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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ERTUGLIFLOZIN AND SITAGLIPTIN IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT This investigation furnishes an analytical method on reverse phase high performance liquid chromatography with PDA detection method for the simultaneous determination ertugliflozin and sitagliptin in bulk and in its tablets. The separation and assay of ertugliflozin and sitagliptin was done using Cosmicsil C8 column (250 mm × 4.6 mm I.D., 5 µm size particle) in isocratic mode of elution . The optimized mobile phase was 0.1 Molar dipotassium hydrogen phosphate and methanol (65:35, v/v). The eluted analytes are monitored at 225 nm wavelength. The method separated ertugliflozin and sitagliptin within a 7 min run time. The parameters proposed by International Conference of Harmonization guidelines were concluded by validation i.e., system suitability , linearity , limit of detection , limit of quantification , selectivity , accuracy precision and robustness . Linearity of ertugliflozin and sitagliptin was in concentration range of 7.5 -22.50 µg/ml and 50-150 µg/ml , respectively by the method . The LOD was 0.087 and 0.071 µg/ml for sitagliptin and ertugliflozin , respectively . The LOQ was 0.291 µg/ml and 0.237 µg/ml for sitagliptin and ertugliflozin, respectively . The developed method was assessed through analysis of sitagliptin and ertugliflozin in the available tablet dosage form . The percent recoveries (± RSD) were 99.60 ± 0.027 and 99.83 ± 0.017 for sitagliptin and ertugliflozin , respectively. The results proved the non interference from the tablet excipients with good recovery and precision . Hence the method can be suggested for the routine quality control analysis of sitagliptin and ertugliflozin.

KEYWORDS : Ertugliflozin, Sitagliptin, RP-HPLC, Method development

INTRODUCTION

Ertugliflozin is approved for treating patients of Type II diabetes by Food and Drug Administration. Ertugliflozin belongs to gliflozins class of drugs. Ertugliflozin is prescribed alone (monotheraphy) or in combination with either metformin or sitagliptin. It has 11-17 hours of half life, bioavailability of 70-90%. It was eliminated 50% and 41% in urine and feces, respectively. Ertugliflozin is a selective inhibitor for Na^{2+}/glucose co transporter 2. Na^{2+}/glucose co transporter 2 inhibitors reduce reabsorption of renal glucose and enhance excretion of glucose in urine, reduce fasting and postprandial levels of glucose in blood. Ertugliflozin also reduces hemoglobin A1C (by about 0.5-1%) and reduces systolic blood pressure (by about 3-6 mm Hg).

Chemically it is (1S,2S,3S,4R,5S)-5-[4-Chloro-3-(4-ethoxybenzyl)phenyl(hydroxymethyl)-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol

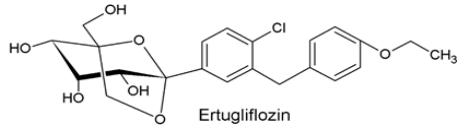


Fig 1: Chemical Structure of ertugliflozin

Sitagliptin is an oral antihyperglycemic agent belonging to dipeptidyl peptidase-4 inhibitor class. Sitagliptin is prescribed in treating diabetes milletus -type 2 either alone or in combined form with metformin or thiazolidinedione. The benefit of sitagliptin is its lower side-effects (e.g., less weight gain, less hypoglycemia) when the blood glucose level are controlled. Sitagliptin has 87% absolute bioavailability. It has 12.4 hours of half life. Within one week of sitagliptin dosage, 135 of it, is eliminated in feces and 87% in urine. Sitagliptin competitively inhibits the enzyme dipeptidyl peptidase 4. This inhibition breaks down the gastrointestinal hormones like incretins glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 which are released in response to a meal. By preventing glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1inactivation, these two incretins are able to increase the secretion of insulin and suppress the glucagon release by pancreas. This makes blood glucose levels towards normal. Chemically it is 1,2,4-triazolo[4,3-a]pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5trifluorophenyl) butyl]-5,6, tetrahydro-3-(trifluoromethyl)-, phosphate (1:1) monohydrate

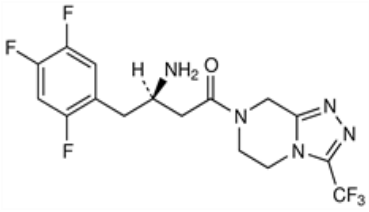


Fig 2: Chemical Structure of sitagliptin

Ertugliflozin and sitagliptin combination is approved by FDA in 2017. This combination is released with name Steglujan™ Tablets (Merck and Co. Inc, Whitehouse station, USA). Steglujan tablet are available in two strengths (Steglujan, 2018).

- 5 mg of ertugliflozin and 100 mg sitagliptin (Steglujan 5/100)
- 15 mg of ertugliflozin and 100 mg sitagliptin (Steglujan 15/100)
- Steglujan is indicated for adults patients aged ≥18 years with type 2 diabetes mellitus. This is given in addition to exercise and diet to get better glycaemic control. Steglujan is used when:
- Metformin and/or a sulphonylurea alone or their combination does not control blood glucose levels.
- Patients already being treated with either ertugliflozin or sitagliptin as separate dosage form, but do not provide enough blood glucose control.

MATERIALS AND METHODS

Materials and Instrumentation:

1. The ertugliflozin and sitagliptin pure standards were procured from Lara Drugs Private Limited (Telangana, India) and used as it is.
2. HPLC grade methanol (Merck specialities Ltd, Hyderabad, India).
3. Dipotassium hydrogen phosphate of analytical reagent grade (Sd. Fine Chemicals Ltd, Mumbai, India).
4. Milli-Q water (Milli-Q water system, Millipore, USA).
5. Steglujan, fixed combination tablet dosage form (Merck and Co. Inc, Whitehouse station, USA) labeled to have 15 mg and ertugliflozin weighing 100mg and sitagliptin, respectively.

6. Waters Alliance HPLC system (2695 Module) attached with photodiode array detector and fitted with degasser, autosampler and column oven.
7. Empower 2 version software used for data analysis and interpretation.
8. Analytical column used is Cosmicsil C8 with dimensions 25 cm × 4.6 mm, particle size 5 µm

Mobile Phase:
The preparation of mobile phase is done by mixing 0.1M dipotassium hydrogen phosphate and methanol in the ratio of 65:35, *v/v*. The same solvent mixture is used as diluent for standard solutions preparation.

Chromatographic Conditions:
The analysis was accomplished at 25°C temperature using above said analytical column and mobile phase. The mobile phase was filtered (via 0.45 µm filter) and degassed (through sonication) prior to using. The process parameters of the RP-HPLC method were set as: wavelength, 225 nm; flow rate, 1 ml/min; injection volume, 10 µl; pH, 3.5; and total run time, 7 min.

Stock Standard Solution:
Ertugliflozin (15 mg) and sitagliptin (100 mg) was truly weighed and dissolved in mobile phase (100 ml) in a volumetric flask (100ml) to make mixed stock solution with concentrations at 150 µg/ml of ertugliflozin and 1000 µg/ml of sitagliptin.

Working Standard Solution:
Following working standard solutions are prepared by accurate dilution of stock standard solution with mobile phase.

Calibration standard solutions: 50, 75, 100, 125 and 150 µg/ml of sitagliptin and 7.5, 11.25, 15, 18.75 and 22.50 µg/ml of ertugliflozin.

For studying validation parameters, the working standard with concentration 15 µg/ml and 100 µg/ml of ertugliflozin and sitagliptin, respectively was prepared.

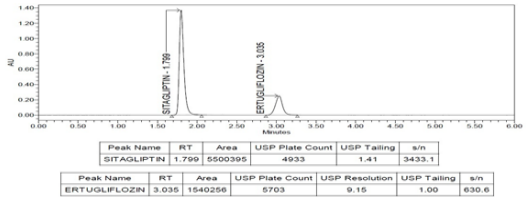
Preparation of sample solution:
Accurate weight of the mixed contents 10 Steglujan tablets equal to 15 mg of ertugliflozin and 100 mg of sitagliptin were transferred to 100 ml volumetric flask and 50 ml of mobile phase was added to it. The content of flask was sonicated for 20 min, completed to volume using mobile phase followed by filtration. The resultant solution is tablet sample stock solution having 150 µg/ml of ertugliflozin and 1000 µg/ml of sitagliptin concentrations. Further, the dilution of stock solution is done to get a test working solution of concentrations 15 µg/ml (ertugliflozin) and 100 µg/ml (sitagliptin) using mobile phase.

Calibration curves of ertugliflozin and sitagliptin:
To a set of five volumetric flasks (10 ml), appropriate volumes of stock standard solution (500, 750, 1000, 1250 and 1500 µg/ml – sitagliptin; 75, 112.5, 150, 187.5 and 225.0 µg/ml – ertugliflozin) were transferred and diluted with mobile to get calibration standards solutions with concentrations 50, 75, 100, 125 and 150 µg/ml of sitagliptin and 7.5, 11.25, 15, 18.75 and 22.50 µg/ml of ertugliflozin. Ten µl aliquot of every solution which is calibrated was injected onto the column. The analysis was performed using described chromatographic conditions. The peak areas of selected drug combination were determined at 225 nm. By plotting drug's peak area *vs* concentration for the calibration curves can be constructed and regression equations for selected drugs were computed.

Determination of ertugliflozin and sitagliptin in tablet dosage form:
Ten µl aliquot of test sample solution (15 µg/ml – ertugliflozin; 100 µg/ml - sitagliptin) prepared in "Preparation of sample solution" section was injected onto the column (n=3). Test sample solutions were analyzed using the process stated under "Calibration curves of ertugliflozin and sitagliptin". From the recorded chromatograms, ertugliflozin and sitagliptin peak areas were calculated. The nominal contents of ertugliflozin and sitagliptin in tablet formulation were quantified using corresponding calibration curve or using regression equation.

RESULTS AND DISCUSSION

Development of RP-HPLC Method:
Four different C8 analytical columns: Supelco, Suncil, Inertsil, and Cosmicsil with dimension 25 cm × 4.6 mm internal diameter, particle size of 5 µm were tested during method development. Based on good peak shape and high resolution, Cosmicsil C8 (25 cm x 4.6 mm, 5 µm) analytical column with 25 °C temperature was selected. From the UV spectra of ertugliflozin and sitagliptin, 225 nm was selected as both drugs showed considerable absorbance. So as to achieve acceptable peak symmetry and separation of ertugliflozin and sitagliptin with good resolution, different combinations of methanol with 0.1% orthophosphoric acid, 0.1M disodium hydrogen phosphate and 0.1M dipotassium hydrogen phosphate buffers of different pHs were tested methodically. In conclusion, a mobile phase solvent mixture having of 0.1M dipotassium hydrogen phosphate and methanol in a ratio of 65:35 *v/v* was preferred to get better resolution, good response and satisfactory peak symmetry. Flow rates between 0.8 and 1.2 ml/min were tested and 1.0 ml/min flow rate was adequate to elute ertugliflozin and sitagliptin inside below 7 min. Using optimized conditions, the retention times for ertugliflozin and sitagliptin are 3.035 min and 1.799 min, respectively. Typical chromatogram is exhibited by Figure 3.



Peak Name	RT	Area	USP Plate Count	USP Tailing	s/n
SITAGLIPTIN	1.799	5500395	4933	1.41	3433.1

Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing	s/n
ERTUGLIFLOZIN	3.035	1540256	5703	9.15	1.00	630.6

Fig 3: Chromatogram of sitagliptin and ertugliflozin using conditions optimized

Validation of the developed RP-HPLC method:
The developed RP-HPLC procedure was validated following International Conference on Harmonization for parameters: system suitability, selectivity, linearity, accuracy, precision, robustness, limit of detection and limit of quantification.

System Suitability:
System suitability was established through injecting 6 replicates of working standard solutions (15 µg/ml – ertugliflozin; 100 µg/ml - sitagliptin). The drug solutions were analyzed for its repeatability of retention time and peak area, and plate count, resolution and peak tailing values of ertugliflozin and sitagliptin. Recommended limits for system suitability parameters are as follows: %RSD of peak area = ≤ 2%; % RSD of retention time = ≤ 2%; Tailing factor = ≤2; plate count = >2000 and resolution = > 1.5. The system suitability parameters values shown in Table 1 are within the suggested limits.

Table 1: System suitability

Sample	RT	PA	PC	TF	RS	RT	PA	PC	TF	RS
Sitagliptin	**Ertugliflozin**									
1	1.797	5513435	5045	1.42	-	3.032	1535425	5777	0.99	9.22
2	1.795	5508506	4948	1.41	-	3.027	1538224	5687	1.00	9.14
3	1.798	5519632	4902	1.42	-	3.029	1540716	5682	1.00	9.1
4	1.795	5533115	4870	1.4	-	3.027	1551801	5588	1.00	9.06
5	1.795	5566938	4865	1.41	-	3.025	1556140	5594	1.00	9.05
Mean	**1.796**	**5528325**	**4926**	**1.412**	**-**	**3.028**	**1544461**	**5666**	**0.998**	**9.114**
SD	**0.0014**	**23473.2879**	**74.2933**	**0.0083**	**-**	**0.0026**	**9011.7857**	**77.9185**	**0.0044**	**0.0691**
RSD	**0.079**	**0.425**	**1.508**	**0.593**	**-**	**0.087**	**0.583**	**1.375**	**0.448**	**0.759**

RT - Retention time; PA – Peak area; PC – Plate count; TF – Tailing factor;
RS – Resolution; SD – standard deviation; RSD – Relative standard deviation

Selectivity:

Selectivity was demonstrated through comparing the chromatograms of working standard solution with concentration of 15 µg/ml (ertugliflozin) and 100 µg/ml (sitagliptin) with chromatograms of blank mobile phase (drug free), placebo blank (contains excipient without drug) and tablet sample solution with 15 µg/ml ertugliflozin and 100 µg/ml sitagliptin concentration. Figures 4-6 represent these chromatograms. No interference peak was seen in the chromatograms of blank mobile phase and placebo blank at ertugliflozin and sitagliptin retention times. The retention times of both drugs in chromatograms of tablet and standard solutions are same. This showed that drug-excipient interaction and drug-drug interaction are absent.

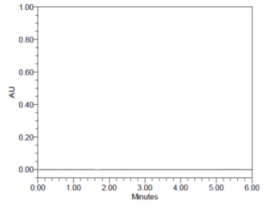


Fig 4: Chromatogram of placebo blank

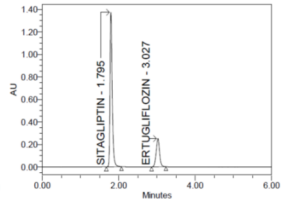


Fig 5: Chromatogram of standard solution

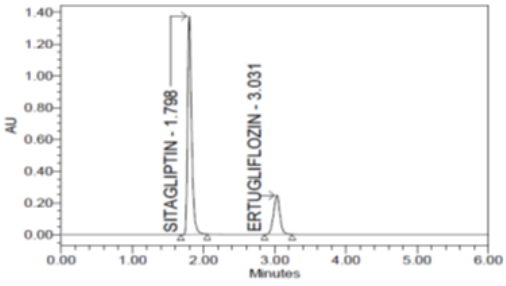


Fig 6: Chromatogram of sitagliptin and ertugliflozin tablet sample solution

LINEARITY:

Linear correlation was observed through mapping drug's peak area against its amount. The relationship was found linear in the range 50–150 µg/ml and 7.5-22.50 µg/ml for sitagliptin and ertugliflozin, respectively. Table 4 has summarized results of linearity. The results show that fine correlation existed between the drug peak area and amount of studied combination of drug. The linearity curves of sitagliptin and ertugliflozin are shown in Figures 7 and 8, respectively.

Table 2: Linearity results for sitagliptin and ertugliflozin

Parameter	Sitagliptin	Ertugliflozin
Linearity (µg/ml)	50-150	7.5-22.5
Regression equation (Y= mx + c)	y = 55308 x - 916.5	y = 10286x - 490.7
Regression coefficient (R2)	0.9998	0.9998

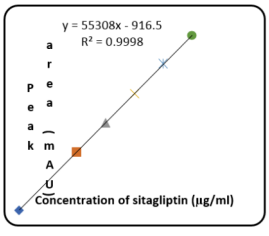


Fig 7: Linearity curve of sitagliptin

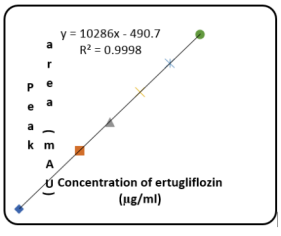


Fig 8: Linearity curve of ertugliflozin

SENSITIVITY:

Measurement of limit of detection (LOD) and limit of quantitation (LOQ) for sitagliptin and ertugliflozin are done at a signal-to-noise ratio of 3 for LOD and 10 for LOQ. The LOD was measured as 0.087 µg/ml (sitagliptin) and 0.071 µg/ml (ertugliflozin). The LOQ was measured as 0.291 µg/ml (sitagliptin) and 0.237 µg/ml (ertugliflozin).The chromatograms at LOD and LOQ levels of proposed method are shown in Figures 9 and 10.

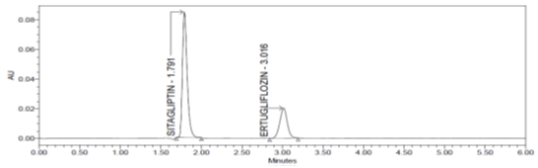
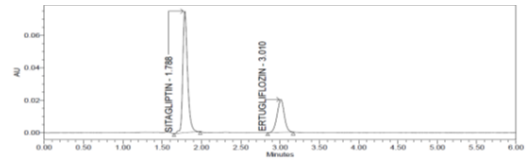


Fig 9: Chromatogram at LOD level for the proposed method



PRECISION:

The determination of the method's precision of the method was performed through six replicate analyses of working standard solution with concentrations 15 µg/ml ertugliflozin and 100 µg/ml sitagliptin. Relative standard deviation of drug peak area was measured to characterize precision. The results for precision (Table 3) are found inside the adequate criteria and allowed the precise analysis of sitagliptin and ertugliflozin. Figure 13 represents these chromatograms.

Table 3: Precision of the method

Sitagliptin	Ertugliflozin		
Amount (µg/ml)	Peak area	Amount (µg/ml)	Peak area
100	5525471	15	1540649
100	5524775	15	1549759
100	5523004	15	1544354
100	5528518	15	1540104
100	5527634	15	1541781
100	5521685	15	1544797
Mean	5525181	Mean	1543574
% RSD	0.047	% RSD	0.232

Accuracy:

Six multiples of working standard solution with concentrations 15 µg/ml ertugliflozin and 100 µg/ml sitagliptin is calculated to show method's accuracy. Characterization of accuracy is achieved by percent assay and mean percent assay of sitagliptin and ertugliflozin . The results for accuracy (Table 4) are found inside the adequate criteria and allowed the accurate analysis of sitagliptin and ertugliflozin.

Table 4: Accuracy of the method

Sitagliptin	Ertugliflozin				
Amount taken (µg/ml)	Amount analyzed (µg/ml)	% Assay	Amount taken (µg/ml)	Amount analyzed (µg/ml)	% Assay
100	99.55	99.55	15	14.93	99.55
100	99.54	99.54	15	15.02	100.14
100	99.50	99.50	15	14.97	99.79
100	99.60	99.60	15	14.93	99.52
100	99.59	99.59	15	14.94	99.63
100	99.48	99.48	15	14.97	99.82
Mean	99.54	99.54	Mean	14.96	99.74
% RSD	0.048	0.048	% RSD	0.231	0.230

RECOVERY STUDY:

The recovery study was done by analyzing preanalyzed tablet samples spiked with pure sitagliptin and ertugliflozin at 3 different concentration levels (50%, 100% and 150%). The recoveries of sitagliptin and ertugliflozin are summarized in Table 5. The recovery ranged from 72% to 89% for sitagliptin and 59% for ertugliflozin. The values demonstrated that the recovery of both drugs was efficient, reproducible and consistent. The excipients in tablet dosage form did not cause indicative interference in analyzing sitagliptin and ertugliflozin. The chromatograms of sitagliptin and ertugliflozin for concentration levels 50%, 100% and 150% are presented in Figure 11-13.

Table 5: Recovery of sitagliptin and ertugliflozin

Spiked Level (%)	Amount of drug (µg/ml)	Recovered(%)	Mean(%)	RSD (%)	
	Added	Found			
Sitagliptin					
50	50	49.75	99.50	99.59	0.102
	50	49.79	99.57		
	50	49.85	99.70		
100	100	99.54	99.54	99.58	0.038
	100	99.60	99.60		
	100	99.61	99.61		
150	150	149.42	99.62	99.63	0.027
	150	149.49	99.66		
	150	149.42	99.61		
Ertuglifloz in					
50	7.5	7.49	99.83	99.84	0.036
	7.5	7.49	99.81		
	7.5	7.49	99.88		
100	15	14.96	99.71	99.84	0.200
	15	15.01	100.07		
	15	14.96	99.74		
150	22.5	22.42	99.65	99.81	0.142
	22.5	22.48	99.89		
	22.5	22.48	99.90		

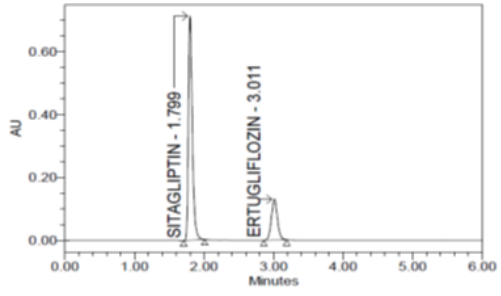


Fig 11: Chromatogram at 50% level of sitagliptin and ertugliflozin

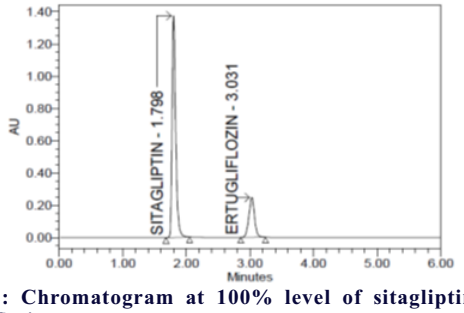


Fig 12: Chromatogram at 100% level of sitagliptin and ertugliflozin

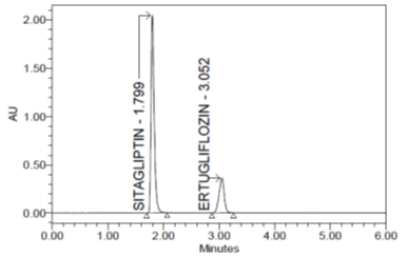


Fig 13: Chromatogram at 150% level of sitagliptin and ertugliflozin

ROBUSTNESS:

The robustness of method was tested by assessing the effect of minor changes in the below conditions:

- Flow rate of mobile phase (± 0.1 ml/min)
- Column temperature (± 2°C)
- pH of mobile phase (± 0.2 units)
- Methanol ratio in the mobile phase (± 5%)
- Detection wavelength (± 2 nm)

In all above said changed conditions, system suitability parameters were measured. For this study, standard solution with concentration 15 µg/ml ertugliflozin and 100 µg/ml sitagliptin was analyzed. Results in Table 6 and 7 proved that the studied minor changes showed no significant effect in the system suitability parameters.

Table 6: Robustness data for sitagliptin

Condition studied	Investigated value	USP plate count	USP Tailing	USP resolution
Column temperature (oC)	23	4405	1.34	-
	27	4559	1.35	-
Flow rate (ml/min)	0.9	1.38	4336	-
	1.1	1.39	4090	-
pH of mobile phase (units)	3.3	5056	1.41	-
	3.7	5027	1.42	-
Methanol ratio in mobile phase (%)	40	4090	1.31	-
	30	4405	1.34	-
Detection wavelength (nm)	223	4906	1.41	-
	227	4903	1.40	-

Table 7: Robustness data for ertugliflozin

Condition studied	Investigated value	USP plate count	USP Tailing	USP resolution
Column temperature (oC)	23	5364	0.98	1073
	27	5722	0.97	11.10
Flow rate (ml/min)	0.9	3959	1.00	9.58
	1.1	5092	0.98	10.41
pH of mobile phase (units)	3.3	5621	0.98	9.15
	3.7	5603	0.99	9.10
Methanol ratio in mobile phase (%)	40	5092	0.98	10.41
	30	5364	0.98	10.73
Detection wavelength (nm)	223	5695	0.99	9.13
	227	5620	1.00	9.06

APPLICATION OF THE METHOD:

After developing and validating the method, the reliability of the method was assessed further by applying the proposed method to Steglujan® tablets. The percent recoveries were 99.60 ± 0.027 and 99.83 ± 0.017 for sitagliptin and ertugliflozin, respectively. These values indicated the accuracy and precision of the method for the analysis of sitagliptin and ertugliflozin in tablets and also showed that common excipients of tablets did not interfere.

Table 8: Analysis of sitagliptin and ertugliflozin in tablets

Drug	Claimed value (mg)	Assayed value (mg)	Recovered value (%)	Mean value (%)	RSD (%)
Sitagliptin	100	99.59	99.59	99.60	0.027
	100	99.58	99.58		
	100	99.63	99.63		

Ertugliflozin	15	14.98	99.84	99.83	0.017
	15	14.98	99.84		
	15	14.97	99.81		

CONCLUSION

Simple, fast and reliable RP-HPLC method was developed for the simultaneous quantification of sitagliptin and ertugliflozin in bulk and combined tablet dosage form. ICH guidelines are followed to validate method. The validation results showed a good performance regarding linearity, sensitivity, accuracy, precision, selectivity and robustness. The method was applied successfully for the quantification of selected drug combination in available tablet form. The common excipients there in tablet dosage form did not hinder with the analysis of sitagliptin and ertugliflozin. As a result, the proposed method can be utilized in regular quality control laboratories.

REFERENCES

1. Francesca C, Simona M, Flavia I, Chiara MAC, Vinsin AS, Gian PS, Teresa M, Andrea G. Spotlight on ertugliflozin and its potential in the treatment of type 2 diabetes: evidence to date. Drug Design, Development and Therapy, 2017, 11, 2905-2919.
2. Abdul-Ghani MA, DeFronzo RA: Inhibition of renal glucose reabsorption: a novel strategy for achieving glucose control in type 2 diabetes mellitus. Endocrinology Practice, 2008, 14 (6), 782-90.
3. Dendup T, Feng X, Clingan S, Astell-Burt T. Environmental risk factors for developing type 2 diabetes mellitus: A systematic review. International Journal of Environmental Research and Public Health, 2018, 15 (1). pii: ijerph15010078.
4. The medical letter on drugs and therapeutics. Ertugliflozin for Type 2 diabetes. JAMA, 2018, 319 (23), 2434-2435.
5. National Prescribing Service (August 2010). Sitagliptin for Type 2 Diabetes. Archived from the original on 18 July 2010. Retrieved 27 August 2010.
6. Amir Q, Michael JB, Linda LH, Mary AF. Oral pharmacologic treatment of type 2 diabetes mellitus: A clinical practice guideline update from the American college of physicians. Annals of Internal Medicine, 2017, 166, 279-290.
7. Karasik A, Aschner P, Katzeff H, Davies MJ, Stein PP: Sitagliptin, a DPP-4 inhibitor for the treatment of patients with type 2 diabetes: a review of recent clinical trials. Current Medical Research and Opinion, 2008, 24 (2), 489-496.
8. Gallwitz B. Review of sitagliptin phosphate: a novel treatment for type 2 diabetes. Vascular Health and Risk Management, 2007, 3 (2), 203-210.
9. Adrian V. Mechanism of Action of DPP-4 Inhibitors—New Insights. The Journal of Clinical Endocrinology & Metabolism, 2012, 97 (8), 2626–2628.
10. Informed Health Online. Fixed-dose combination of ertugliflozin and sitagliptin (Steglujan) for the treatment of type 2 diabetes: Overview. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG), 2018. Available at https://www.ncbi.nlm.nih.gov/books/NBK525775/
11. Steglujan- ertugliflozin and sitagliptin tablet, film coated. 2018. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=c2c553d8-5a9d-4366-bf53-8bdb5a876d19
12. Miller S, Krumins T, Zhou H. Ertugliflozin and Sitagliptin Co-initiation in Patients with Type 2 Diabetes: The VERTIS SITA Randomized Study. Diabetes Therapy, 2018, 9 (1), 253-268.
13. Pratley RE, Eldor R, Raji A, Golm G, Huyck SB, Qiu Y, Sunga S, Johnson J, Terra SG, Mancuso JP, Engel SS, Lauring B. Ertugliflozin plus sitagliptin versus either individual agent over 52 weeks in patients with type 2 diabetes mellitus inadequately controlled with metformin: The VERTIS FACTORIAL randomized trial. Diabetes, Obesity and Metabolism, 2018, 20 (5), 1111-1120.
14. Q2 (R1) Validation of analytical procedures, Proceedings of the International Conference on Harmonisation (ICH), Geneva, 2005.