ORIGINAL ARTICLE

A Novel UPLC Method for the Estimation of Antidiabetic Drugs in Bulk and its Tablet dosage form

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Abstract

A highly sensitive, precise, and accurate ultra-performance liquid chromatography method was developed and validated for the determination of ertugliflozin and sitagliptin in the combined formulation. Chromatographic separation was carried out on the DIKMA Endeversil C18 column (2.1 mm × 50 mm, 1.7 µm) using the mobile phase of KH_2PO_4:methanol 45:55 v/v. The common wavelength of absorption of ertugliflozin and sitagliptin was found to be 225 nm. The flow rate was maintained at 0.3 mL/min, with 2 µL injection volume. The retention time of ertugliflozin and sitagliptin was found to be 0.41 min and 0.535 min. % relative standard deviation of the ertugliflozin and sitagliptin was found to be 0.681 and 0.218, respectively. %recovery was obtained as 100.146% and 100.27% for ertugliflozin and sitagliptin, respectively. Limit of detection (LOD), limit of quantification (LOQ) values obtained from regression equations of ertugliflozin and sitagliptin were 2.91, 2.96, and 10.04, 10.09, respectively. Regression equation of ertugliflozin is y = 1329.8 × −228.7 (0.9997) and y = 1294.4.× –40.1 of sitagliptin (0.9998). The proposed method was validated in terms of linearity, precision, accuracy, specificity, LOD, LOQ, and robustness. The method was successfully applied to the estimation of ertugliflozin and sitagliptin tablet dosage forms.

Key words: Ertugliflozin, international conference on harmonization guidelines, sitagliptin, ultra-performance liquid chromatography, validation

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic, progressive, incompletely understood metabolic condition primarily characterized by hyperglycemia. Type 2 diabetes is due to insufficient insulin production from beta cells in the setting of insulin resistance.[1] Without treatment, type 2 diabetes can cause various health problems, such as heart disease, kidney disease, and stroke. Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters, which inhibits renal glucose reabsorption and reductions in plasma glucose in patients with T2DM with IUPAC name (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 [Figure 1]. Sitagliptin is a new oral hypoglycemic dipeptidyl peptidase-4 inhibitor class of drug that inhibits the breakdown of glucagon and increases the insulin release in patients with type 2 diabetes with IUPAC name 7-[(3R)-3-amino-1-[3-(trifluoromethyl)-5H,6H,7H,8H-[1,2,4] triazolo [4,3-a] pyrazin-7-yl]-4-(2,4,5-trifluoro phenyl) butan-1-one [Figure 2]. Steglujan (ertugliflozin 15 mg and sitagliptin 100 mgn) is a member of the antidiabetic drug, developed by Merck Sharp and Dohme Company for the treatment of type 2 diabetes. It is available in the market as a film-coated tablet dosage form. Ertugliflozin and sitagliptin can be used alone or in combination therapy.[2-4] The literature review reveals that very few analytical methods have been reported for the determination of ertugliflozin and sitagliptin using various analytical techniques.[5-11] It was found that no suitable

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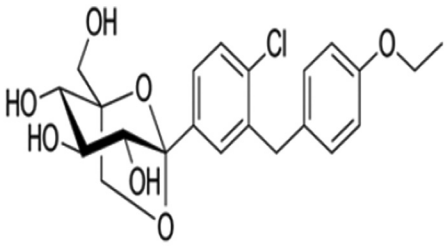

Figure 1: Chemical structure of ertugliflozin

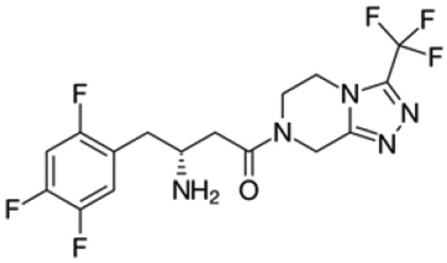

Figure 2: Chemical structure of sitagliptin

validated method was available from the literature for the determination of Steglujan by ultra-performance liquid chromatography (UPLC). The objective of the present study is to develop a novel, accurate, precise, economic method for the simultaneous estimation of ertugliflozin and sitagliptin according to the International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Materials

The reference sample of sitagliptin and ertugliflozin was obtained as a gift sample from Inception Source, Hyderabad, India. The reagents and solvents used (acetonitrile, methanol, potassium dihydrogen orthophosphate, and orthophosphoric acid were of AR grade obtained from Merck Chemicals, Mumbai, India). An ultrasonic device, a sensitive balance, and a pH meter were used for the preparation of solutions.

Instrumentation and chromatography conditions

Waters Acquity UPLC system (Waters, Milford, MA, USA) equipped with a quaternary gradient pump, autosampler, column oven, and photodiode array detector and empower 2 software. The chromatographic separation was performed on the DIKMA endeversil C18 column (2.1 mm × 50 mm, 1.7 µm particle size) at an ambient column temperature. The samples were eluted using a mobile phase of buffer (potassium dihydrogen orthophosphate): Methanol (45:55v/v) and samples were degassed by ultrasonication for 30 min and filtered through 0.45 µm membrane filter. The measurements were carried out with an injection volume of 2 µL; the flow rate was set to 0.3 mL/min, and detection was carried out at 225 nm. All determinations were done at ambient column temperature (30°C).

Preparation of phosphate buffer pH 3

Take an equivalent amount of 0.1 M potassium dihydrogen orthophosphate 1000 mL volumetric flask and makeup with HPLC water and degassed in an ultrasonic water bath for 10 min and pH was adjusted with OPA up to 3 then filtered through 0.45 µ filter under vacuum filtration.

Preparation of mobile phase

0.1% OPA buffer: 1 mL of orthophosphoric acid was diluted to 1000 mL with HPLC-grade water. Mobile phase was prepared by accurately measuring 450 mL of phosphate buffer and 550 mL of methanol was mixed and degassed in an ultrasonic water bath for 10 min and then filtered through 0.45 µ filter under vacuum filtration. The mobile phase was used as the diluent.

Preparation of stock solution and standard solution

Accurately weigh and transfer 15 mg of ertugliflozin and 100 mg of sitagliptin working standard into a 100 mL clean dry volumetric flask add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further, pipette 1 mL of the above stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent.

Preparation of sample solution

Accurately weigh and transfer steglujan tablet powder equivalent to 15 mg of ertugliflozin and 100 mg of sitagliptin sample into a 100 mL clean dry volumetric flask add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further, pipette 1 mL of the above stock solutions into a 10 mL volumetric flask and dilute up to the mark with diluent.

RESULTS

Method development and optimized chromatographic conditions

During the optimization of the method, different columns with different dimensions were tried and finally, satisfactory separation was obtained on the DIKMA Endeversil C18 column (2.1 mm × 50 mm, 1.7 µm) column. Methanol, water, acetonitrile, and different buffers were used simultaneously

as mobile phase and buffer (potassium dihydrogen orthophosphate):methanol (45:55 v/v) was found to be more suitable for better separation of Steglujan under analysis. Isocratic mode of elution with different ratios of organic to aqueous phases was tried to achieve appropriate separation of analytes in a reasonable run time. Flow rate of 0.3 mL/min was optimum at 225 nm. The column temperature was set at 30°C. Under optimized experimental conditions, all the peaks were well defined and free from tailing and providing good resolution. The concern of small deliberate changes in the flow rates and wavelength on results was evaluated as a part of robustness.

Validation of the analytical method

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.[12-16]

System suitability test

The system suitability parameters were determined by preparing standard solutions of ertugliflozin (15 ppm) and sitagliptin (100 ppm); the solutions were injected 6 times; and parameters such as peak tailing, resolution, and USP plate count were calculated.

A sharp and symmetrical peak with a retention value of 0.41 and 0.535 for ertugliflozin and sitagliptin at 225 nm was observed [Figures 3 and 4]. For two of them, the peak symmetries were <1.5; the theoretical plate numbers were >2000; and the relative standard deviation (%RSD) of areas of six standard injections of ertugliflozin and sitagliptin was <2. The system suitability results are shown in Table 1.

Specificity

The specificity of the method was carried out to check whether there is any interference exists between any impurities with analyte peaks. The specificity was performed by injecting blank, placebo, and standard solutions of drugs. In the placebo chromatogram, there were no peaks observed at the retention times of ertugliflozin and sitagliptin, indicating that the method was specific.

Linearity

The linearity of the method was established by determining the peak areas of different concentrations of ertugliflozin and sitagliptin over a range of 3.75–22.5 µg/mL and 25–150 µg/mL, respectively. Six replicates of each concentration were independently prepared and injected into UPLC system. The linearity was determined by calculating a regression line from the plot of peak area and concentration of the drug. The calibration curve was linear with an average correlation

Table 1: System suitability data of ertugliflozin and sitagliptin

S. No.	Ertugliflozin		Sitagliptin	
	RT	Peak area	RT	Peak area
1	0.407	14,472	0.533	96,836
2	0.415	14,556	0.541	96,486
3	0.421	14,592	0.543	96,435
4	0.429	14,643	0.552	96,856
5	0.437	14,569	0.555	96,456
6	0.441	14,768	0.557	96,786
	Average	14,600		96,642.5
	SD	99.416		202.95
	Percentage RSD	0.681		0.21

SD: Standard deviation, RSD: Relative standard deviation

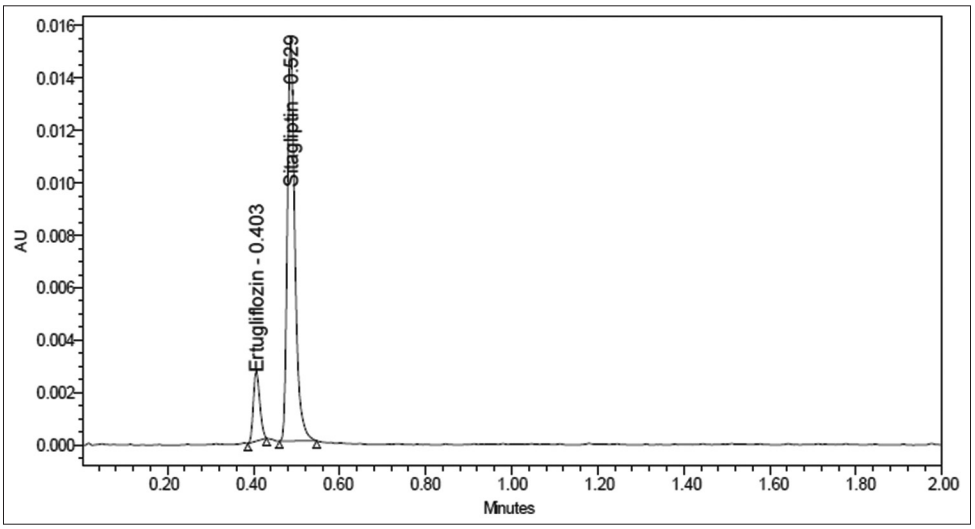

Figure 3: Chromatograms of ertugliflozin and sitagliptin standard solution

coefficient of R^2 0.999 [Figure 5]. The summary of the results is shown in Table 2.

Precision

The precision was evaluated at three levels, repeatability, reproducibility, and intermediate precision. Each level of precision was investigated by six replicate injections of concentrations 15 µg/mL and 100 µg/mL of ertugliflozin and sitagliptin, respectively. The peak areas of all injections were taken and standard deviation, %RSD was calculated. The result of precision was expressed as % of RSD and is tabulated in Tables 3 and 4.

Accuracy

The accuracy of the method was performed by recovery studies. Acknowledged amount of pure drug concentrations was spiked in the placebo at three different levels, that is, 50%, 100%, and 150%. Each sample was injected thrice. The mean recovery was calculated. Results of recovery data are shown in Tables 5 and 6.

LOD and LOQ

Estimation of LOD and LOQ considered the acceptable signal-to-noise ratios 3:1 and 10:1, respectively, for ertugliflozin and sitagliptin. The LOD's were found to be 2.91 and 2.96. The LOQ's were found to be 10.01 and 10.09 for ertugliflozin and sitagliptin, respectively [Figures 6 and 7].

Robustness

UPLC conditions were slightly modified to evaluate the analytical method's robustness. These changes included the flow rates and wavelength. The robustness of the method was genuine when small, deliberate changes such as flow rate

Table 2: Linearity data of ertugliflozin and sitagliptin

Ertugliflozin		Sitagliptin	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
3.75	4862	25	32,573
7.5	9757	50	64,978
11.25	14,527	75	96,453
15	19,678	100	128,673
18.75	24,836	125	162,527
22.5	29,087	150	195,462
Correlation coefficient	0.9997	Correlation coefficient	0.9998

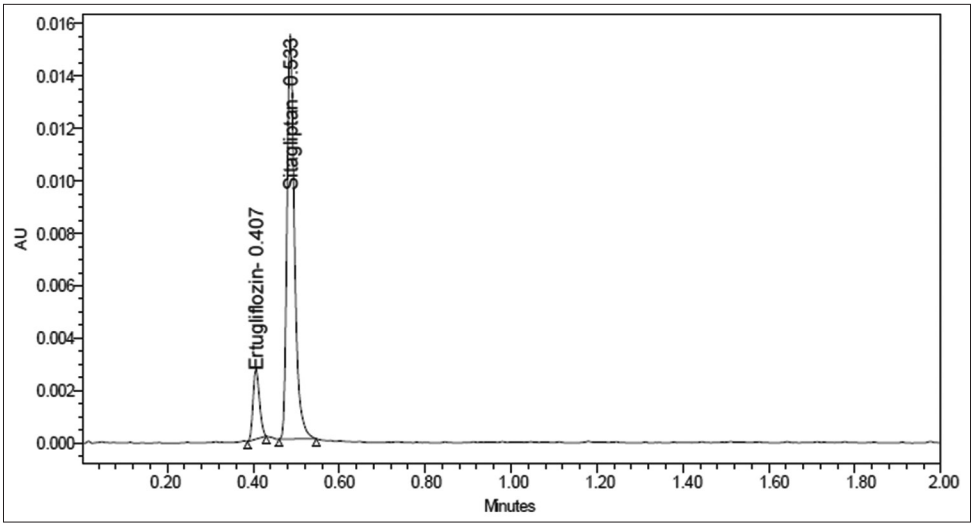

Figure 4: Chromatograms of ertugliflozin and sitagliptin sample solution

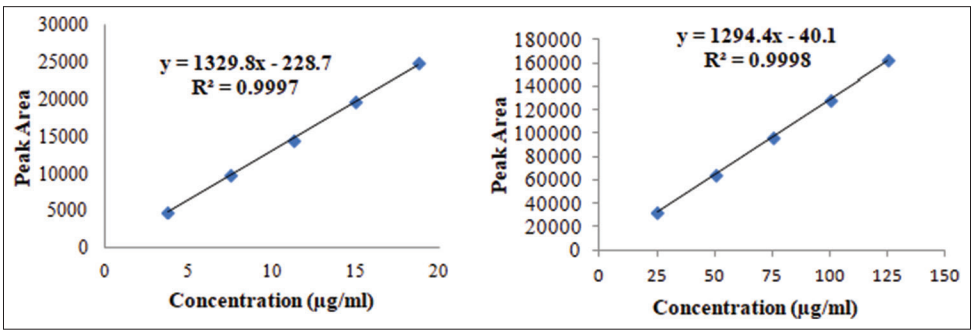

Figure 5: Ertugliflozin and sitagliptin calibration curves

Table 3: Method precision data of ertugliflozin and sitagliptin

S. No.	Ertugliflozin			Sitagliptin		
	RT	Peak area	Percentage assay	RT	Peak area	Percentage assay
1	0.407	14,572	99.38	0.531	96,756	100.32
2	0.415	14,697	100.23	0.542	96,245	99.79
3	0.421	14,656	99.95	0.545	96,786	100.35
4	0.429	14,678	100.10	0.550	96,458	100.01
5	0.437	14,618	99.69	0.538	96,542	100.10
6	0.441	14,767	100.71	0.554	96,753	100.31
Average			100.01			100.146
SD			0.4581			0.2218
Percentage RSD			0.4580			0.2215

SD: Standard deviation, RSD: Relative SD

Table 4: Intermediate precision data of ertugliflozin and sitagliptin

S. No.	Ertugliflozin			Sitagliptin		
	RT	Peak area	Percentage assay	RT	Peak area	Percentage assay
1	0.403	14,672	100.06	0.529	96,836	100.40
2	0.414	14,756	100.63	0.535	96,485	100.04
3	0.419	14,582	99.45	0.542	96,435	99.98
4	0.425	14,643	99.86	0.547	96,856	100.42
5	0.431	14,669	100.04	0.553	96,786	100.35
6	0.441	14,668	100.03	0.557	96,456	100.01
Average			100.01			100.02
SD			0.3806			0.2102
Percentage RSD			0.3805			0.2098

SD: Standard deviation, RSD: Relative SD

Table 5: Accuracy data of ertugliflozin

Sample number	Spiked level (%)	Sample area	µg/mL added	µg/mL found	Percentage recovery	Percentage mean recovery
1	50	7316	7.50	7.48	99.73	99.99
2		7324	7.50	7.49	99.86	
3		7356	7.50	7.53	100.40	
1	100	14,615	15.00	14.95	99.66	100.03
2		14,785	15.00	15.12	100.80	
3		14,597	15.00	14.93	99.53	
1	150	22,192	22.50	22.70	100.88	100.72
2		22,098	22.50	22.61	100.48	
3		22,175	22.50	22.68	100.80	

and wavelength were performed at 100% test concentration. Results are shown in Table 7. It can be concluded that the variation in flow rate and wavelength has not affected the method significantly.

Analytical performance summary data are shown in Table 8.

DISCUSSION

In the present work, an attempt was made to provide a novel, precise, accurate, and economical UPLC method. It was successfully applied for the determination of ertugliflozin and sitagliptin in tablet dosage forms without

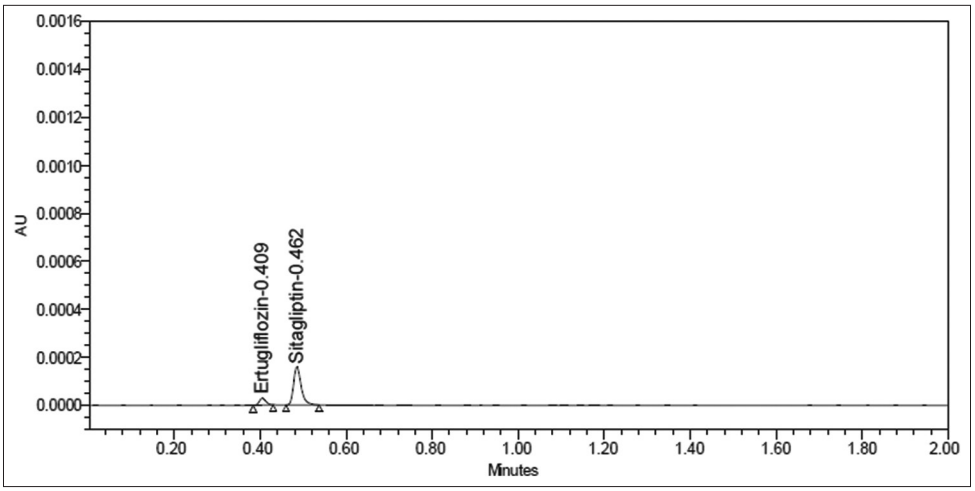

Figure 6: Limit of detection chromatogram of ertugliflozin and sitagliptin

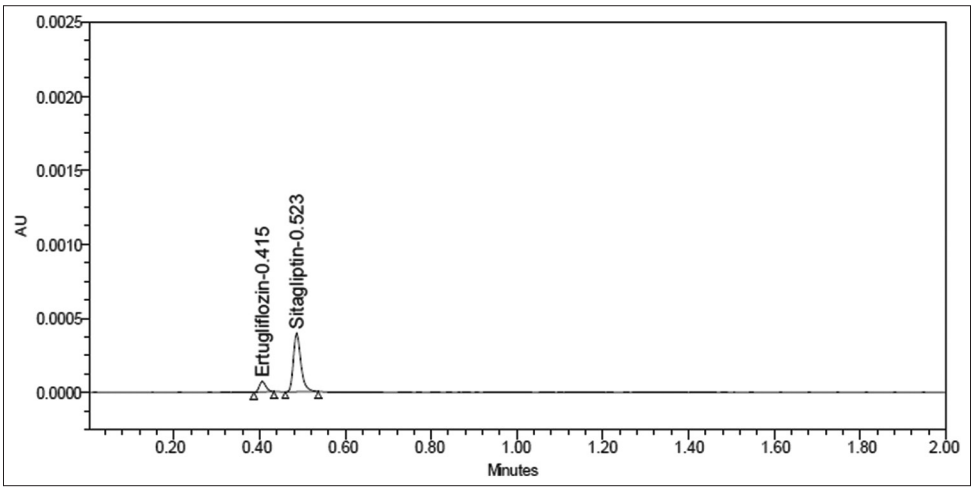

Figure 7: Limit of quantification chromatogram of ertugliflozin and sitagliptin

Table 6: Accuracy data of sitagliptin

Sample number	Spiked level (%)	Sample area	µg/mL added	µg/mL found	Percentage recovery	Percentage mean recovery
1	50	47,862	50.00	49.62	99.24	100.01
2		48,685	50.00	50.48	100.96	
3		48,137	50.00	49.91	99.82	
1	100	96,019	100.00	99.55	99.55	99.98
2		96,551	100.00	100.73	100.11	
3		96,368	100.00	99.92	99.92	
1	150	145,076	150.00	150.42	100.28	100.05
2		144,502	150.00	149.82	99.88	
3		144,697	150.00	150.02	100.01	

the interference of other constituents. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection were done based on peak parameters (height, tailing, theoretical plates, capacity factor, run time, etc.). The chromatographic separation was obtained on the DIKMA endeversil C18 column (2.1 mm × 50 mm, 1.7 µm) and mobile phase of buffer (potassium dihydrogen orthophosphate):methanol (45:55 v/v) with 0.3 mL/min flow rate. The optimum wavelength for the detection of drugs was at 225 nm. The

Table 7: Robustness data ertugliflozin and sitagliptin

S. No.	Parameter	Ertugliflozin		Sitagliptin	
		Change	USP plate count	Change	USP plate count
1	Flow rate (mL/min)	0.27	9626.92	0.27	9132.29
2		0.30	9725.92	0.30	9256.39
3		0.33	9865.39	0.33	9352.29
4	Wavelength variation (nm)	220	9762.23	220	9214.27
5		225	9725.92	225	9256.39
6		230	9767.76	230	9232.23

Table 8: Analytical performance summary data of ertugliflozin and sitagliptin

Validation parameter	Results		Acceptance criteria
	Ertugliflozin	Sitagliptin	
Accuracy (%Recovery) (*n*=9)	Mean recovery 100.24%	Mean recovery 100.01%	Mean assay - 98–102% Percentage RSD should be <2
Precision (*n*=6)	Mean assay-100.146% Percentage RSD-0.3745	Mean assay-100.27% Percentage RSD-0.1068	
Linearity	y=1329.8x–228.7 R^2=0.9997	y=1294.4x–42.1 R^2 = 0.9998	R^2=0.999
LOD-S/N ratio	2.91	2.96	3
LOQ-S/N ratio	10.04	10.09	10

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

proposed method was validated as per ICH guidelines 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. The average retention time for ertugliflozin and sitagliptin was found to be 0.41 and 0.535 min. The calibration was linear in the concentration range of 3.75–22.5 µg/mL for ertugliflozin and 25–150 µg/mL for sitagliptin. Regression equation of ertugliflozin is y = 1329.8 × −228.7 (0.9997) and y = 1294.4 × –40.1 of sitagliptin (0.9998). System precision and method precision were evaluated by six replicate injections of both standard and sample solutions which were prepared and analyzed on the same day and on 3 different days. %RSD of the ertugliflozin and sitagliptin were found to be 0.681 and 0.218, respectively. The % RSD reported was found to be <2%. The low values of % RSD indicate that the method was precise. The accuracy of the method was performed by recovery studies. The mean %Recovery was obtained as 100.146% and 100.27% for ertugliflozin and sitagliptin, respectively at 50%, 100%, and 150%. The robustness of the proposed method was determined by modifying the parameters such as flow rate 0.27 mL/min and 0.33 mL/min) and wavelengths 220 nm and 230 nm. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. LOD and LOQ values obtained from regression equations of ertugliflozin and sitagliptin were 2.91, 2.96, and 10.04, 10.09, respectively.

CONCLUSION

Nowadays pharmaceutical industries as well as analytical laboratories are in search of new ways to reduce the cost and time for the analysis of drugs and improve the quality of their product. UPLC can be regarded as a new direction for liquid chromatography. UPLC improves three areas of liquid chromatography: speed, resolution, and sensitivity. The UPLC method was developed and validated as per ICH guidelines for the effective separation of ertugliflozin and sitagliptin, ensuring accurate quantification with high precision. The results showcased a highly sensitive, specific, and reliable method, devoid of any interference from other substances. Consequently, it can be easily adopted for routine quality control for monitoring the assay in the API, in-process samples, and the finished tablet formulation.

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