Journal of Pharmaceutical Research International

34(20B): 22-26, 2022; Article no.JPRI.84234
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Validated RP-HPLC Method Development for Estimation of Ertugliflozin and Sitagliptin in Bulk and Dosage Forms

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2022/v34i20B35829

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84234

Original Research Article

Received 02 January 2022
Accepted 05 March 2022
Published 09 March 2022

ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Ertugliflozin and Sitagliptin in bulk and tablet dosage form. Chromatogram was run through Std Discovery C18150 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1% OPA (2.2ph): Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% Ortho Phosphoric Acid (OPA). Temperature was maintained at 30°C. Optimized wavelength selected was 218 nm. Retention time of Ertugliflozin and Sitagliptin were found to be 2.228min and 2.880min. %RSD of the Ertugliflozin and Sitagliptin were and found to be 0.4 and 0.7 respectively. %Recovery was obtained as 99.23% and 100.01% for Ertugliflozin and Sitagliptin respectively. LOD, LOQ values obtained from regression equations of Ertugliflozin and Sitagliptin were 0.20, 0.61 and 0.46, 1.40 respectively. Regression equation of Ertugliflozin is y = 43997x + 2639, and y = 46501.x + 13112 of Sitagliptin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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Keywords: Ertugliflozin; Sitagliptin; RP-HPLC.

1. INTRODUCTION

Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters (SGLT), more specifically the type 2 diabetes which is responsible for about 90% of the glucose reabsorption from glomerulus with IUPAC name(1*S*,2*S*,3*S*,4*R*,5*S*)-5-[4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl]-1-(hydroxyleth yl)-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol . Sitagliptin is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs with IUPAC name 3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one. This enzyme-inhibiting drug is to be used either alone or in combination with metformin or a thiazolidinedione for control of type 2 diabetes mellitus. Ertugliflozin and Sitagliptin were used in combined dosage form to treat type 2 diabetes.

Literature review reveals that very few analytical methods have been reported for the determination of Ertugliflozin and Sitagliptin by using various analytical techniques like HPLC, LCMS, LCMSMS [1-15]. It was found that no suitable validated method was available from the literature for determination of bioavailability and bioequivalence of Ertugliflozin and Sitagliptin in biological samples. The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Ertugliflozin, Sitagliptin in bulk ant tablet dosage form.

2. METHODS

2.1 Chemicals and Reagents

Ertugliflozin and Sitagliptin pure drugs (API) were obtained as gift sample from SS Pharma, Combination Ertugliflozin and Sitagliptin tablets (QTERN) were purchased from local market, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

2.2 Instruments

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Ertugliflozin and Sitagliptin solutions.

2.3 Preparation of Solutions

Diluent: Based up on the solubility of the drugs, diluent was selected, acetonitrile and water taken in the ratio of 50:50.

Preparation of Standard stock solutions: Accurately weighed 3.75 mg of Ertugliflozin, 25 mg of Sitagliptin and transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (150µg/ml of Ertugliflozin and 1000µg/ml of Sitagliptin).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15µg/ml Ertugliflozin of and 100µg/ml of Sitagliptin).

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (150µg/ml of Ertugliflozin and 1000µg/ml of Sitagliptin).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10 ml volumetric flask and made up with diluent.(15µg/ml of Ertugliflozin and 100µg/ml of Sitagliptin).

Preparation of buffer: **0.1% OPA Buffer:** 1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

3. RESULTS

3.1 Method Development and Optimization

Method development was done by changing various method parameters like mobile phase

ratios, column, flow rate, wavelength etc. Chromatographic separation was achieved with Mobile phase in the ratio of 60% 0.1% OPA buffer: 40% Acetonitrile. Water and Acetonitrile in the ratio 50:50 was used as Diluent. Chromatographic separation was achieved on Discovery C18 (4.6 x 150mm, 5µm) column with 1 ml/min flow rate at 218 nm wave length.

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

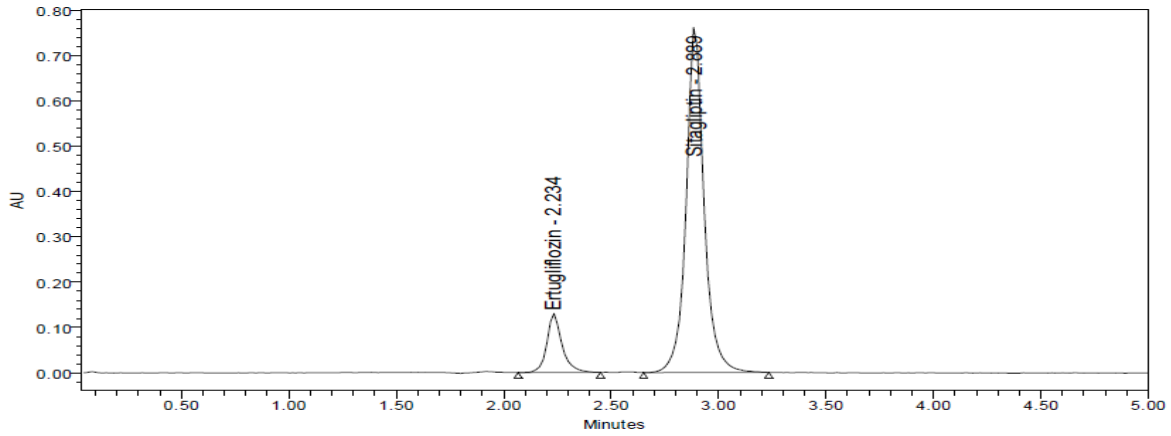

Fig. 1. Optimized Chromatogram for Ertugliflozin and Sitagliptin

Table 1. System suitability parameters for Ertugliflozin and Sitagliptin

S no	Ertugliflozin			Sitagliptin			
Inj	Rt(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.226	4965	1.11	2.873	6256	1.07	4.5
2	2.227	5222	1.15	2.878	6114	1.07	4.6
3	2.227	5190	1.16	2.878	5864	1.07	4.5
4	2.228	5222	1.16	2.879	5878	1.07	4.5
5	2.230	4888	1.14	2.880	5917	1.07	4.5
6	2.231	5413	1.14	2.881	5874	1.06	4.5

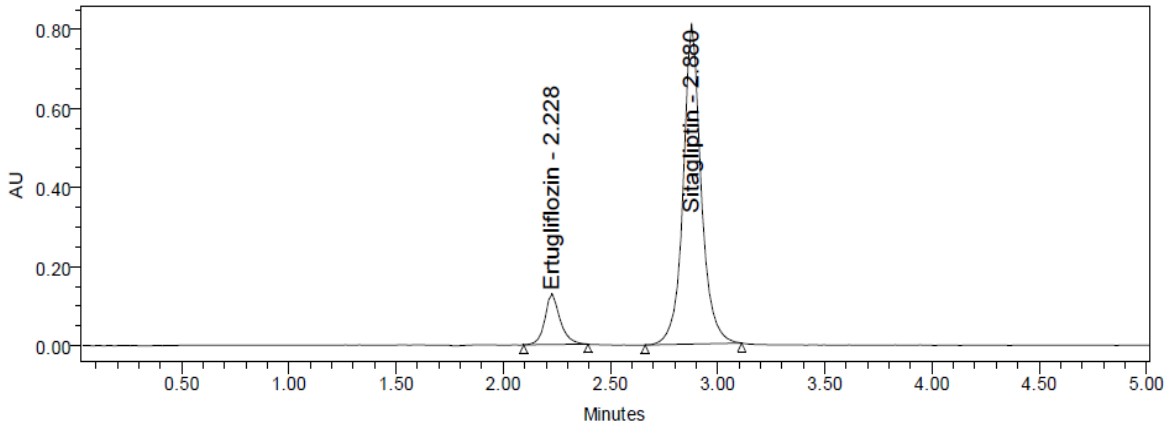

Fig. 2. System suitability Chromatogram for Ertugliflozin and Sitagliptin

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. Various parameters were tabulated.

Table 2. Summary of various parameters

Parameters		Ertugliflozin	Sitagliptin	LIMIT
Linearity				
Range(µg/ml)		3.75-22.5µg/ml	25-150 µg/ml	
Regression coefficient		0.999	0.999	
Slope(m)		43997	46501	
Intercept(c)		2639	13112	R< 1
Regression equation (Y=mx+c)		y = 43997x + 2639	y = 46501x + 13112	
Assay (% mean assay)		99.61%	99.69%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.5	0.4	NMT 2.0%
Method precision %RSD		0.4	0.7	NMT 2.0%
Accuracy %recovery		99.23%	100.01%	98-102%
LOD		0.20	0.46	NMT 3
LOQ		0.61	1.40	NMT 10
	FM	0.5	0.2	
Robustness	**FP**	0.2	0.2	%RSD NMT 2.0
	MM	0.6	0.6	
	MP	0.9	1.0	
	TM	0.5	0.5	
	TP	0.7	1.1	

4. DISCUSSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Ertugliflozin and Sitagliptin in bulk and tablet dosage form. Retention time of Ertugliflozin and Sitagliptin were found to be 2.228min and 2.880min. %RSD of the Ertugliflozin and Sitagliptin were and found to be 0.4 and 0.7 respectively. %Recovery was obtained as 99.23% and 100.01% for Ertugliflozin and Sitagliptin respectively. LOD, LOQ values obtained from regression equations of Ertugliflozin and Sitagliptin were 0.20, 0.61 and 0.46, 1.40 respectively. Regression equation of Ertugliflozin is y = 43997x + 2639, and y = 46501.x + 13112 of Sitagliptin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

5. CONCLUSION

The proposed research work is highly specific and prior over other developed methods reported previously. Chromatographic conditions were improved. Hence the proposed method has significant advantages over previously reported methods in-terms of selectivity, sensitivity, linearity, reproducibility, accuracy. Therefore the developed method can be used in quality control laboratories for estimation of Ertugliflozin and Sitagliptin in bulk and tablet dosage form.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/84234