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Development and validation of RP-HPLC method for simultaneous estimation of Ertugliflozin and Sitagliptin in bulk drug and tablet dosage form

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Ertugliflozin, RP-HPLC, Sitagliptin, Tablet

Abstract: To treat type 2 diabetes, in a combined tablet dosage form the ertugliflozin and sitagliptin were administered. Considering the less complication and readily availability of HPLC, the main objective of present study was to develop a new, precise, accurate, linear, robust, and economical RP-HPLC method for the simultaneous estimation of ertugliflozin and sitagliptin in tablet dosage form. Effective chromatographic separation of Ertugliflozin and Sitagliptin was achieved on Kromasil C18 (5 µm 250 mm X 4.6 mm) and the mobile phase containing Methanol and 0.1% OPA in water isocratic elution mode at a flow rate of 1.0mL/min. with column temperature at 30 °C and the injection volume was 20 µL at column temperature at 30°C. At an isosbestic wavelength of 212 nm, ertugliflozin and sitagliptin were found to have retention times of 5.30 min. and 2.05 min., respectively. The method was proven to be precise (%RSD 2%), accurate (>90%), and specific for the simultaneous measurement of both drugs in tablets. As a result, the suggested method with excellent specificity, accuracy, precision, linearity and robustness as well as economical was useful for the regular quality control analysis of ertugliflozin and sitagliptin tablets.

Introduction

Ertugliflozin, chemically known as (1S,2S,3S,4R,5S)-5-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-1-(hydroxymethyl)-6, 8-dioxabicyclo (3,2,1) octane-2,3,4-triol; (2S)-5-oxopyrrolidine-2-carboxylic acid, is a selective inhibitor of sodium-dependent glucose cotransporters (SGLT), more specifically type 2 diabetes (Fediuk et al., 2020). A new dipeptidyl peptidase-4 (DPP-4) inhibitor drug with the chemical name (R)-3-Amino-1-(3-(Trifluoromethyl)-5,6-Dihydro- (1,2,4) Triazolo (4,3-A) Pyrazin-7(8h)-yl)-4-(2,4,5-Trifluorophenyl) Butan-1-One is sitagliptin. Sitagliptin is an inhibitor of the protease dipeptidyl peptidase-4 (DPP-4), which breaks down the incretin GLP-1. GLP-1 levels that are elevated or sustained can enhance the pancreas's ability to secrete insulin by blocking DPP-4. Sitagliptin reduces hepatic glucose overproduction while increasing insulin production. In order to address decreased insulin levels brought on by beta-cell malfunction and the liver's unchecked synthesis of glucose, sitagliptin only functions when blood sugar levels are raised (Davis et al., 2010). To treat type 2 diabetes, ertugliflozin and sitagliptin were administered in a combined dosage form. There have been a few reported validated analytical techniques for estimating ertugliflozin and sitagliptin by RP-HPLC method (China et al., 2019; Rajeswari et al., 2022; Venkateswara et al., 2018; Raju et al., 2021; Vilas et al., 2022). It was found that no economically validated method was available from the literature for simultaneous estimation of Ertugliflozin and sitagliptin in bulk and tablet dosage form. The goal of the present research is to develop and validate the economical RP-HPLC method for simultaneous estimation of ertugliflozin and sitagliptin in bulk drugs and tablet dosage forms.

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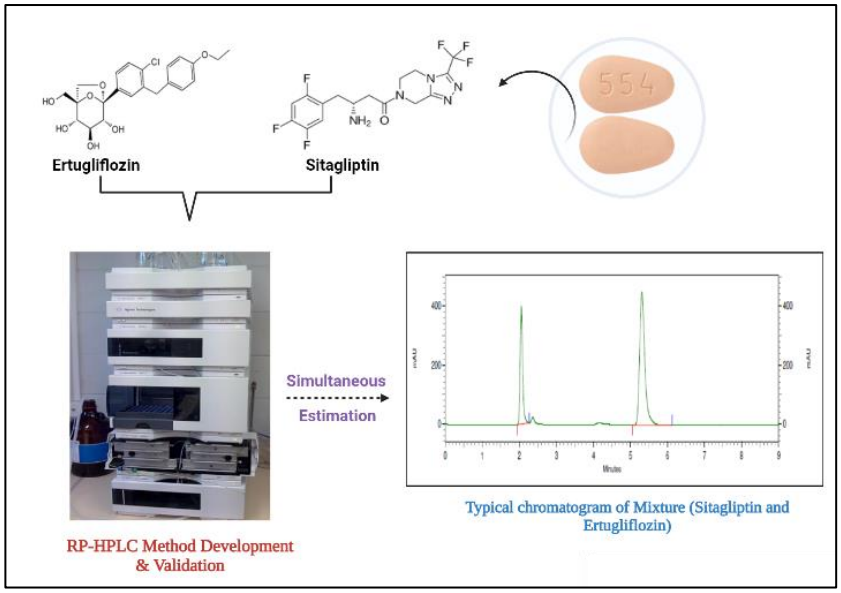

Figure 1. Simultaneous estimation of Ertugliflozin and Sitagliptin in tablet dosage form

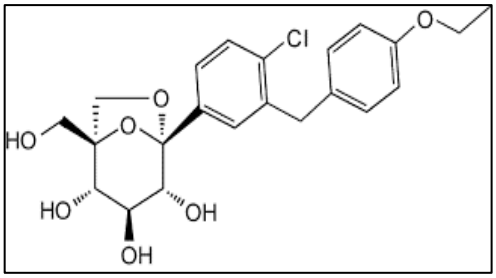

Figure 2a. Structure of Ertugliflozin

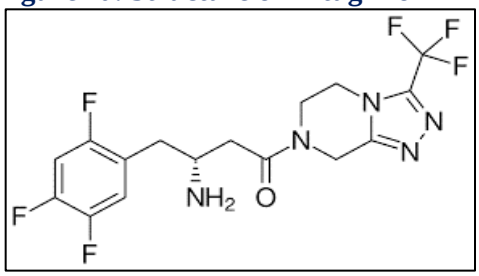

Figure 2b. Structure of Sitagliptin

Material and Method

Material

Ertugliflozin, Sitagliptin were purchased from Vidisha Analytical. Steglujan® tablets containing Ertugliflozin 15 mg and Sitagliptin 100 mg in the ratio of 1:6.67 were purchased from the market. HPLC-grade methanol and acetonitrile were purchased from Merck Specialities Pvt. Ltd (Mumbai, India). HPLC grade Milli-Q water was purchased from Siddhi Lab. Solvents, chemicals, and reagents of HPLC grade were used throughout the validation of the analytical method.

Selection of wavelength

To get the ultraviolet-visible (UV-vis) spectra, both the drugs ertugliflozin and sitagliptin were dissolved in

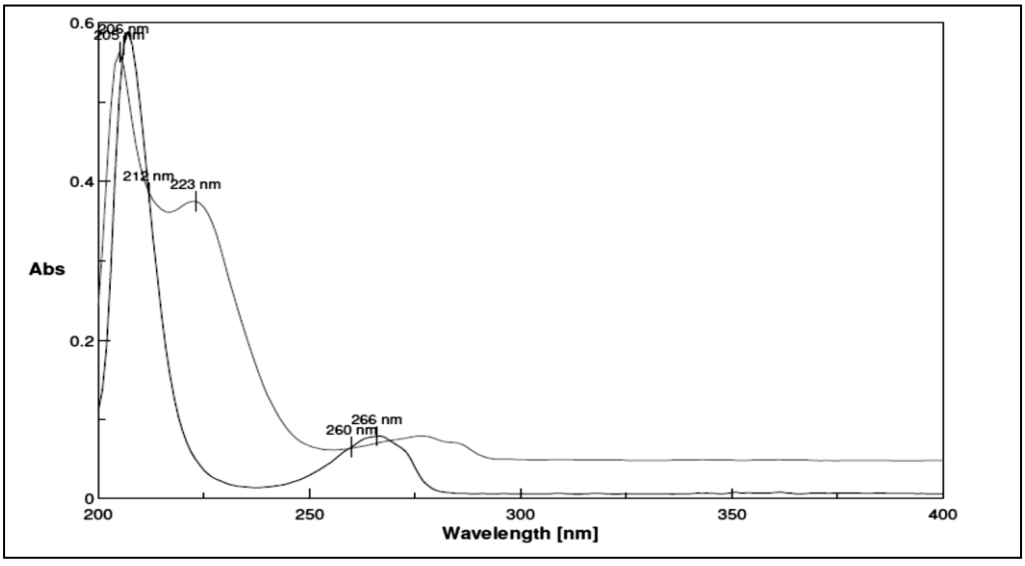

Figure 3. UV spectrum of Sitagliptin & Ertugliflozin

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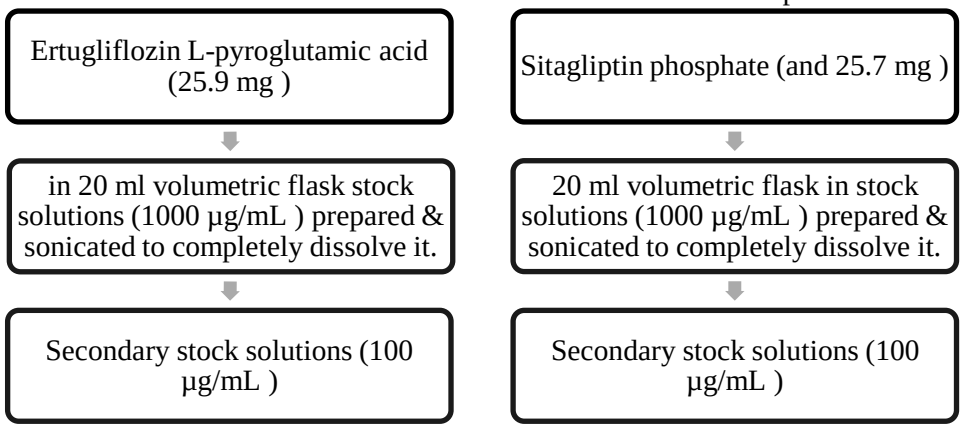
methanol separately to prepare primary standard stock solutions (500 µg/mL each). These solutions were then further diluted to prepare secondary stock solutions (at a concentration of 20 µg/mL each). Methanol was utilized as a blank. A UV spectrophotometer was used to scan a standard solution of sitagliptin and ertugliflozin (20 µg/mL each) in wavelength spectral mode between 400 and 200 nm. For both drugs, absorption maxima were found. At 212 nm, Sitagliptin and ertugliflozin displayed Q-point.

Instrumentation and Chromatographic Conditions

HPLC (1260 Infinity II, Agilent) equipped with quaternary pump (DEAX02386) and Detector (DEACX16446) was used. This system was operated by Openlab EZ Chrome for controlling the instrument parameters. Chromatographic separation of Ertugliflozin and Sitagliptin was achieved on Kromasil C18 (250 mm X 4.6 mm, 5 µm) and the mobile phase containing Methanol and 0.1% OPA in water isocratic elution mode at flow rate of 1.0mL/min. with column temperature at 30 °C and the injection volume was 20 µL.

Standard and sample solutions preparation

Standard solutions preparation



Sample solutions preparation

Sample solution prepared by Weighing the powder material (from 20 tablets) equivalent to 100 mg of Sitagliptin and 15 mg of Ertugliflozin. Transfer it to a 100 mL volumetric flask that has been thoroughly cleaned and dried, add 70 ml of methanol, sonicate it for 15 minutes, and then level off the volume by adding more methanol. Filter the solution with an appropriate 0.45-syringe filter, and then dilute the filtrate (3.35 ml) to 50 ml with diluent to make 10 µg/mL of ertugliflozin and 67 µg/mL of sitagliptin.

Method validation

Stability of Analytical Solution

The stability of the standard and test sample solutions was studied. The stability study was carried out in normal laboratory conditions. After being kept in a normal illuminated laboratory for conditions 12 and 24 hours, the solution was analyzed.

Specificity

Following solution was prepared and injected to prove the specificity nature of the method. Blank (Mobile phase), Placebo (Placebo solution prepared using 312.34 mg of placebo material containing Lactose, Starch, Magnesium stearate, Talc, crospovidone Which is equivalent to 100 mg of Sitagliptin and 15 mg of Ertugliflozin in methanol), Sitagliptin and Ertugliflozin Standard solution mixture, Tablet test sample solution.

Linearity and range

12.95 mg Ertugliflozin L-pyroglutamic acid (Equivalent to 10 mg of Ertugliflozin) and 32.13 mg Sitagliptin phosphate (Equivalent to 25 mg of Sitagliptin) were weighed accurately and transferred separately into 50 ml volumetric flask, added 30 ml of methanol in each and sonicated to dissolve the standard completely and diluted each solution up to the mark with methanol to prepare 200 µg/mL and 500 µg/mL stock solution of Ertugliflozin and Sitagliptin respectively. Working standard solutions were prepared as a result of diluting the aforementioned solutions 1, 5, 10, 12.50, 15 for Ertugliflozin and 6.75, 33.51, 67.01, 83.77, 100.02 µg/mL for Sitagliptin. Each level was injected three times, and the mean area was computed. Ertugliflozin and Sitagliptin concentration vs peak area response plots were constructed.

Accuracy

Tests for accuracy will be conducted at three levels of accuracy: 50%, 100%, and 150% of the working concentration of the sample solution. The solution was prepared in triplicate for each accuracy level. Calculated the percent recovery, the mean percent recovery, the percent RSD for both the overall recovery and each level.

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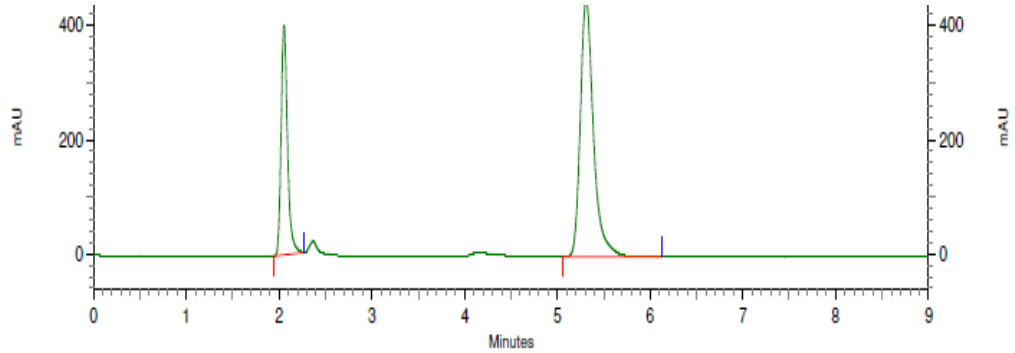
Figure 4. Typical chromatogram of Mixture (Sitagliptin and Ertugliflozin)



Precision

The precision of the method was calculated using %RSD. By examining the concentrations of 6 samples, the developed method's intra-day and inter-day batch precision was examined. By conducting the analysis on a different day, intermediate precision was done to ensure that results could be replicated. sample prepared using the sample preparation protocol.

Sensitivity

As per ICH Q2R1 guidelines, the approach based on the calibration curve was used to calculate the residual standard deviation of a regression line and determine the Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Robustness

By purposefully changing chromatographic conditions including flow rate, wavelength and column oven temperature, the robustness of the method was assessed. The column oven temperature was changed by ± 2ºC, the flow rate by ±0.1 mL/min, and the wavelength varied by ±3nm. For the purpose of determining robustness, the impact of such changes on peak resolutions, tailing factors and theoretical plates was assessed.

System suitability

A Pharmacopeial requirement known as "system suitability" is used to assess if the chromatographic system is suitable to perform the desired analysis. Data was collected from five replicate injections of the standard drugs solution during the tests, and the outcomes were recorded. 10 µg/mL of Ertugliflozin and 67 µg/mL of Sitagliptin are the working concentrations. The marketed formulation contains Ertugliflozin (15 mg) and Sitagliptin (100 mg) in the ratio of 1:6.67, hence concentration is selected in this ratio.

Results and Discussion

Method development

The development of an RP-HPLC method for simultaneous analysis of ertugliflozin and sitagliptin began using a number of mobile phase ratios composed up of methanol and 0.1% OPA in water. Finally, effective chromatographic separation of Ertugliflozin and Sitagliptin was achieved on Kromasil C18 (250 mm X 4.6 mm, 5 µm) and mobile phase Methanol and 0.1% OPA in water in the ratio 75:25 in an isocratic elution mode at a flow rate of 1 mL/min. The retention time for Ertugliflozin and Sitagliptin were found to be at 5.30 min and 2.05 min respectively.

Method Validation

Solution stability

Table 1. Results of Solution Stability

Analyte	Time point	Test sample solution		Standard solution	
		Area	% Absolute difference	Area	% Absolute difference
Ertugliflozin	Initial	7469485	NA	7484047	NA
	12 Hours	7440294	0.39	7453170	0.41
	24 Hours	7423172	0.62	7440318	0.58
Sitagliptin	Initial	17845913	NA	17594023	NA
	12 Hours	17768956	0.43	17668406	0.42
	24 Hours	17698942	0.82	17464513	0.74

Specificity

Ertugliflozin and Sitagliptin did not interfere with the R.T. in the blank or placebo sample. Peak purity was found to be NLT 0.95 and within limits for Standard solution and test solution. As a result, the devised chromatographic method met the specificity requirements.

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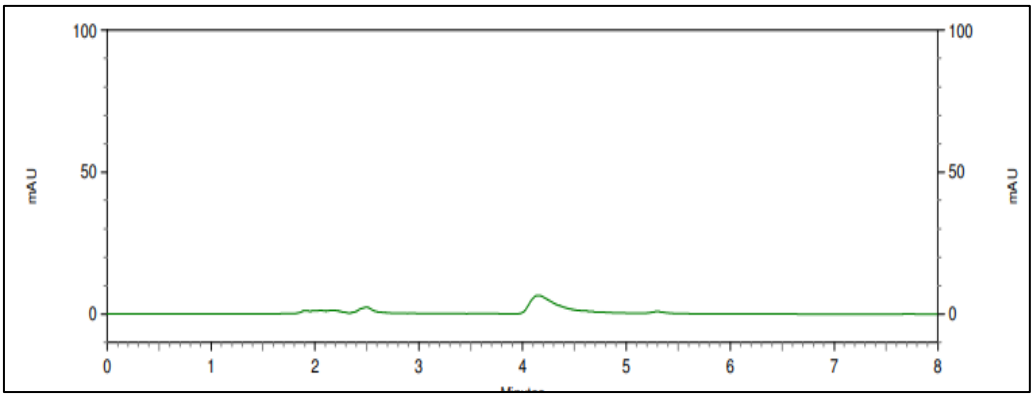

Figure 5. Typical chromatogram of Blank solution

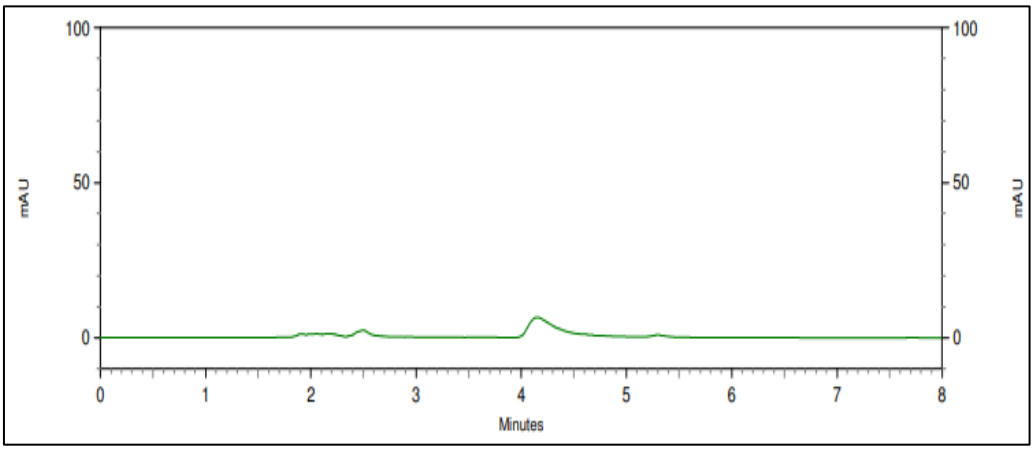

Figure 6. Typical chromatogram of Placebo solution

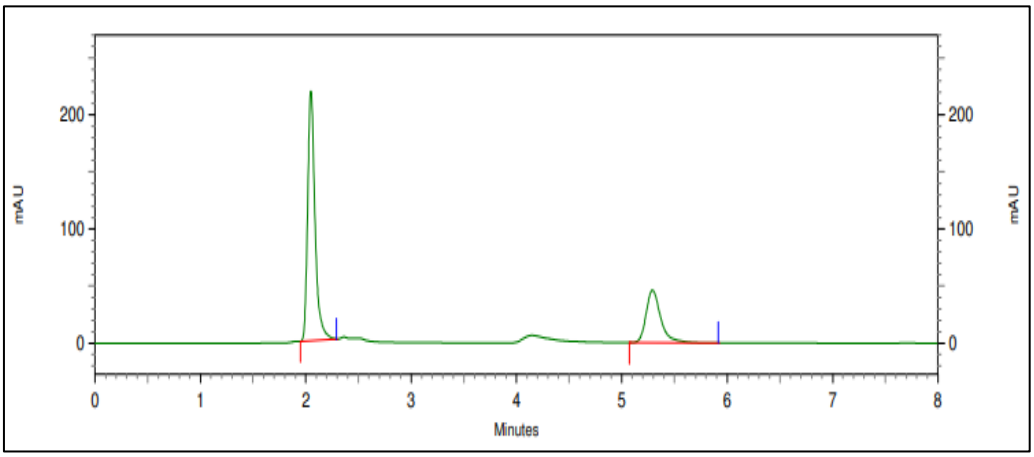

Figure 7. Typical chromatogram of Peak purity of Standard solution

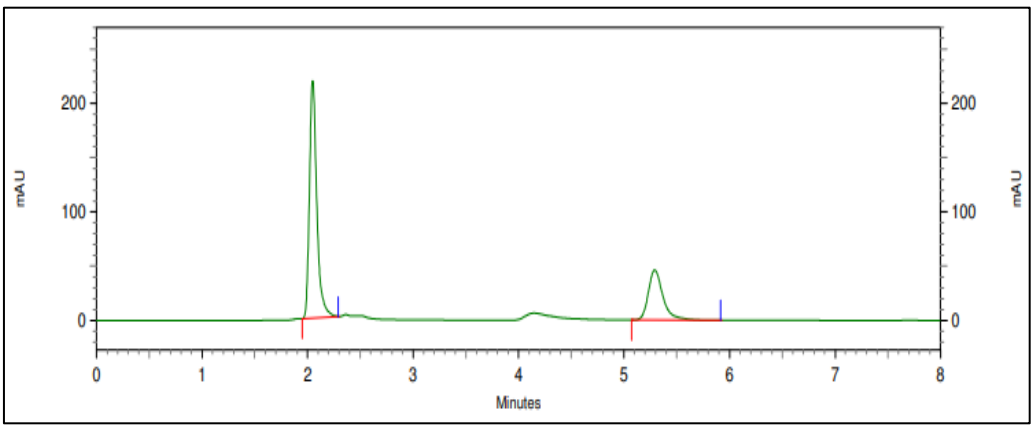

Figure 8. Typical chromatogram of Peak purity of Test sample solution

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Table 2. Peak purity of Standard and Test solution

	Peak Name	Retention Time	Peak Purity
Standard	Ertugliflozin	5.29	0.984
	Sitagliptin	2.05	0.989
Test	Ertugliflozin	5.29	0.988
	Sitagliptin	2.05	0.993

Table 3. Result for Ertugliflozin of Intra- day and Inter- Day Precision of test sample assay (Where, A refer as Ertugliflozin, B refer as Sitagliptin)

Analyte	Sample	Repeatability					Intermediate precision (Inter-Day)				
		Test Sample (mg)	Area	% Assay	Mean (±SD)	% RSD	Test Sample (mg)	Area	% Assay	Mean (±SD)	% RSD
A	1	460.3	7319703	98.98	99.87 (±0.46)	0.466	460.6	7310953	98.79	99.14 (±0.85)	0.861
	2	460.5	7324901	99.00			460.4	7364965	99.57		
	3	460.2	7244034	97.97			460.5	7240245	97.86		
	4	460.5	7320094	98.94			460.5	7420987	100.30		
	5	460.4	7320397	98.96			460.6	7368128	99.57		
	6	460.5	7349417	99.34			460.4	7302768	98.73		
B	1	460.3	17339724	99.04	99.14 (±0.88)	0.893	460.6	17479455	99.77	99.34 (±1.27)	1.284
	2	460.5	17413456	99.41			460.4	17648921	100.78		
	3	460.2	17240842	98.49			460.5	17503462	99.93		
	4	460.5	17142103	97.87			460.5	17247920	98.47		
	5	460.4	17445034	99.62			460.6	17495421	99.86		
	6	460.5	17584601	100.39			460.4	17025032	97.22		

Linearity and range

The standard calibration curve has been discovered to be linear over the concentration range of 1.0 -15.0 µg/mL and 6.75 - 100.02 µg/mL for ertugliflozin and sitagliptin, respectively. For ertugliflozin and sitagliptin, the correlation coefficient (R^2) derived from linear regression analysis was 0.999. Based on mean peak and concentration, the calibration curve's equations for ertugliflozin and sitagliptin were y =744986.547 x + -19560.762 and y =266629.117 x + -4185.341, respectively.

Precision

Precision is expressed as % RSD and % RSD NMT 2% is considered to be acceptable. Since the % assay and % RSD results were well within the acceptable range, the method is reproducible and accurate. The proposed method was found to have an overall % RSD for intra-day and inter-day precision of less than 2%.

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Table 4. Result for Repeatability Plus Inter-day

Repeatability Plus Inter-day	Mean (±SD)	%RSD
Ertugliflozin	99.00 (±0.669)	0.676
Sitagliptin	99.23 (±1.05)	1.060

Accuracy/recovery

The accuracy of analytical method is expressed as % recovery and it determines the degree of closeness between the obtained values to the true values. The overall % recovery was observed to be in the range of 98-102% for both Ertugliflozin and Sitagliptin. % RSD for each level and overall recovery were found to be <2%. Recovery of analytical procedure was found well within the acceptance limit at all 3 levels.

Table 5. Result and statistical data of Accuracy of Ertugliflozin and Sitagliptin

Analyte	Level (%)	Added Conc (µg/mL)	Area	Recovered Conc. (µg/mL)	% Recovery	Mean % Recovery	% RSD
Ertugliflozin	50	5.07	3734613	5.08	100.12	99.13	0.984
		5.12	3699102	5.03	98.17		
		5.02	3659130	4.97	99.11		
	100	10.09	7388094	10.04	99.54	99.06	0.470
		10.03	7312560	9.94	99.03		
		10.03	7280970	9.89	98.61		
	150	15.16	11089716	15.07	99.44	99.46	0.694
		15.10	10978213	14.92	98.78		
		15.05	11093405	15.08	100.16		
Sitagliptin	50	33.62	8746713	33.47	99.55	99.69	1.220
		33.57	8644675	33.08	98.55		
		33.52	8844037	33.84	100.97		
	100	67.19	17740273	67.89	101.04	99.89	1.000
		67.03	17409812	66.62	99.39		
		67.09	17398201	66.58	99.24		
	150	100.66	26439702	101.18	100.52	99.87	0.561
		100.71	26204756	100.28	99.57		
		100.66	26179158	100.18	99.53		

Sensitivity

Ertugliflozin and Sitagliptin have been shown to have Limit of Detection (LOD) values of 0.091 µg/mL and 0.960 µg/mL, respectively. The Limit of Quantitation (LOQ) values for ertugliflozin and sitagliptin, however, were determined to be 0.275 µg/mL and 2.910 µg/mL, respectively. These results are sufficient for accurately and precisely measuring and detecting ertugliflozin and sitagliptin.

Robustness

As shown in Table 6, minor variations in wavelength, flow rate, and column oven temperature did not significantly affect retention time, theoretical plate, or asymmetry. This led to the conclusion that the analytical approach was reliable and self-sufficient to analyse the two drugs simultaneously and that the developed method was found to be well within the limits.

System suitability

It was found that the resolution was 17.76, indicating well-resolved peaks. The theoretical plate, peak resolution, and tailing factor were all found to be within the permissible range, i.e., the % RSD was 2.0%, Theoretical plates exceeded 2000 and the tailing factor (asymmetry) was less than two. It was found that the suggested validated analytical approach satisfies the system the suitability requirements.

Conclusion

The assay of commercially available formulations was conducted using the aforementioned method, and the average assay results for ertugliflozin and sitagliptin were 99.64% and 100.12%, respectively. On placebo and blank samples who were not interfered with during the retention times of these drugs, a specificity study was conducted. Thus, this method is specific. Linearity study was carried out between 50 % to 150% levels, R^2 value was found to be 0.99999 for both Ertugliflozin and Sitagliptin. From the results shown in the recovery table, the value was found 99.13%, 99.06%, 99.46 % for 50%, 100% and 150% which were between 98-102% which indicates the

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Table 6. Result of Robustness of Ertugliflozin and Sitagliptin

Change in Parameter	Analyte	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 nm (215 nm)	Ertugliflozin	5.30	7045296	1.37	7569
	Sitagliptin	2.04	11352511	1.37	4465
Wavelength by -3 nm (209 nm)	Ertugliflozin	5.32	9127511	1.36	7560
	Sitagliptin	2.05	27171188	1.36	4502
Flow rate by +10% (1.10 ml/min)	Ertugliflozin	4.85	6807843	1.36	7169
	Sitagliptin	1.86	16549726	1.37	4177
Flow rate by -10% (0.90 ml/min)	Ertugliflozin	5.91	8321079	1.40	7800
	Sitagliptin	2.27	20277309	1.44	4556
Column oven temp by +2ºC (32 ºC)	Ertugliflozin	5.29	7460423	1.42	7585
	Sitagliptin	2.05	16830875	1.40	4512
Column oven temp by -2ºC (28^0C)	Ertugliflozin	5.29	7390590	1.43	7696
	Sitagliptin	2.05	17293012	1.42	4376

Table 7. Result of System suitability [*Data expressed as mean (±SD), n=5]

Analytes	Conc.	Area (±SD) *	Peak resolution	% RSD	Theoretical plates (±SD)*	Tailing Factor
Ertugliflozin	10 µg/mL	7441908 (±23889.04)	17.76	0.32	7646 (±15.53)	1.4
Sitagliptin	67 µg/mL	17702952 (±107046.36)	-	0.6	4472 (±12.71)	1.37

method is accurate. The relative standard deviation values for repeatability and intermediate precision studies were less than 2%. %RSD for Repeatability for both Sitagliptin and Ertugliflozin was obtained as 0.893% & 0.466% respectively. In Intermediate precision %RSD was calculated for two drugs and obtained as 1.284 % and 0.861 % respectively for Sitagliptin and Ertugliflozin. LOD, LOQ values obtained from regression equations of Ertugliflozin and Sitagliptin were 0.091 µg/ml, 0.960 µg/ml and 0.275 µg/ml, 2.910 µg/ml respectively. The robustness result for changes in wavelength, flow rate and column oven temperature were found to be within acceptable limits. As a result, the suggested method is useful for the regular quality control analysis of ertugliflozin and sitagliptin and is specific, accurate, precise, linear and robust.

Conflict of Interest

We do not have conflict of interest.

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