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Development and validation of stability-indicating RP-HPLC method for the simultaneous determination of ertugliflozin pidolate and metformin hydrochloride in bulk and tablets

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Abstract

Background: In the present study, an improved simple, specific, rapid, sensitive, precise, accurate and stability-indicating RP-HPLC method for the simultaneous estimation of ertugliflozin pidolate and metformin hydrochloride in bulk and tablets was developed and validated. The separation of ertugliflozin pidolate and metformin HCl was achieved isocratically on Kromasil C18 column (150 mm × 4.6 mm, 5 μm) using 0.1% *ortho*-phosphoric acid buffer (pH 2.7):acetonitrile (65:35% *v/v*) as mobile phase, pumped at a flow rate of 1 ml/min and column temperature of 30 ± 2 °C. HPLC grade water:ACN (1:1) was used as diluent. About 10 μl of standard solution of the drugs was injected, and the eluted analytes were detected at 224 nm.

Results: Metformin HCl was eluted at 2.170 min and ertugliflozin pidolate at 2.929 min with a run time of 5.0 min. Linearity of the developed method was observed in the concentration range of 0.9375–5.625 μg/ml for ertugliflozin pidolate and 62.5–375 μg/ml for metformin HCl with a correlation coefficient of 0.999 for both the drugs. LOD for ertugliflozin pidolate and metformin HCl were 0.025 μg/ml and 0.87 μg/ml respectively. LOQ for ertugliflozin pidolate and metformin HCl were 0.076 μg/ml and 2.63 μg/ml.

Conclusion: The developed RP-HPLC method for the simultaneous estimation of ertugliflozin pidolate and metformin HCl in bulk and tablets was simple, rapid, sensitive, accurate, precise, linear, and stability indicating. Hence, the developed method could be used for the routine quality control of the drugs in bulk and tablets.

Keywords: Stability-indicating RP-HPLC, Ertugliflozin pidolate, Metformin HCl, Kromasil C18 column

Background

Globally, about 463 million people are suffering from type-2 diabetes mellitus [1] which is manifested by polydipsia, polyuria, and polyphagia and requires a lifetime treatment with antidiabetic drugs [2]. The treatment goals involve the achievement of glycemic control and reducing the diabetes-associated cardiovascular risk. Patients suffering from recent onset of diabetes are treated with metformin, an insulin sensitizer. The risk of hypoglycemia is insignificant with metformin and drug interactions are less making it a highly safe and acceptable first-line of drug for the treatment of early type-2 diabetes mellitus [3].

The pathogenesis of type-2 diabetes mellitus is multiplex, involving several organs, and treatments using a combination of drugs with different mechanisms of action effectively controls the plasma glucose levels [4]. Sodium glucose co-transporter type-2 (SGLT-2) inhibitors

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are the new option for the treatment of type-2 diabetes mellitus. These agents act by inhibiting SGLT-2 transporter in the kidneys, thereby promoting the excretion of glucose in urine and reducing the plasma glucose levels [5]. Ertugliflozin pidolate, a SGLT-2 inhibitor, is used in the treatment of Type-2 diabetes mellitus with a pKa of 11.98 and log P of 2.21. The drug is soluble in ethanol, sparingly soluble in ethyl acetate and acetonitrile, and very slightly soluble in water [6, 7]. Metformin hydrochloride is a biguanide anti-diabetic with a pKa of 12.33 and log P of − 0.92. It is freely soluble in water, sparingly soluble in alcohol, and practically insoluble in acetone and dichloromethane [6, 8]. The structures of ertugliflozin pidolate and metformin HCl are shown in Figs. 1 and 2.

Ertugliflozin pidolate and metformin hydrochloride in combination efficiently reduce the elevated HbA_{1c} levels in type-2 diabetic patients [6]. A simultaneous method development deals with the analysis of a combination of drugs and is useful for the analysis of two or more drugs of a formulation without their separation. The method is rapid and cost-effective as the reagents used are common for the analysis of the drugs [9]. Extensive literature study revealed that two methods were reported for the simultaneous estimation of both the selected drugs in bulk and formulations by RP-HPLC [10, 11]. In the present study, an improved stability-indicating RP-HPLC method for the simultaneous estimation of ertugliflozin pidolate (ERTU) and metformin HCl (MET) in bulk and tablets was designed to develop and validate.

Methods

Reference standard of ertugliflozin pidolate was obtained as gift sample from Ajanta Pharma, Mumbai and metformin HCl from Laurus Labs, Hyderabad. Water, methanol, acetonitrile of HPLC grade, and ortho phosphoric acid of analytical reagent grade from Merck, Mumbai were used for the present study. The commercially available ertugliflozin pidolate and metformin HCl tablet formulation Segluromet (Merck Sharp & Dohme Corp) with a label claim of ERTU 7.5 mg, MET 500 mg was used.

Instrumentation

Waters HPLC (Separation module 2965) chromatographic system equipped with PDA-detector 2487 with Empower-2 software, Shimadzu digital weighing balance (ATX 224), Bio-Technics ultra-Sonicator (BTI-48), Elico pH meter (LI 120), and Millipore vacuum filter pump (XI 5522050) were used for the development of the method. A 0.22-μm Nylon filter and a 0.45-μm Polytetrafluoroethylene (PTFE) filter of Merck Millipore were used for filtration.

Chromatographic conditions

The separation of ertugliflozin pidolate and metformin HCl was achieved using Kromasil C18 column (150 mm × 4.6 mm, 5 μm), 0.1% *ortho*-phosphoric acid buffer (pH 2.7):acetonitrile (65:35%, *v*/*v*), flow rate of 1 ml/min, an injection volume of 10 μl, at a λ max of 224 nm, and column temperature 30 ± 2 °C using HPLC grade water: ACN (1:1) as diluent.

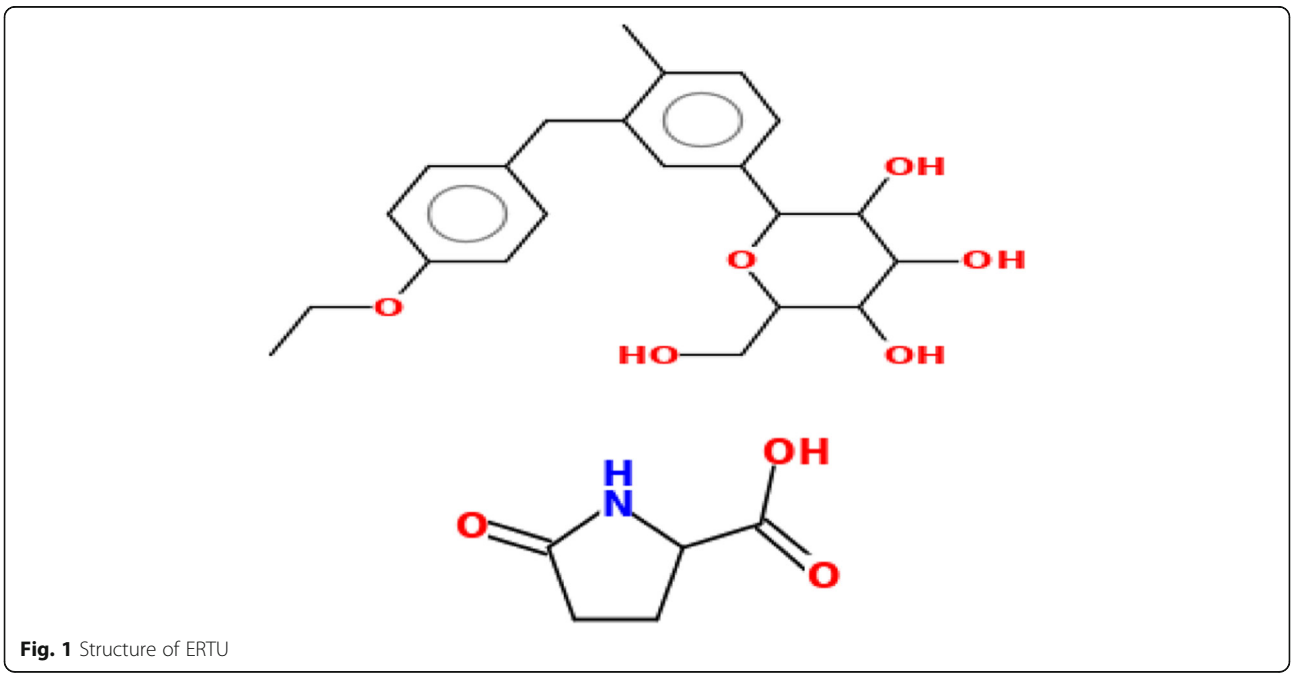

Fig. 1 Structure of ERTU

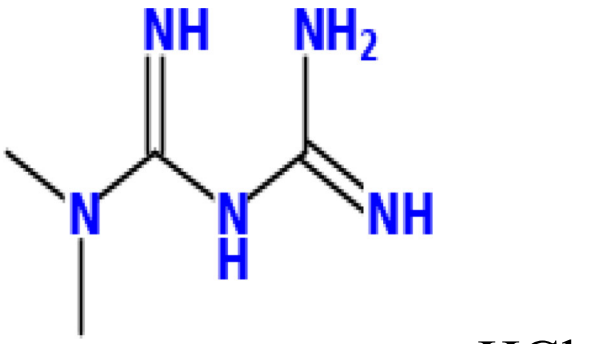

Fig. 2 Structure of MET

Preparation of 0.1% *ortho*-phosphoric acid buffer (pH 2.7)

About 1 ml of *ortho*-phosphoric acid was diluted to 1 l with HPLC grade water in a 1-L volumetric flask. The solution was degassed by ultra-sonicating for 5 min, and the resultant solution was filtered through a 0.45-μm PTFE filter. The pH of the prepared buffer was checked using a pH meter.

Preparation of standard solution of ERTU and MET

Standard stock solution was prepared by accurately weighing and transferring 3.75 mg of ERTU and 250 mg of MET bulk drugs into a 100-ml volumetric flask. About 10 ml of the diluent was added, and the solution was ultra-sonicated for 10 min to dissolve the drugs completely. The final volume was made up with HPLC grade water:ACN (1:1), and the solution was filtered through a 0.45-μm PTFE filter (ERTU (37.5 μg/ml) and MET (2500 μg/ml)).

Accurately, 1 ml of standard stock solution was transferred into a 10-ml volumetric flask, and the volume was made up with the diluent to prepare working standard solution (ERTU (3.75 μg/ml) and MET (250 μg/ml)).

Preparation of sample solution of ERTU and MET

About 20 tablets of Segluromet were weighed and powdered finely. Tablet powder equivalent to 3.75 mg of ERTU and 250 mg of MET was weighed and transferred into a 100-ml volumetric flask and dissolved completely by sonicating for 25 min using10 ml of the diluent. The final volume was made up with the diluent and filtered through 0.45-μm PTFE filter (Stock solution: ERTU (37.5 μg/ml) and MET (2500 μg/ml)).

Accurately 1 ml of sample stock solution was diluted to 10 ml in a 10-ml volumetric flask with the diluent (Working sample solution: ERTU (3.75 μg/ml) and MET (250 μg/ml)).

Validation [12]

The developed RP-HPLC method was validated as per ICH guidelines. The parameters validated are Specificity, Forced degradation studies, Accuracy, Precision (Intra-day precision, Inter-day precision), Linearity, Limit of Detection (LOD), Limit of quantitation (LOQ), Solution stability, Robustness, and System suitability parameters.

Specificity

Specificity of the developed RP-HPLC method was established by injecting 10 μl each of the blank, working standard, and sample solutions.

Forced degradation studies [13–15]

The stability of the developed method was established by performing forced degradation studies of the drug in the presence of acid, alkali, H_2O_2, temperature, UV light, and HPLC grade water.

Acid degradation

Degradation under acidic condition was evaluated by treating 1 ml of standard stock solution of ERTU and MET with 1 ml of 2N HCl and refluxed for 30 min at 60 ± 2 °C. The resulting solution was diluted to 10 ml with the diluent.

Alkali degradation

Under alkaline conditions, degradation was studied by refluxing 1 ml of standard stock solution of ERTU and MET with 1 ml of 2N NaOH for 30 min at 60 ± 2 °C. The stressed solution was made up to 10 ml with the diluent.

Oxidative degradation

About 1 ml of standard stock solution of ERTU and MET was subjected to oxidative degradation by refluxing with 20% *v*/*v* H_2O_2 in a 10-ml volumetric flask for 30 min at 60 ± 2 °C and made up with the diluent.

Thermal degradation

Thermal stability of the drugs was evaluated by placing the standard stock solution in the oven at 105 ± 2 °C for 6 h. About 1 ml of the stressed solution was diluted to 10 ml with the diluent.

Photolytic degradation

Photolytic degradation was studied by exposing the standard solution of ERTU and MET to UV light in the UV chamber for 7 days. The resulting stressed solution was diluted to 10 ml with the diluent.

Neutral degradation

Neutral degradation was carried out by refluxing 1 ml of standard stock solution with 1 ml of HPLC grade water

Table 1 Preliminary trial runs

Trial	Column	Mobile phase	Observation
1	BDS C18 (250 mm × 4.6 mm, 5 μm)	Methanol: 0.1 % OPA (40:60)	Only MET was eluted with less theoretical plates
2	BDS C18 (250 mm × 4.6 mm, 5 μm)	ACN: KH_2PO_4 (40:60)	Only MET was eluted
3	Agilent C18 (250 mm × 4.6 mm, 5 μm)	ACN: 0.1 % OPA (60:40)	Peak symmetry of both the peaks was good but with increased retention time
4	Kromasil C18 (150 mm × 4.6 mm, 5 μm)	ACN: 0.1 % OPA (50:50)	Peak symmetry was good but MET eluted at 1.6 min
5	Kromasil C18 (150 mm × 4.6 mm, 5 μm)	ACN: 0.1 % OPA (65:35)	Peak symmetry was good with system suitability parameters in limits

in a 10-ml volumetric flask at 60 ± 2 °C for 6 h. The volume was made up with the diluent.

About 10 μl of each of the solutions exposed to different stress conditions were injected separately into the column, and the chromatograms were recorded to evaluate the stability of the drugs.

Accuracy

Accuracy was established by injecting about 10 μl of ERTU and MET at 80, 100, and 120% levels into the column, and the procedure was repeated thrice. Mean percent recovery of three levels was determined using the peak areas at each level.

Precision

Precision of the optimized method was determined by injecting six samples of working standard solution of ERTU and MET into the column on the same day for intra-day precision and on two continuous days for inter-day precision; % RSD was calculated.

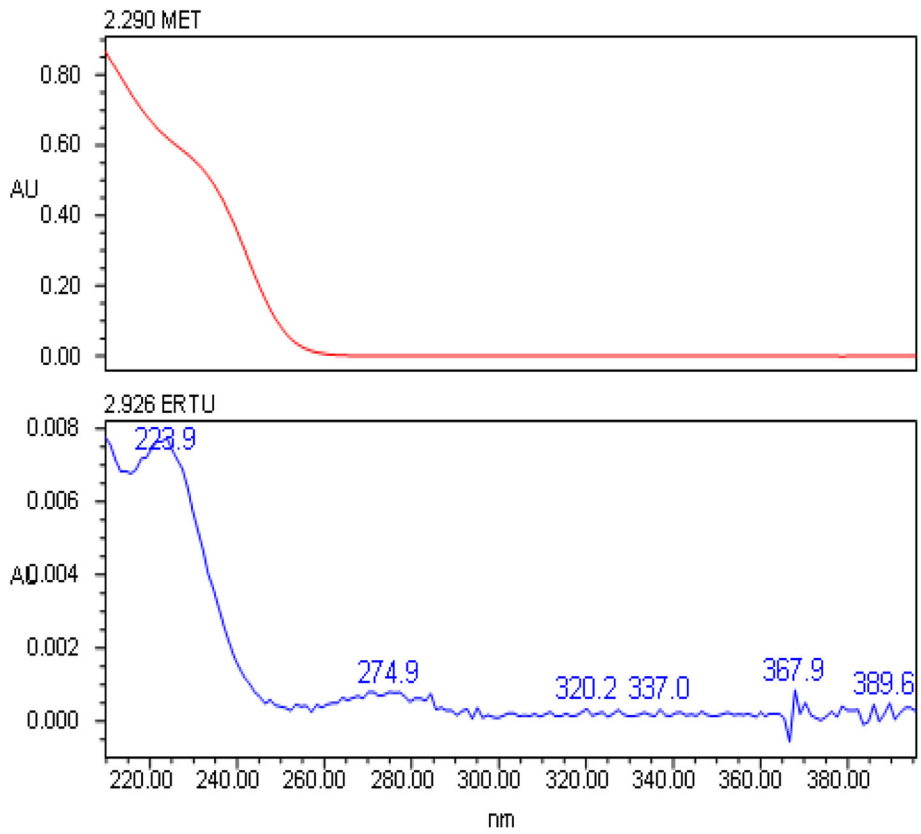

Fig. 3 UV spectrum of ERTU and MET

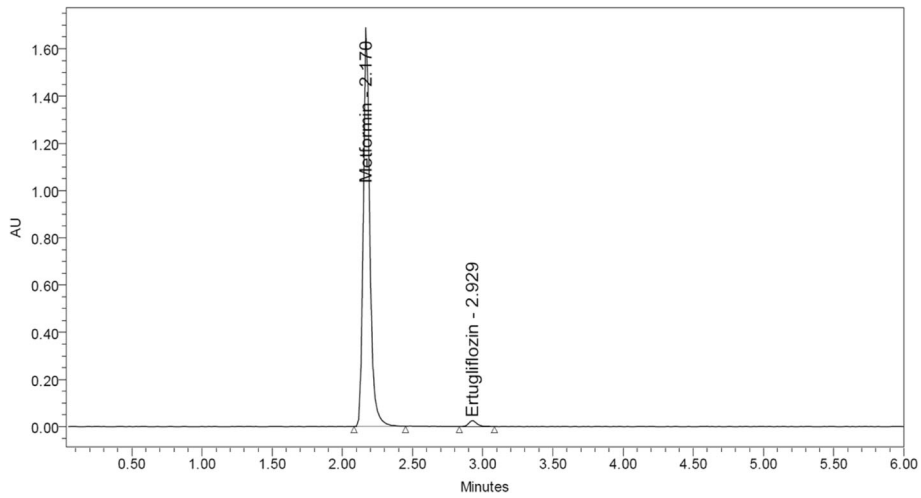

Fig. 4 Optimized chromatogram of ERTU and MET

Linearity

Linearity of the developed method was evaluated by injecting ERTU in the concentration range of 0.9375–5.625 μg/ml and MET in the range of 62.5–375 μg/ml. Calibration curve was constructed by plotting peak area on the *y*-axis against concentration (μg/ml) on the *x*-axis. The correlation coefficient of the calibration curve was calculated by using the method of least squares in MS Office Excel 2007.

LOD and LOQ

LOD and LOQ were calculated using the formulae based on the standard deviation of the *y*-intercept of regression lines and the slope of the calibration curve.

$$\mathbf{LOD}=\mathbf{3.3}\,X\,\frac{\sigma}{S} \qquad \mathbf{LOQ}=\mathbf{10}\,X\,\frac{\sigma}{S}$$

where σ is the standard deviation and S is the slope of the curve.

Stability of standard solution of ERTU and MET

Stability of the standard solution of ERTU and MET was conducted by placing the solution in a volumetric flask at 30 ± 2 °C for 24 h. At three time points 0, 12, and 24 h, about 10 μl of the stored solution was injected into the column to calculate the percent (%) assay difference of the drug.

Robustness

Robustness of the developed RP-HPLC method was evaluated by making minor changes in the flow rate (0.9 to 1.1 ml/min), percentage of acetonitrile in the mobile phase (30 to 40%) and temperature (25 to 35 °C). The parameters evaluated were % RSD of peak areas, theoretical plates, tailing factor, and resolution.

System suitability testing

Suitability of the system was evaluated by injecting working standard solution into the column to evaluate parameters like % RSD of peak areas, theoretical plates, tailing factor, and resolution in the optimized chromatographic conditions.

Table 2 Forced Degradation of ERTU and MET

Stressor	ERTU				MET			
	Purity angle	Purity threshold	% Assay	% Degraded	Purity angle	Purity threshold	% Assay	% Degraded
Acid	1.493	1.966	92.45	7.55	1.448	1.801	92.29	7.71
Alkali	1.766	2.146	94.56	5.44	1.402	1.752	93.07	6.93
Oxidative	1.693	2.130	95.42	4.58	1.348	2.270	94.47	5.53
Thermal	1.936	2.341	96.86	3.14	2.753	2.927	97.07	2.93
Photolytic	1.659	2.009	98.30	1.70	2.489	2.786	98.21	1.79
Neutral	1.780	2.134	98.98	1.02	2.358	2.915	99.10	0.90

Table 3 Accuracy (% recovery) of ERTU and MET

ERTU					MET			
% Spiked level	Fixed sample concentration (μg/ml)	Amount Spiked (μg/ml)	Statistical Analysis		Fixed sample concentration (μg/ml)	Amount Spiked (μg/ml)	Statistical Analysis	
			Mean % Recovery ± SD	% RSD			Mean % Recovery ± SD	% RSD
80	3.75	3.0	100.04 ± 0.306	0.306	250	200	99.7 ± 0.54	0.54
100	3.75	3.75	99.99 ± 0.135	0.135	250	250	99.91 ± 0.57	0.57
120	3.75	4.50	99.87 ± 0.64	0.604	250	300	100.65 ± 0.47	0.47

Assay

The working sample solution was injected six times into the column, and % assay was calculated by using the formula:

$$\%\text{Assay} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Dilution of standard}}{\text{Dilution of sample}} \times \frac{P}{100} \times \frac{\text{Avg.wt}}{\text{LC}} \times 100$$

where Avg. wt is the average weight of tablets, P is the percentage purity of working standard, and LC is the label claim of the drugs

Results

Based on the solubility studies, the diluent selected was HPLC grade water:ACN (1:1), ERTU being sparingly soluble in water and MET freely soluble in water.

Method optimization

Method was optimized by trial and error method to obtain a chromatogram with good resolution, acceptable number of theoretical plates, and tailing factor. To optimize the method, preliminary trial runs were performed by changing mobile phase and column type (Table 1). Buffer selected for the present analysis was 0.1% *ortho*-phosphoric acid, as ERTU (566 Da) and MET (165.62 Da) being regular samples with basic nature requiring the control of pH by the addition of buffer in the mobile phase. The pH of the buffer should be ± 1.5 U of the pKa value to retain the drug in single state which avoids peak splitting [16]. pH of the buffer selected was 2.7 as the pKa of ERTU was 11.98 and MET was 12.33 to retain both the selected drugs in completely ionized state in order to avoid peak splitting.

PDA detector has an advantage of simultaneously collecting the chromatograms in the entire UV range during a single run [16]. When the drug samples were scanned between 200 and 400 nm, the ideal λ max was found to be 224 nm. UV spectrum is shown in Fig. 3. C18 columns are rugged, highly retentive, and widely available [16]. Optimal separation and peak shapes were obtained on Kromasil C18 column with dimensions of 150 mm × 4.6 mm, 5 μm indicating that it was suitable for the simultaneous estimation of ERTU and MET. Theoretical plates are an important characteristic of the column which indicates the ability of the column to produce sharp, narrow peaks for achieving good resolution. Under optimized test conditions, a column with a length of 150 mm and a particle diameter of 5 μm produces theoretical plates of 10,000–12,000 [16]. In the optimized trial, the observed system suitability parameters were theoretical plates (11,025 (MET), 11,261 (ERTU)), tailing factor (1.2 (MET), 1.1 (ERTU)) and resolution of 7.7 for both the drugs reflecting that the selected column was ideal for the estimation of the drugs.

In the trial runs, for the ideal separation of the selected drugs, mixtures of solvents like methanol and acetonitrile with or without buffers (0.1% *ortho*-phosphoric acid and KH_2PO_4) in different proportions were tried on C18 column. In the optimized method, the mobile phase selected was 0.1% *ortho*-phosphoric acid (pH 2.7):acetonitrile (65:35% *v*/*v*), as the resolution and peak shape of ERTU and MET were good with optimum system suitability parameters. The flow rate was optimized based on the peak resolution and minimal consumption of the mobile phase, and the flow rate selected was 1.0 ml/min. MET and ERTU were eluted at 2.170 min and 2.929 min confirming that MET (log P – 0.92) was more polar over

Table 4 Linearity of ERTU

Concentration [*x*] (μg/ml)	Mean peak area* [*y*] (AU)
0	0
0.9375	24,802
1.875	49,541
2.8125	74,657
3.75	99,130
4.6875	125,714
5.625	145,645
Linear regression equation ($y = mx + c$)	$y = 26{,}223x + 460.9$
Slope (m)	26,223
Intercept (c)	460.9
Correlation coefficient (R^2)	0.999

*Average of three determinations

Table 5 Linearity of MET

Concentration [*x*] (μg/ml)	Mean peak area* [*y*] (AU)
0	0
62.5	1,374,565
125	2,780,573
187.5	4,158,469
250	5,566,797
312.5	6,877,296
375	8,152,632
Linear regression equation ($y = mx + c$)	$y = 21{,}857x + 31{,}878$
Slope (*m*)	21,857
Intercept (*c*)	31,878
Correlation coefficient (R^2)	0.999

*Average of three determinations

ERTU (log *P* 2.21). Optimized chromatogram is shown in Fig. 4.

The developed method was validated as per ICH guidelines. Good association was observed between the retention times of the standard and sample peaks confirming the drugs in the tablet were ERTU and MET. Additional peaks were absent in the chromatograms indicating no interference of excipients with the drugs at the retention times, and the developed method was specific for the simultaneous estimation of the drugs in tablet formulation.

The important part of validation of a stability-indicating method is to assess the presence of impurities under the main analyte peak. The analyte peak should be checked for its purity/homogeneity, which is usually evaluated by determining the purity angle and purity threshold. According to the ICH Q2 (R1) guidelines, in forced degradation studies, purity threshold should be greater than purity angle and % degradation should be less than 20 to consider the method as stable [12]. The developed method was specific and stable for the simultaneous estimation of the drugs. The % degradation of ERTU and MET were 1.02–7.55 and 0.90–7.71, respectively. The co-elution of degradants with the drugs was absent. The purity threshold for both the drugs (1.966–2.341 (ERTU), 1.752–2.927 (MET)) was found to be greater than the purity angle (1.493–1.936 (ERTU), 1.348–2.753 (MET)) in the presence of 2N HCl, 2N NaOH, 20 % *v*/*v* H_2O_2 at 60 ± 2 °C for 30 min, in the oven at 105 ± 2 °C for 6 h, in the UV chamber for 7 days and in the presence of HPLC grade water at 60 ± 2 °C for 30 min.

In forced degradations studies, purity threshold was found to be greater than purity angle for both the drugs inferring the absence of co-elution of degradants with the drugs, and the analyte peaks were pure. Percent degradation of less than 10 for both the drugs demonstrates that the developed method was specific and stable (Table 2). Accuracy is presented in terms of % recovery and should be 98–102 [12]. Accuracy was demonstrated at 80, 100, and 120% of the target concentration, and the percent recovery for ERTU was 99.27–100.60, and MET was 99.11–101.13 (Table 3) reflecting that the developed method was accurate. The % RSD is used to express the precision of the method, and ICH-stated limits are that % RSD should be not more than 2.0 for intra-day precision and inter-day precision [12]. The % RSD of intra-day precision was 0.25 and 0.42 (Table 7) for ERTU and MET. % RSD of inter-day precision was 0.70 and 0.56 (Table 7) for ERTU and MET implying that the deviation was less among repeated results and the developed method was more precise.

The linearity is expressed in terms of correlation coefficient (R^2) and should be not less than (NLT) 0.999 [12]. The developed method was found to be linear in the concentration range of 0.9375–5.625 μg/ml for ERTU (Table 4) and 62.5–375 μg/ml for MET (Table 5)

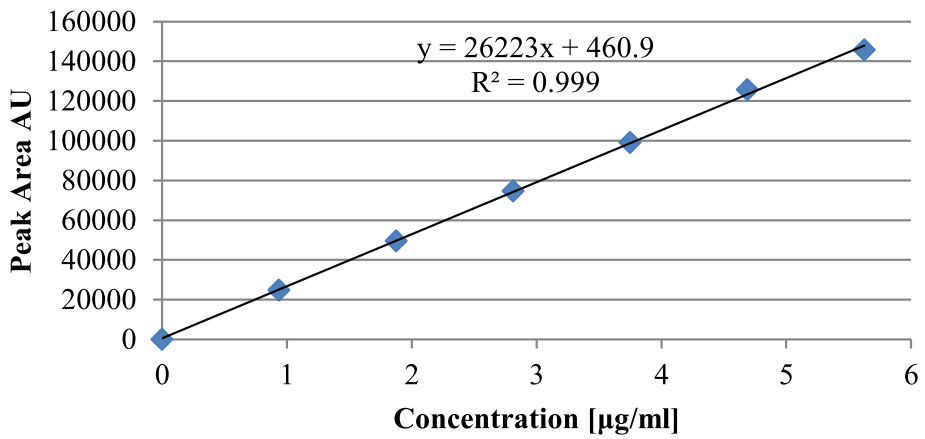

Fig. 5 Calibration curve of ERTU

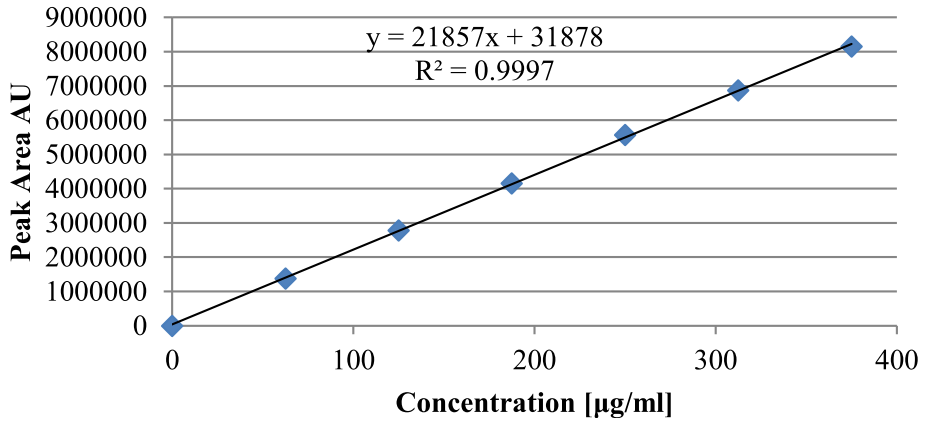

Fig. 6 Calibration curve of MET

with a correlation coefficient of 0.999 for both the drugs indicating the linear relationship between the peak area and concentration of the drugs (Figs. 5 and 6). LOD for ERTU and MET were 0.025 µg/ml and 0.87 µg/ml (Table 7) respectively indicating that the developed method was suitable for the estimation of 0.1 µg of the drugs. LOQ for ERTU and MET were 0.076 µg/ml and 2.63 µg/ml (Table 7). The stability of standard solution is expressed in terms of % assay difference, and it should be no more than (NMT) 2.0 [12]. In the present study, % assay difference was 0.76 for 12 h and 1.74 for 24 h for ERTU and was 0.83 and 1.51 for MET (Table 7). The % assay difference of ERTU and MET reflect that the solution was stable for 24 h at 30 ± 2 °C.

System suitability parameters are used for expressing the robustness of the method. To consider the method is robust, % RSD should be less than 2.0, theoretical plates should be more than 2000, tailing factor should be less than 2.0, and resolution should be more than 2.0 [12]. The system suitability parameters observed were % RSD 0.531–1.377 (ERTU), 0.382–1.293 (MET), theoretical plates 11,564–13,396 (ERTU), 9463–11,751 (MET), tailing factor 1.14–1.26 (ERTU), 1.14–1.33 (MET), and resolution of 6.78–9.2 for ERTU and MET reflecting that the developed method was robust for the simultaneous determination of ERTU and MET (Table 6). In the present study, the % RSDs 0.531 (ERTU) and 0.382 (MET), theoretical plates 11,679 (ERTU) and 9947 (MET), tailing factors 1.23 (ERTU) and 1.25 (MET), and resolution of 7.21 for ERTU and MET indicate that the system was suitable for the simultaneous analysis of ERTU and MET (Table 7). On the application of the developed method to tablets, the mean amount of ERTU and MET present in the tablets were found to be 7.45 ± 0.16 mg and 497.4 ± 0.97 mg against the labeled claim of 7.5 mg (ERTU) and 500 mg (MET), reflecting that the method was suitable for the simultaneous estimation of ERTU and MET in tablet formulation (Table 7).

Discussion

The stability-indicating RP-HPLC method is an analytical procedure that is capable of discriminating between the major active pharmaceutical ingredients from any degradation/decomposition products formed under

Table 6 Robustness of ERTU and MET

Parameter	Modified condition	% RSD of peak area (NMT 2.0 %)		Theoretical plates* *N* (> 2000)		Tailing factor* (< 2.0)		Rs* (> 2.0)
		ERTU	MET	ERTU	MET	ERTU	MET	
Flow rate (ml/min)	0.9	0.893	1.209	12,959	10,911	1.17	1.17	7.96
	1.0	0.531	0.382	11,679	9947	1.23	1.33	7.21
	1.1	0.949	1.113	11,564	9463	1.21	1.16	7.63
ACN ratio in mobile phase Buffer: ACN (% *v/v*)	70:30	1.377	1.039	11,949	11,751	1.26	1.15	9.2
	65:35	0.531	0.382	11,679	9947	1.23	1.33	7.21
	60:40	1.360	1.293	12,741	9485	1.22	1.14	6.78
Temperature (°C)	25	0.912	1.278	13,396	9470	1.14	1.20	7.03
	30	0.531	0.382	11,679	9947	1.23	1.33	7.21
	35	0.574	0.993	13,396	9516	1.21	1.17	7.01

* All the values were expressed as mean of six determinations

Table 7 Summary of validation parameters of ERTU and MET

Parameter	Results		ICH limits
	MET	ERTU	
Retention time (min)	2.170	2.929	-
System suitability parameters			
% RSD	0.382	0.531	NLT 2.0
Theoretical plates	9947	11679	MT 2000
Tailing factor	1.25	1.23	NMT 2.0
Range (μg/ml)	62.5–375	0.9375–5.625	-
Linearity (R^2)	0.999	0.999	NLT 0.999
% Recovery	99.11–101.13	99.27–100.60	98–102
% RSD			
Intra-day precision	0.42	0.25	NMT 1.0
Inter-day precision	0.56	0.70	NMT 2.0
LOD (μg/ml)	0.87	0.025	-
LOQ (μg/ml)	2.63	0.076	-
% Assay difference at 24 h	1.51	1.74	NMT 2.0
% Assay	99.48	99.31	-

defined storage conditions. Stability-indicating assay method development studies the effect of stressors on a drug which helps in understanding the stability of the drug during storage conditions and analysis. Few methods were reported for the simultaneous estimation of ERTU and MET by RP-HPLC. In the present method, MET and ERTU were eluted at 2.170 and 2.929 min with a resolution of 7.21. The present method was developed using 0.1% *ortho*-phosphoric acid (pH 2.7): ACN as the mobile phase. The developed method was found to be sensitive and cost-effective with the reduced ratio of organic solvent in the mobile phase.

Conclusion

Improved stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of ertugliflozin and metformin. The present method was developed using 0.1% *ortho*-phosphoric acid (pH 2.7): acetonitrile as the mobile phase. The method was found to be sensitive and cost-effective with the reduced ratio of organic solvent in the mobile phase, decrease in linearity range, LOD and LOQ, and retention times compared to the best method reported. The developed method could be suitable for routine analysis of the drugs in bulk and tablet formulation.

Abbreviations

ERTU: Ertugliflozin pidolate; MET: Metformin hydrochloride; PTFE: Polytetrafluoroethylene; LOD: Limit of detection; LOQ: Limit of quantitation; % RSD: Relative standard deviation; ACN: Acetonitrile; NMT: Not more than; NLT: Not less than; min: Minutes; MT: More than; ICH: International Conference on Harmonization; Rs: Resolution; SD: Standard deviation; SGT-2: Sodium glucose co-transporter type-2; °C: Degree Celsius; μg: Microgram; ml: Milliliters; mg: Milligrams; %: Percentage; *v/v*: Volume/volume; RP: Reverse phase; HPLC: High-performance liquid chromatography

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Authors' contributions

The authors have read and approved the manuscript. SB and SK designed the study. SK performed the experiment and analyzed and reviewed the data. SB supervised the experiment, reviewed the data, and supported for writing the manuscript.

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