CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1220228

Available online at: http://www.iajps.com Research Article

A NEW STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ERTUGLIFLOZIN AND SITAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM ITS VALIDATION AS PER ICH GUIDELINES

P. Venkateswara Rao[1*], A. Lakshmana Rao[2], and S.V.U.M Prasad[3]

[1]Department of Pharmaceutical Analysis, Vikas College of Pharmacy, Vissannapeta -521215 and Ph.D Research Scholar, JNTUK, Kakinada.India.

[2]Principal,V. V. Institute of Pharmaceutical Sciences, Gudlavalleru-521 356, India.

[3]Program Director, School of Pharmacy, JNTUK, Kakinada-533003, India.

Abstract:

A new RP-HPLC method for the quantitative determination of Ertugliflozin and Sitagliptin *was developed and validated as per ICH guidelines. The drugs were injected into* Std Azilent *column (150×4.6, 5 µm), maintained at ambient temperature and effluent monitored at* 240 *nm.The mobile phase consisted of Buffer* (Potassium di hydrogen Ortho Phosphate)*: Acetonitrile (70:30 V/V). The flow rate was maintained at 1.0 ml/min. The calibration curve for* Ertugliflozin and Sitagliptin *were linear from 3.75-22.5µg/ml and 25-150µg/ml respectively (r2 for* Ertugliflozin *= 0.9992,r2 for* Sitagliptin *= 0.9995). Retention time was 3.203min (*Ertugliflozin*), 2.106min (*Sitagliptin *). Accuracy was in the range of 99.67-99.90% for both drugs. Precision was 0.1% and 0.2% for* Ertugliflozin and Sitagliptin, *LOD and LOQ are 0.43ug/ml and 1.31ug/ml for* Ertugliflozin and *, 0.74ug/ml and 2.24ug/ml for* Sitagliptin. *The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of* Ertugliflozin and Sitagliptin *in bulk and pharmaceutical dosage forms. When applied for tablet assay, drug content was within 99.18.-99.13 % of labeled content. Forced degradation studies indicated the suitability of the method for stability studies.*

Keywords: *Ertugliflozin and Sitagliptin, RP-HPLC Method, Simultaneous estimation, Validation as per ICH guidelines, Forced degradation studies.*

Corresponding author:

P.Venkateswara Rao,

Associate Professor,

Department of Pharmaceutical analysis

Vikas College of Pharmacy, Vissannapeta-521215

Ph.D Research Scholar, JNTUK, Kakinada India

***Mobile**: +91-9949963007*

***Email:** venkats0425@gmail.com,*

QR code

Please cite this article in press P.Venkateswara Rao et al., ***A new stability indicating Rp-Hplc method for Simultaneous Estimation of Ertugliflozin and Sitagliptin in Bulk and Pharmaceutical Dosage Form Its Validation as Per ICH Guidelines,*** *Indo Am. J. P. Sci, 2018; 05(04).*

INTRODUCTION:

Type 2 diabetes mellitus (T2DM) is a global pandemic, as evident from the global cartographic picture of diabetes by the International Diabetes Federation [1]. Diabetes mellitus is a chronic, progressive, incompletely understood metabolic condition chiefly characterized by hyperglycemia. Impaired insulin secretion,resistance to tissue actions of insulin, or a combination of both are thought to be thecommonest reasons contributing to the pathophysiology of T2DM, a spectrum of disease originally arising from tissue insulin resistance and gradually progressing to a state characterized by complete loss of secretory activity of the beta cells of the pancreas.T2DM is a major contributor to the very large rise in the rate of non-communicable diseases [2-3].

Ertugliflozin is chemically known as ertugliflozin L-pyroglutamic acid is (1S,2S,3S,4R,5S)-5-(4-chloro-3-(4ethoxybenzyl)phenyl)-1-(hydroxymethyl)-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol, compound with (2S)-5oxopyrrolidine-2-carboxylicacid.The molecular formula is $C_{27}H_{32}ClNO_{10}$ and the molecular weight is 566.00. 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[4]. Administration of ertugliflozin increases urinary glucose excretion which leads to a negative balance and osmotic diuresis. Thus, this antidiabetic agent has been reported to significantly reduce the body weight and blood pressure of diabetic patients [5].

Sitagliptin is chemically 3-amino-1-[3-(trifluoromethyl)6,8dihydro5H[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one;phosphoric acid;hydrate. It has a molecular formula of $C_{16}H_{15}F_6N_5O{\bullet}H_3PO_4{\bullet}H_2O$ with molecular weight 523.324. Sitagliptin is potent and highly selective inhibitor of dipeptidyl peptidase-4 (DPP-4). Sitagliptin is the first of a new class of drugs for the treatment of type II diabetes, a well known hypoglycemic drug in the present therapy. It reduces blood glucose concentration by enhancing the effect of incretins and there by leading to a significant increase in insulin secretion [6-7]. Indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus when treatment with both ertugliflozin and sitagliptin is appropriate [8-9].

Though several methods are reported in literature for the estimation of Sitagliptin with other drugs combination [10-17] and individually, no methods are reported for estimation of ertugliflozin and sitagliptin in combination.The objective of the present study is to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Ertugliflozin and Sitagliptin and validate the method with forced degradation studies according to ICH guidelines.

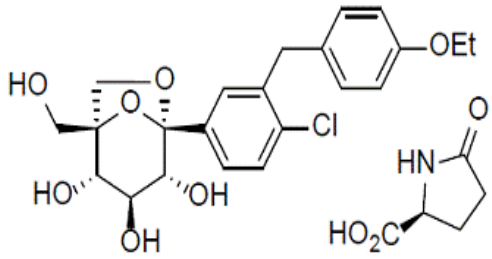

Fig 1: Chemical structure of Ertugliflozin

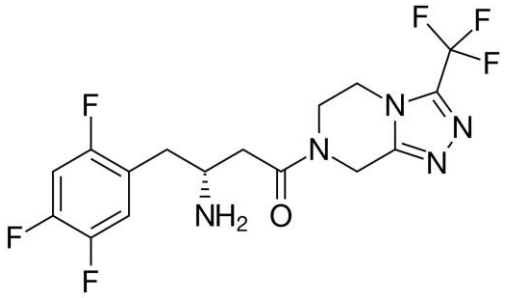

Fig 2: Chemical structure of Sitagliptin

MATERIALS AND METHODS:

Chemicals and solvents:

The reference sample of Ertugliflozin and Sitagliptin was obtained as a gift sample from Merck & Co., and Steglujan tablet containing Ertugliflozin 15mg and Sitagliptin 10mg (Merck & Co.,) was procured from US market. Water (HPLC grade) purchased from Rankem and acetonitrile (HPLC grade), ortho phosphoric acid (AR grade), sodium hydroxide (pure), hydrogen peroxide (pure) was purchased from Merck Limited, 0.45µm Nylon filter was from Zodiac life sciences.

Instrumentation:

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, Electronics Balance-Denver p^H meter -BVK enterprises, India Ultrasonicator-BVK enterprises.

Chromatography conditions:
The chromatographic separation was performed on Standard Azilent (4.6 x 150mm, 5µm particle size) at an ambient column temperature. The samples were eluted using Buffer (Potassium dihydrogen Ortho Phosphate) : Acetonitrile (70:30v/v) as the mobile phase at a flow rate of 1ml/min the mobile phase and samples were degassed by ultrasonication for 30 min and filtered through 0.45µm Nylon (N66) 47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1 mL/min, and PDA detection was carried out at 240 nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Ertugliflozin and Sitagliptin were recorded under optimized chromatographic conditions.

Preparation of Buffer and Mobile Phase:
0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Orthophosphoric acid solution

0.1% OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water

Preparation of mobile phase:
700 ml (70%) of phosphate buffer and 300 ml of Acetonitrile (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent: Based up on the solubility of the drugs, diluents was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard Solutions:
Stock solution of Ertugliflozin:
Standard stock solution of Ertugliflozin was prepared by dissolving 3.5 mg of Ertugliflozin in 25 ml of diluent (Acetonitrile and Water, 50:50v/v) in a 25 ml clean dry volumetric flask separately and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 150µg/ml of Ertugliflozin. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Ertugliflozin.
Stock solution of Sitagliptin:
Standard stock solution of Sitagliptin was prepared by dissolving 25 mg Sitagliptin in 25 ml of diluent (Acetonitrile and Water, 50:50v/v) in a 25 ml clean dry volumetric flask separately and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 1000 µg/ml of Sitagliptin respectively.The above standard stock solutions suitably diluted with diluents to obtain various concentrations of Ertugliflozin and Sitagliptin

Working Standard Solution of Ertugliflozin:
Working standard solution of Ertugliflozin was prepared by taking 1 ml of stock solutions of Ertugliflozin in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 15µg/ml of Ertugliflozin.
Working Standard Solution of Sitagliptin:
Working standard solution of Sitagliptin was prepared by taking 1 ml of stock solutions of Sitagliptin in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 100µg/ml of Sitagliptin.

Preparation of Sample Solutions of Ertugliflozin and Sitagliptin:
Five tablets were accurately weighed and powdered and tablet powder equivalent to 15mg of Ertugliflozin and 100mg of Sitagliptin was taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above sample solution was filtered and suitably diluted to get a concentration of 150µg/ml of Ertugliflozin and 1000µg/ml of Sitagliptin.

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions:
During the optimization cycle, different columns with different lengths and internal diameters were tried namely, Discovery C18 column, Kromocil column, and Azilent column but finally satisfactory separation was obtained on Azilent (4.6 x 150mm, 5µm) column. Water, Methanol, Acetonitrile and different types of buffers were examined simultaneously as organic modifiers and Buffer (Potassium di hydrogen Ortho Phosphate)*:* Acetonitrile *(*70:30 V/V) was found to be more suitable for better separation of Ertugliflozin and Sitagliptin under investigation. Isocratic mode of elution with different ratios of organic to aqueous phases was tried in order to achieve proper separation of the cited analytes in a reasonable run time. It was found that pH higher than 4.59 was not suitable as due to improper separation of the analyzed compounds. pH was adjusted at 3 for the best separation of Ertugliflozin in a reasonable run time (<10 min) and with good resolution between all peaks. Flow rate of 1 ml/ min was optimum. Quantization was achieved with UV-detection at 240nm. The column temperature

was set at 30^{O}C. Optimized method was providing good resolution and peak shape for Ertugliflozin and Sitagliptin. Under above described experimental conditions, all the peaks were well defined and free from tailing. The concern of small deliberate changes in the mobile phase composition and flow rates on results were evaluated as a part of testing for methods robustness.

Validation of Method Developed:
The proposed method was validated according to the ICH guidelines for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System suitability test:
The system suitability parameters were determined by preparing standard solutions of Ertugliflozin (15ppm) and Sitagliptin (100ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were calculated.

Specificity:
The specificity of the method was carried out to check whether there is any interference of any impurities with the retention time of analyte peaks. The specificity was performed by the injecting blank, Placebo and standard solutions of drugs.

Precision:
Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Ertugliflozin (15µg/mL) and Sitagliptin (100µg/mL), have been analyzed by injecting them into a HPLC column on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on three different days by different operators. The peak area ratios of all injections were taken and standard deviation, % relative standard deviation (RSD) was calculated.

Accuracy:
Accuracy is tested by the standard addition method at different levels: 50, 100 and 150%. A known amount of the standard drug was added to the blank sample at each level. Each sample was injected thrice The mean recovery of Ertugliflozin and Sitagliptin were calculated and accepted with 100±2%.

Linearity:
The linearity of the method was established by determining the absorbance of different concentrations of Ertugliflozin and Sitagliptin over a range of 3.75-22.5µg/ml and 25-0.150µg/ml respectively. Six replicates of each concentration were independently prepared and injected in to HPLC system. The linearity was determined by calculating a regression line from plot of peak area ratio of drug and is versus concentration of the drug. Regression analysis was computed for Ertugliflozin and Sitagliptin. The method was evaluated by determination of correlation coefficient and intercept values according to ICH guidelines.

Limit of Detection and Limit of Quantification:
Limit of detection (LOD) and limit of quantification (LOQ) of Saxagliptine and Dapagliflozin were determined by calibration curve method. Solutions of Saxagliptine and Dapagliflozin were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using the following equations:

$$LOD=3Xn/B$$

$$LOQ= 10x\ N/B$$

Where N is residual variance due to regression; B is the slope.

Robustness:
HPLC conditions were slightly modified to evaluate the analytical method robustness. These changes included the flow rate, column temperature and the Acetonitrile proportion in the mobile phase.

Degradation Study:
Alkaline, acidic, oxidative stress, thermal, water and direct exposure to UV were carried out. No internal standard was added in the forced degradation study.

Acid Degradation Studies:
To one ml of stock s solution Ertugliflozin and Sitagliptine, one ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600C. The resultant solution was diluted to obtain 15µg/ml & 100µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:
To one ml of stock solution Ertugliflozin and Sitagliptine, one ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}C. The resultant solution was diluted to obtain 15µg/ml&100µg/ml solution and 10 µl were injected

into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative Studies:

To one ml of stock solution of Ertugliflozin and Sitagliptine, one ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 15µg/ml & 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidation:

To one ml of stock solution of Ertugliflozin and Sitagliptine, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 15µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 1 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 15µg/ml&100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 150µg/ml and 1000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1day or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 15µg/ml and 100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 15µg/ml and 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Validation of Method Developed:

The proposed method was validated according to the ICH guidelines31 for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System suitability:

The Retention time of Ertugliflozin and Sitagliptine using optimum conditions was 3.203 min and 2.106 min respectively. For two of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Ertugliflozin and Sitagliptine was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in **Table 1.**

Table 1: System suitability parameters for Ertugliflozin and Sitagliptin

S. no	Ertugliflozin				Sitagliptin				
Inj	RT(min)	USP Plate Count	Tailing	Area	RT(min)	USP Plate Count	Tailing	Area	Resoluution
1	3.188	6022	1.09	4294213	2.100	5371	1.20	377112	7.5
2	3.188	6051	1.07	4297170	2.102	5365	1.20	377288	7.4
3	3.197	5944	1.12	4297220	2.104	5123	1.20	374762	7.5
4	3.201	5867	1.08	4292142	2.105	5061	1.19	375094	7.6
5	3.204	5974	1.08	4297103	2.112	5024	1.17	378661	7.7
6	3.210	6032	1.06	4278079	2.115	5273	1.18	377650	7.7
MEAN				4292655	MEAN			376761	
standard deviation				7431.8	standard deviation			1521.6	
%RSD				0.2	%RSD			0.4	

Specificity:

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized Chromatogram of Ertugliflozin and Sitagliptine is shown in **Fig.4** clearly shows the ability of the method to assess the analyte in the presence of other excipients.

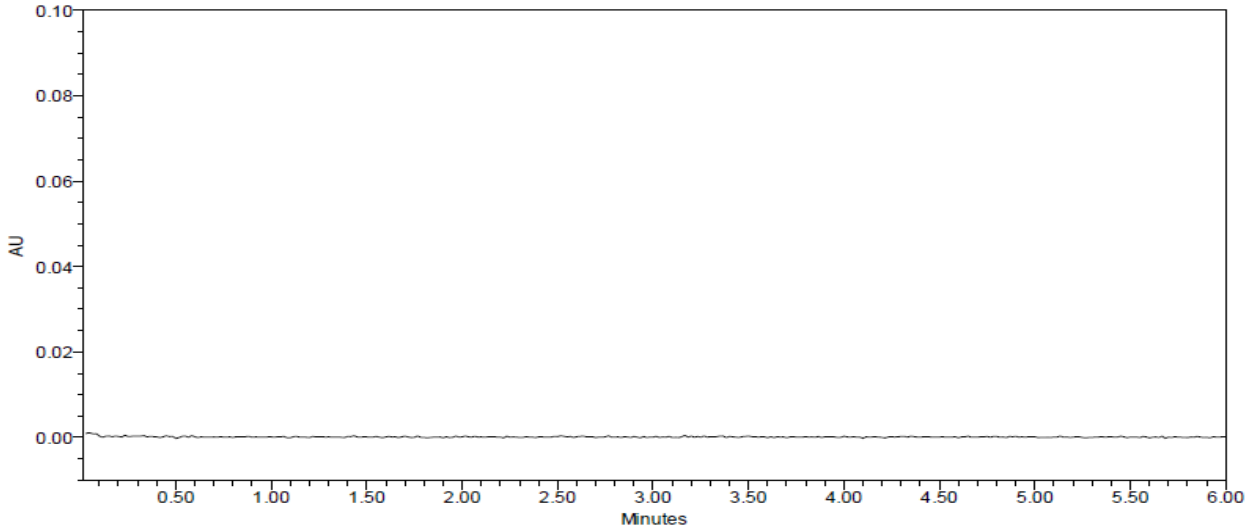

Fig 3: Blank Chromatogram

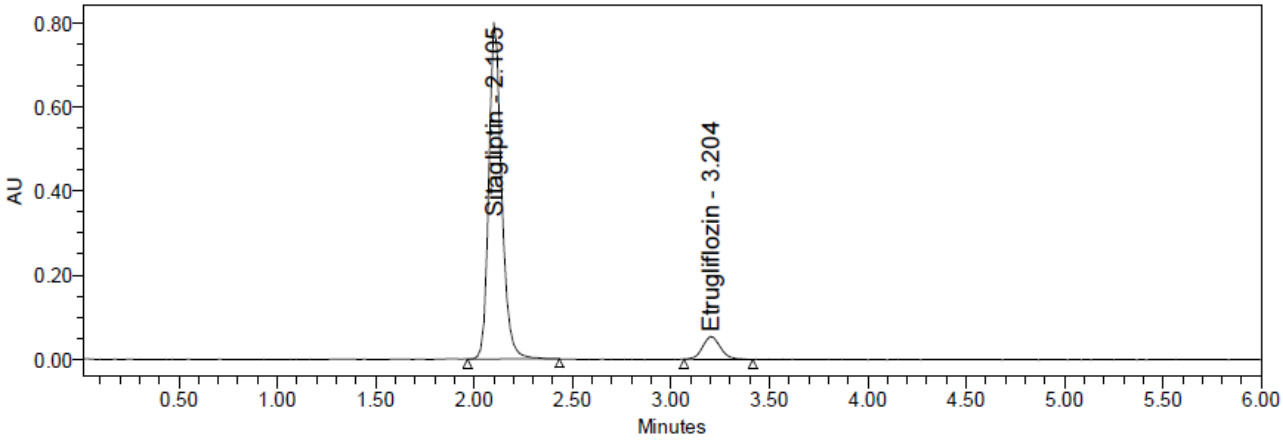

Fig 4: Optimized Chromatogram Ertugliflozin and Sitagliptin.

Precision:

System Precision:

One dilution of both the drugs in six replicates was injected into HPLC system & was analyzed and the results were found within the acceptance limits (RSD<2).

Method Precision (Repeatability):

Six replicate injections of a known concentration of sample preparation of Ertugliflozin (15µg/mL) and Sitagliptin (100µg/mL) have been analyzed by injecting them into a HPLC column on the same day. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in **Table 2**.

Table 2: System precision data of Ertugliflozin and Sitagliptin

S.No	Injection number	Retention time of Ertugliflozin	Area of Ertugliflozin	Retention time of Sitagliptin	Area of Sitagliptin
1	Injection 1	3.167	373573	2.084	4256686
2	Injection 2	3.203	374129	2.107	4254357
3	Injection 3	3.206	373730	2.108	4259188
4	Injection 4	3.207	373943	2.113	4254120
5	Injection 5	3.211	373933	2.116	4257632
6	Injection 6	3.215	375047	2.119	4276343
	Mean		374059		4259721
	Standard deviation		520.5		8370.1
	% RSD		0.1		0.2

Ruggedness:

Intermediate precision was accessed injecting sample preparation of of Ertugliflozin (15µg/mL) and Sitagliptin (100µg/mL) in six replicates in to HPLC column on the same day and on consecutive days and in different laboratories by different analysts . Results were found within the acceptance limits (RSD<2) as shown in the **Tables 3,4**.

Table 3: Ruggedness data of Ertugliflozin

Concentration (15 µg/mL)	Analyst-1		Analyst-2		Laboratory -1		Laboratory-2	
	RT	Area	RT	Area	RT	Area	RT	Area
Injection 1	3.197	368398	3.188	377112	3.237	370676	3.237	370544
Injection 2	3.198	368857	3.188	377288	3.239	370627	3.239	370442
Injection 3	3.199	370732	3.197	374762	3.239	370395	3.240	369183
Injection 4	3.201	369155	3.201	375094	3.240	372987	3.241	369269
Injection 5	3.204	367556	3.204	378661	3.245	370976	3.244	371673
Injection 6	3.204	370177	3.210	377650	3.251	371774	3.251	369794
Mean		369146		376761	3.237	371239	3.237	370151
Standard deviation		1161.9		1521.6		981.3		937.9
% RSD		0.3		0.4		0.3		0.3

Table 4: Ruggedness data of Sitagliptin

Concentration (100 µg/mL)	Analyst-1		Analyst-2		Laboratory -1		Laboratory-2	
	RT	Area	RT	Area	RT	Area	RT	Area
Injection 1	2.100	4264949	2.100	370544	2.118	4153380	2.120	4280152
Injection 2	2.102	4243876	2.102	370442	2.120	4155806	2.120	4272862
Injection 3	2.104	4264228	2.104	369183	2.122	4150588	2.122	4266229
Injection 4	2.105	4278501	2.105	369269	2.122	4119764	2.122	4284055
Injection 5	2.106	4249209	2.112	371673	2.124	4130178	2.123	4260724
Injection 6	2.106	4243174	2.115	369794	2.133	4137075	2.124	4275598
Mean		4257323		370151	2.118	4141132	2.120	4273270
Standard deviation		368398		937.9		14476.2		8674.6
% RSD		368857		0.3		0.3		0.2

Robustness:

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (75:25), mobile phase plus (65:35), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. The result of robustness study of the developed assay method was established in **Table 5.**

Table 5: Robustness data for Ertugliflozin and Sitagliptin.

S.NO	Condition	%RSD of Ertugliflozin	%RSD of Sitagliptin
1	Flow rate (-) 1.1ml/min	0.4	0.2
2	Flow rate (+) 1.3ml/min	0.3	0.4
3	Mobile phase (-) 75B:25A	0.8	1.0
4	Mobile phase (+) 65B:35A	1.3	0.5
5	Temperature (-) 25°C	1.5	0.4
6	Temperature (+) 35°C	1.7	0.5

Accuracy:

A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Ertugliflozin and Sitagliptin was achieved between 99.67% and 99.90% respectively. The results are given in **Tables 6,7.**

Table 6: Accuracy table of Ertugliflozin

S NO	Accuracy level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Statistical Analysis
1	50%	7.5	7.484061	99.79	Mean = 99.87 .SD =0.19 %RSD = 0.19
2		7.5	7.497311	99.96	
3		7.5	7.468201	99.58	
4	100%	15	14.90247	99.35	Mean = 99.29 SD =0.5 %RSD = 0.5
5		15	14.88807	99.25	
6		15	14.88902	99.26	
7	150%	22.5	22.49854	99.99	Mean = 99.94 SD =0.07 %RSD = 0.07
8		22.5	22.49191	99.96	
9		22.5	22.46683	99.85	

Table 7: Accuracy table of Sitagliptin

S NO	Accuracy level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Statistical Analysis
1	50%	50	49.53313	99.07	Mean = 99.13 . SD =0.132 %RSD = 0.13
2		50	49.64345	99.29	
3		50	49.52552	99.05	
4	100%	100	99.84408	99.84	Mean = 99.78 . SD =0.0675 %RSD = 0.07
5		100	99.70917	99.71	
6		100	99.77721	99.78	
7	150%	150	149.555	99.70	Mean = 99.66 . SD = 0.2726 %RSD = 0.27
8		150	149.8553	99.07	
9		150	149.0463	99.29	

Linearity:

The linearity of the method was established by determining the absorbance of different concentrations of Ertugliflozin and Sitagliptin over a range of 3.75-22.5µg/ml and 25-150µg/ml respectively. The results are given in **Tables 8** and **Fig. 5,6**.

Table 8: Linearity table for Ertugliflozin and Sitagliptin.

S. NO	Ertugliflozin		Sitagliptin	
	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
1	0	0	0	0
2	3.75	106567	25	1215100
3	7.5	197094	50	2179127
4	11.25	295390	75	3225672
5	15	376237	100	4296959
6	18.75	484397	125	5325244
7	22.5	573322	150	6338079

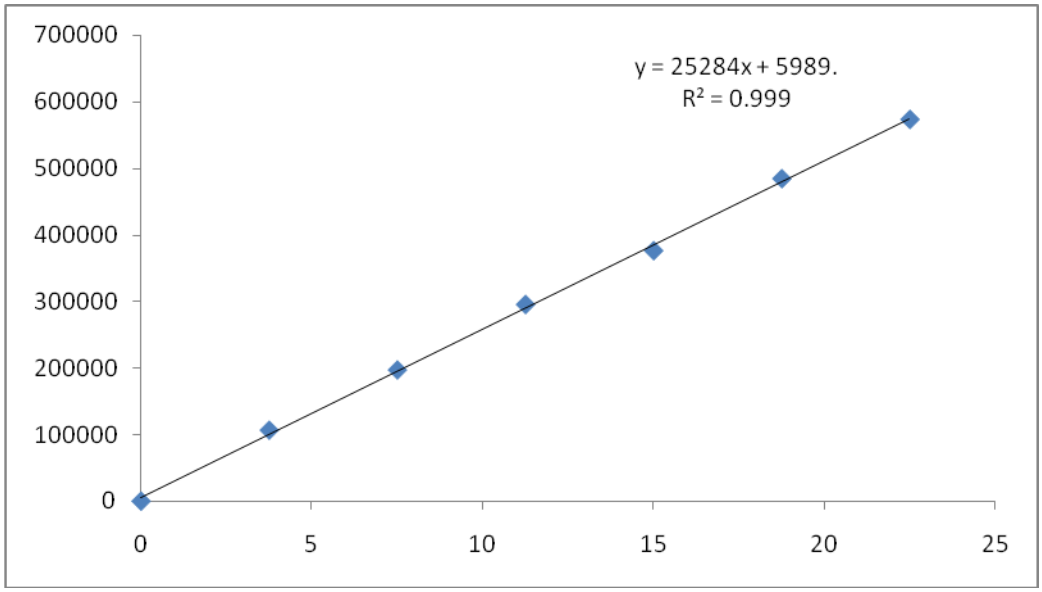

Fig.5: Calibration curve of Ertugliflozin

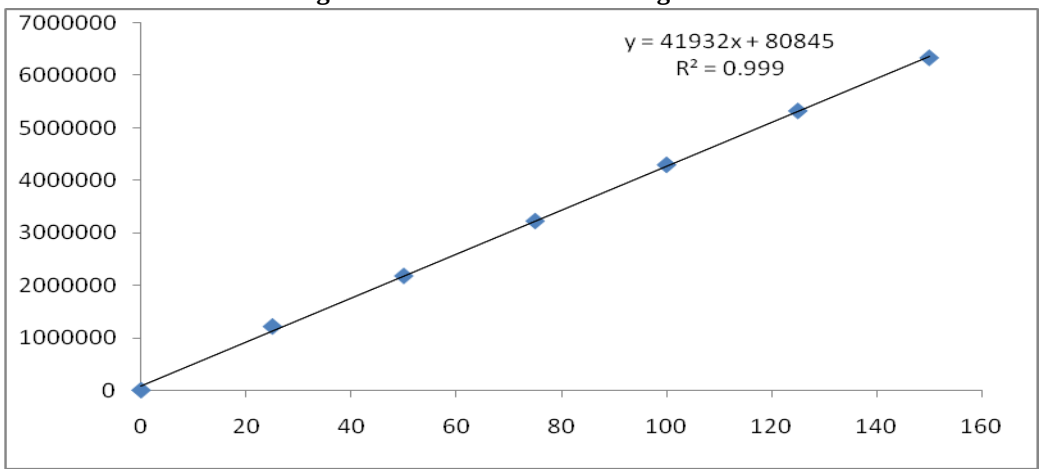

Fig. 6: Calibration curve of Sitagliptin

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The limit of detection and limit of quantification were evaluated by serial dilutions of Ertugliflozin and Sitagliptin stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Ertugliflozin and Sitagliptin was found to be 0.43µg/mL and 0.74 µg/mL respectively and the LOQ value 1.30µg/mL and 2.24µg/mL respectively.

Assay:

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out (**Table 9)**.

Table 9: Assay data of Ertugliflozin and Sitagliptin combination marketed formulation

Drug	Labelled claim(mg)	Drug found(mg)	% Purity
Ertugliflozin	15	14.88	99.18%
Sitagliptin	100	99.13	99.13%

Table 10: Forced Degradation studies of Ertugliflozin and Sitagliptin

Degradation Condition	Degradation (%)	
	Ertugliflozin	Sitagliptin
Acid	4.80	4.44
Alkali	3.74	3.98
Oxidation	2.10	2.78
Thermal	1.83	2.41
UV	1.54	1.30
Water	0.75	0.14

Forced degradation studies:
The assay method was used to test the drug stability by conducting forced degradation studies for the drug substances under various stress conditions. Stress degradation studies were carried out for acid hydrolysis (2N HCl heated for 30 min at 60°C), alkali hydrolysis (2 N NaOH heated for 30 min at 60°C), oxidative degradation (20%H_2O_2 heated at 60°C for 30 min) and thermal degradation (samples placed in an oven at 105°C for 6 h). For photolytic stress studies, samples were exposed to UV light by keeping them in a UV chamber for 7 days. Results are shown in **Tables 10**.

DISCUSSION:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Ertugliflozin and Sitagliptin in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Buffer (Potassium dihydrogen Ortho Phosphate)*:* Acetonitrile (70:30 V/V) with 1.0 ml/min flow rate was quite robust.

The optimum wavelength for detection was 240 nm at which better detector response for drug was obtained. The average retention time for Ertugliflozin and Sitagliptin were found to be 3.203 and 2.106 min. The calibration was linear in concentration range of 3.75-22.5mcg/ml for Ertugliflozin and 25-150mcg/ml for Sitagliptin**.** The low values of % RSD indicate the method is precise and accurate.
Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days. These results show the accuracy and reproducibility of the assay. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % RSD. reported was found to be less than 2 %.The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. There was no significant difference in the results achieved by the proposed method.

CONCLUSION:

The proposed method for the assay of the popular anti- diabetic drugs Ertugliflozin and Sitagliptin in the commercially available tablet formulation is simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the API, in-process samples, and the finished tablet formulation.

ACKNOWLEDGEMENT:

Authors wish to thank Merck & Co., for providing the gift samples of Ertugliflozin and Sitagliptin to carry out our present work and also thankful to the management of Vikas College of pharmacy, Vissannapeta for providing the required facilities and for their constant encouragement.

REFERENCES:

1. Leonor Guariguata, Tim Nolan, Jessica Beagleyet al, IDF Diabetes Atlas, International Diabetes Federation, 2013, ISBN: 2-930229-85-3, www.idf.org/diabetesatlas.
2. Chaudhury, Arun et al. “Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management.” *Frontiers in Endocrinology* 8 (2017): 6. *PMC*. Web. 19 Feb. 2018.
3. Miao Z, Nucci G et al.: Pharmacokinetics, metabolism, and excretion of the antidiabetic

agent ertugliflozin (PF-04971729) in healthy male subjects. Drug Metab Dispos. 2013 Feb;41(2):445-56. doi: 10.1124/dmd.112.049551. Epub 2012 Nov 20. [PubMed:23169609]

4. Cinti F, Moffa S, Impronta F, Cefalo CM, Sun VA, Sorice GP, Mezza T, Giaccari A: Spotlight on ertugliflozin and its potential in the treatment of type 2 diabetes: evidence to date. Drug Des Devel Ther. 2017Oct3; 11:2905-2919. doi:10.2147/DDDT.S114932. eCollection 2017. [PubMed:29042751]
5. Ghazala K, Dinesh S, Agrawal YP, Neetu S, Avnish J, Gupta AK. Simultaneous estimation of metformin and sitagliptin in tablet dosage form. Asian J Biochem Pham Res. 2011; 2:223–9.
6. Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: Results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther.
7. Plosker GL, Sitagliptin: A review of its use in patients with type 2 diabetes mellitus, Drugs.2014Feb;74(2):22342.doi:10.1007/s40265 013https://www.ncbi.nlm.nih.gov/pubmed/24407 560.
8. Validation of Analytical Proceudures, ICH Harmonised Tripatite Guidelines,Q2 B 1997.
9. Ramalingam, P. et al. "Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Sitagliptin and Simvastatin in Tablets." *Indian Journal of Pharmaceutical Sciences* 76.5 (2014): 407–414.
10. Meher Vijay Dalawai et al, Development and validation of stability indicating assay method by HPLC for the analysis of sitagliptin phospahte in bulk drug substances, Journal of Chemical and Pharmaceutical Research, 2015, 7(10):781-787.
11. Vasanth P M et al, Method development and validation of sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form , Der Pharmacia Lettre, 2013, 5 (5):168-174.
12. Hanan A.etval, Merey, Nesrin K. Ramadan, Sherine S. Diab, Azza A. Moustafa Chromatographic methods for the simultaneous determination of binary mixture of Saxagliptin HCl and Metformin HCl, Bulletin of Faculty of Pharmacy, Cairo University, Volume 55, Issue 2, 2017, 311-317
13. S.N. Konari, Stability indicating validated RP-HPLC technique for the analysis of multicomponent anti-diabetic drug combos in pharmaceutical dosage forms, Karbala International Journal of Modern Science 1 (2015) 39-48.
14. V. Deepthi, Stability-indicating RP- HPLC method for analysis of sitagliptin in the bulk drug and it's pharmaceutical dosage form, https://www.pharmatutor.org/articles/stability-indicating-rp-hplc-method-analysis-sitagliptin-bulk-drug-pharmaceutical-dosage-form
15. R. Lavanya MD. Yunoos ,Development and Validation of RP-HPLC method for the estimation of Sitagliptin Phosphate in Bulk and itsTablet Dosage Form; J. Adv. Pharm. Edu. & Res. 2013: 3(4):475-479.
16. Mohamed Karam Qassas, A Validated HPLC Stability Indicating Method for the Determination of Sitagliptin in Bulk Drug Substance and Tablets, Int. J. Pharm. Sci. Rev. Res., 32(1), 2015; 33, 194-19.
17. Hitesh P. Inamdar, RP-HPLC method for simultaneous determination of metformin hydrochloride, rosiglitazone and sitagliptin – application to commercially available drug products, IJPSR, 2012; Vol. 3(9): 3267-3276, http://dx.doi.org/10.13040/IJPSR.0975-8232.3 (9).3267-76.