 4. The mean recovery is well within the acceptance limit, hence the method is accurate.

Table 4 Recovery studies of Ertugliflozin

%Concentration (at specification Level)	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

*Average of six or three determinations, Mean ± Standard Deviation

3.2.6. Precision

System precision

The system precision of the method was established by six replicate injections of the standard solution containing Ertugliflozin The percentage RSD were calculated and presented in Table 5. From the data obtained, the developed RP-HPLC method was found to be precise.

Method precision

The method precision of the method was established by carrying out the analysis of Ertugliflozin (n=6) using the proposed method. The low value of the relative standard deviation showed that the method was precise. The results obtained were presented in table 5.

Table 5 Precision studies of Ertugliflozin in dosage forms

	System Precision		**Method Precision**	
	Ertugliflozin		Ertugliflozin	
S.No.	**Retention time**	**Peak Area**	**Retention time**	**Peak Area**
1	2.277	1665847	2.274	1678541
2	2.255	1658989	2.258	1685985
3	2.265	1659845	2.267	1685745
4	2.255	1665964	2.270	1685987
5	2.253	1659863	2.264	1698526
6	2.252	1665986	2.265	1685943
Avg		1662749		1686788
Stdev		3501.766		6463.466
%RSD		0.210601		0.383182

3.2.7. Specificity

Specificity of the method was determined by injecting the diluted placebo. There was no interference of placebo with the principle peak, hence the developed analytical method was specific for Ertugliflozin in tablet dosage form.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature and refrigerator temperature for 24h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were up to 24h at room temperature and refrigerator temperature.

3.2.8. Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010 A4T), Water Alliance HPLC 2695 by different operators using different columns of similar type like Hypersil C_{18} column and Phenomenex C18 column. Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The results of ruggedness were presented in table 6. The results of robustness were presented in table 7.

Table 6 Method ruggedness of Ertugliflozin in dosage forms

%Assay*(n=6)	**%RSD of Assay(n=6)**
Day -1 , Analyst-1, Instrument-1&Column-1	
99.92 ± 0.142	0.633
Day -2 , Analyst-2, Instrument-2&Column-2	
100.39 ± 0.321	0.562

*Average of six determinations, mean ± Standard Deviation

Table 7 Method robustness of Ertugliflozin in dosage Forms

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1658242	2.312	6569	1.24
Less Flow rate of 0.9 mL/min	1854215	2.458	6865	1.35
More Flow rate of 1.1 mL/min	1758468	2.032	6254	1.32
More Organic phase	16514236	2.034	6747	1.73
Less organic phase	1742536	2.610	6514	1.41

4. Conclusion

The proposed RP-HPLC method for the estimation of Ertugliflozin in tablet dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in determination of Ertugliflozin in bulk form and pharmaceutical dosage form. Hence the present RP-HPLC method is suitable for the quality control of the raw material, formulation and dissolution studies. The method validation shows satisfactory data for all the method validation parameter tested.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflicts of interest.

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