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Development and Validation of New Analytical RP-HPLC Method for the Estimation of Antidiabetic Drugs Metformin Hydrochloride and Ertugliflozin in Combined Pharmaceutical Dosage Form

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ABSTRACT: Metformin Hydrochloride and Ertugliflozin is combination of Antidiabetic drug in tablet Segluromet ® Tablet (500/7.5 mg), a member Antidiabetic drug, is a recent drug developed by Merck Sharp & Dohme Company for the treatment of Type 2 diabetes. A new sensitive and rapid HPLC method was developed for the determination of Metformin Hydrochloride and Ertugliflozin in pharmaceutical dosage forms; it was validated according to International Conference on Harmonization and Food and Drug Administration guidelines. The analysis was performed on the HPLC system equipped with a using Kromasil C18 (5 µm), (250 mm x 4.6 mm column, with of Buffer (0.1 % v/v Phosphoric acid in water) and Acetonitrile(ACN) in the ratio 60 : 40 v/v with at a flow rate of 1 mL/min , column temperature 25°C , total run time was 20 min, injection volume 20 µl, and detection was performed at the a wavelength (λ) of 235 nm. the calibration plot gave linear relationship over the concentration range of Metformin Hydrochloride 350, 450, 500, 550 and 600 µg/ml, and Ertugliflozin 6.80, 8.74, 9.72, 10.69 and 11.66 µg/ml, respectively. The accuracy of the proposed method was determined by recovery studies and was found to be Metformin Hydrochloride 98.5 % to 101.3 % and Ertugliflozin 98.2 % to 100.3%. The Limit of Detection were 0.0003 and 0.0009 µg/ml for Metformin Hydrochloride and Ertugliflozin, respectively and the Limit of Quantitation were 0.0037 and 0.0112 µg/ml for Metformin Hydrochloride and Ertugliflozin, respectively. % Relative Standard Deviation of the determination of precision was <2%. The results of robustness and solutions stability studies were within the acceptable limits as well the main features of the developed method are low run time and retention time of around 1.8 min for Metformin Hydrochloride (Met) and 3.8 min for Ertugliflozin (Ertu).

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INTRODUCTION

Drug Profile

Metformin Hydrochloride: Metformin, chemically 1-carbamimidamido-N, N dimethyl methanimidamide (**Figure 1**) is a biguanide antihyperglycemic agent used for treating non-insulin dependent diabetes mellitus (NIDDM). It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Molecular formula: $C_4H_{11}N_5$-HCl., Molecular Weight: 165.63 g/mol [1-3].

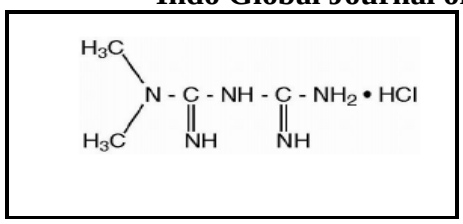

Figure. 1 Metformin Hydrochloride

Ertugliflozin: Ertugliflozin is an oral, selective inhibitor of sodium glucose co-transporter-2 (SGLT2) which inhibits renal glucose reabsorption and results in urinary glucose excretion (UGE) and reductions in plasma glucose and haemoglobin A1c (Estimated Blood Sugar) in patients with type 2 diabetes mellitus (T2DM). It possesses a high selectivity for SGLT2 versus SGLT1 and other glucose transporters (GLUT1-4).

Ertugliflozin is a new chemical entity with a chemical name of (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 2**). Ertugliflozin is included in the drug product as a co crystal with L-pyroglutamic acid (L-PGA), known as Ertugliflozin L-PGA. Molecular formula: $C_{27}H_{32}ClNO_{10}$. Ertugliflozin L-PGA Relative molecular mass: 566.00 g/mol. Ertugliflozin molecular mass: 436.9 g/mol [1-3].

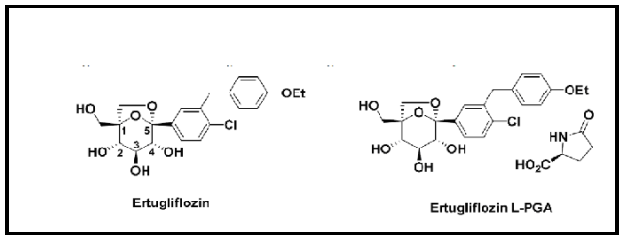

Figure 2. Ertugliflozin and Ertugliflozin L-PGA

Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the International conference harmonization guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [4–9].

Literature survey revealed a few methods reported for determination for Ertugliflozin and Metformin in Pharmaceutical preparation [14-21].

In this research, a new sensitive and rapid HPLC method was developed for the determination of Ertugliflozin and Metformin Hydrochloride in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

MATERIALS AND METHODS

Instrumentation

Chromatographic HPLC system equipped with a Kromasil C18 (5 µm), 250 mm x 4.6 mm column.

Chemicals and Reagents

Acetonitrile, Phosphoric acid, Methanol, Water were of HPLC Grade.

Chromatographic Conditions

Mobile Phase (Buffer (0.1 % v/v Phosphoric acid in water) and Acetonitrile (ACN) in the ratio 60: 40 v/v with a flow rate of 1 mL/min. the detection was performed at the wavelength (λ) of 235 nm, injection volume 20 µl, run time 20 min, and column temperature 25°C Diluent –HPLC Grade water.

Preparation of Standard Solution

Weigh accurately and transfer about 9.72 mg of Ertugliflozin L-PGA (Equivalent to 7.5 mg Ertugliflozin) and 500 mg of Metformin Hydrochloride standard into 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve, cool. Dilute to volume with diluent and mix. Transfer 5 ml of this solution to a 50 ml volumetric flask and dilute with diluent to volume and mix well.

Preparation of Sample Solution

Weigh accurately and transfer Approx. 595 mg of synthetic mixture Equivalent to 9.72 mg of Ertugliflozin L-PGA (Equivalent to 7.5 mg Ertugliflozin) and 500 mg of Metformin Hydrochloride standard into 100 ml volumetric flask, add 50 ml of diluent and sonicate for 15 min with intermittent shaking. Dilute to volume with diluent and mix. Filter a portion of this solution using 0.45 µ PVDF Syringe filter, transfer 5 ml of this solution to a 50 ml volumetric flask and dilute with diluent to volume and mix well.

Method Validation

The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD) robustness, and solution stability [5-7].

a) **Specificity:** Specificity is one of the significant features of HPLC, and it refers to the ability of the analytical method to discriminate between the analyte and the other components in the complex mixture [7]. Specificity of the method was evaluated by injecting 20 µL solutions of standard, sample, blank, and placebo separately.
b) **Linearity:** To evaluate the linearity and range of the method, Direct standard solutions were prepared by diluting the standard stock solution with the diluent in different concentrations Metformin Hydrochloride: 350, 450, 500, 550 and 600 µg/ml, and Ertugliflozin 6.80, 8.74, 9.72, 10.69 and 11.66 µg/ml which cover 70%, 90%, 100%, 110% and 120%, of the target concentration, respectively. Three replicate injections from each concentration were analysed under the same conditions. Linear regression analysis was used to evaluate the

linearity of the calibration curve by using the least square linear regression method.

c) Sensitivity: Limit of detection (LOD)/limit of quantitation (LOQ) of Metformin Hydrochloride and Ertugliflozin were determined by measuring the signal-to-noise ratio. limit of detection (LOD) is the concentration that gives a signal-to-noise ratio of approximately 3: 1, while the limit of quantification (LOQ) is the concentration that gives a signal-to-noise ratio of approximately 10 :1 with %RSD (n = 3) of less than 10%.

d) Accuracy: The accuracy of the assay method was determined by recovery studies at three concentration levels (70%, 100%, and 120%), i.e., 350, 500, and 600 µg/ml for Metformin Hydrochloride and Ertugliflozin 6.8, 9.72 and 11.66 µg/ml and three samples from each concentration were injected. percentage recovery of Metformin Hydrochloride and Ertugliflozin added and RSD were calculated for each of the three replicate samples.

e) Precision: The system precision and method precision (repeatability) of the proposed methods were determined by several measurements of standard solution and sample solution, respectively [7-9]. System precision was established by six measurements of the standard solution at the 100% concentration levels on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on the same day [9-12]. The RSD of obtained results was calculated to evaluate repeatability results.

f) Robustness: Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for example:
(i) Column temperature: ±2°C
(ii) Flow rate: ±0.2 mL/min.
Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD and percent of recovery.

g) Stability of Analytical Solutions: The stability of analytical solutions was determined by analysing the standard and sample preparations at Initial, 12 Hr and 24 Hr at ambient room temperature 25°C. Three injections from each solution were analysed, and the average of the peak and the RSD were calculated.

RESULTS AND DISCUSSION

Method Development and Optimization

Several physical and chemical properties of Metformin Hydrochloride and Ertugliflozin were obtained from the literature. The analytical method was developed to select preliminary reversed phase HPLC method chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure. For this purpose, a series of trials were performed by varying the ratio of include trials.

Table 1. Results of method optimization

Column Used	Mobile Phase	Flow Rate	Wavelength	Observation
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Water : Methanol (60:40)	1.0 ml/min	235 nm	Improper Peak Shape observed for both drugs
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Water : Methanol (50:50)	1.0 ml/min	235 nm	Improper Peak Shape observed for both drugs
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Water: Acetonitrile (50:50) Diluent Water : Methanol (60:40)	1.0 ml/min	235 nm	Improper Peak shape and more retention time observed for Ertugliflozin
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Water : Acetonitrile (50:50) Diluent : Mobile Phase	1.0 ml/min	235 nm	Improper peak shape and more run time
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Water : Acetonitrile (50:50) Diluent : Water HPLC Grade	1.0 ml/min	235 nm	Peak shape Improper for Metformin Hydrochloride
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Buffer: Acetonitrile (70:30) Buffer : 0.68 gms of KH_2PO_4 in 1000 ml of volumetric flask in dilute with water Diluent: HPLC Grade water	1.0 ml/min	235 nm	No Ertugliflozin peak eluted in this mobile phase combination
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Buffer: ACN. (50:50) Buffer (0.1 %v/v H_3PO_4)	1.0 ml/min	235 nm	Good Resolution and good peak shape but the method further optimized to make less Retention time of Main Peak
Kromasil C18 (5 µm) (250 mm x 4.6 mm)	Buffer: ACN. (60:40) Buffer (0.1 %v/v H_3PO_4)	1.0 ml/min	235 nm	Optimized method with good resolution and good peak shape and less retention time

Optimizing the chromatographic conditions on the Kromasil C18 (5 µm),(250 mm x 4.6 mm column. the results of method optimization are summarized in **Table 1**. the mobile phase consisting of Buffer (0.1 % v/v Phosphoric acid in water) and ACN (Acetonitrile) in the ratio 60: 40 v/v with a flow rate of 1 mL/min, injection volume 20 µl, run time 20 min, and column temperature 25°C at wavelength (λ) 235 was optimized as the best chromatographic conditions for the entire study where Metformin Hydrochloride and Ertugliflozin was eluted forming symmetrical peak shape, resolution and suitable analysis time with retention time about 1.8 min for Metformin Hydrochloride (Met) and 3.8 min for Ertugliflozin (Ertu) (**Figure. 3**).

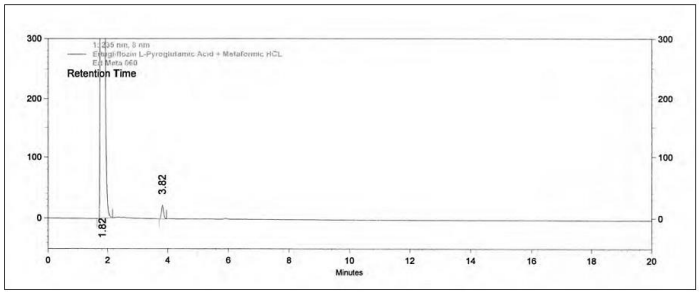

Figure 3. Chromatogram of Metformin Hydrochloride and Ertugliflozin standard solution

Method Validation

Specificity. Specificity was evaluated by comparing the chromatograms of mobile phase blank, placebo solution, standard solution, and sample solution (Metformin Hydrochloride and Ertugliflozin). For this purpose, 20 µl from solutions mobile phase blank, standard solution (API) , and sample solution were injected into the HPLC system separately, and the chromatogram results are shown in **Figures 4–5a,5b,5c,5d**. It can be observed that there no coeluting peaks at the retention time of Metformin Hydrochloride and Ertugliflozin interference. This result indicates that the peak of the analyte was pure and this confirmed the Specificity of the method.

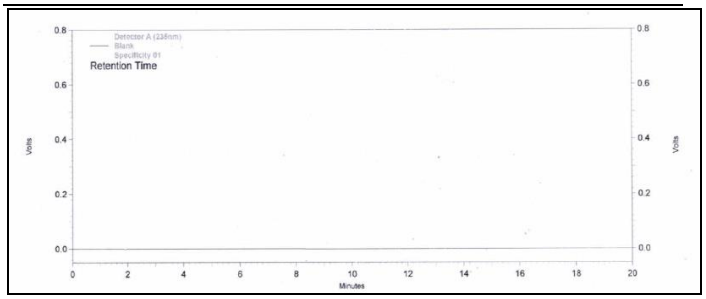

Figure 4. Chromatogram of Blank solution

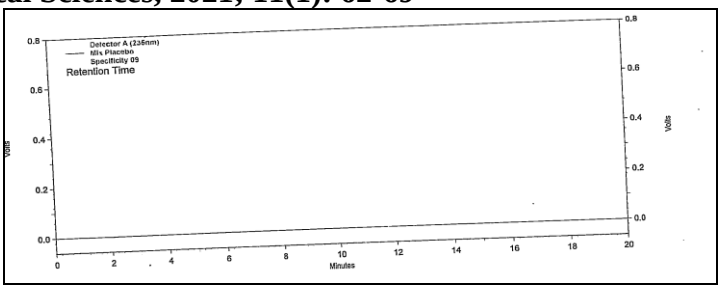

Figure 5a: Chromatogram of placebo solution.

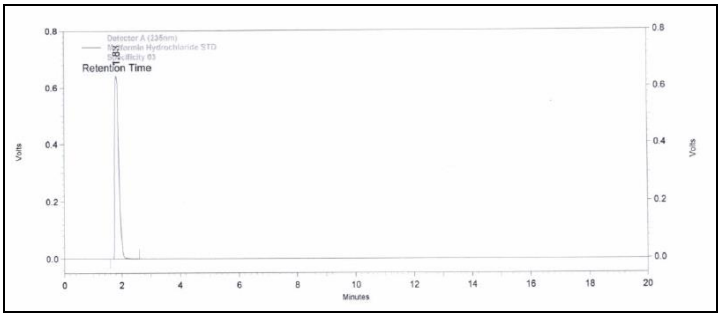

Figure 5b: Chromatogram of Metformin API solution.

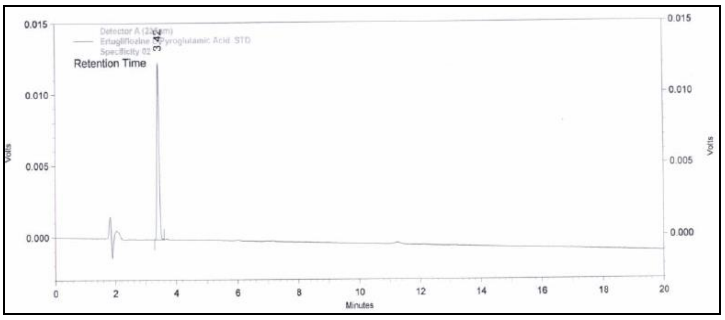

Figure 5c. Chromatogram of Ertugliflozin API solution

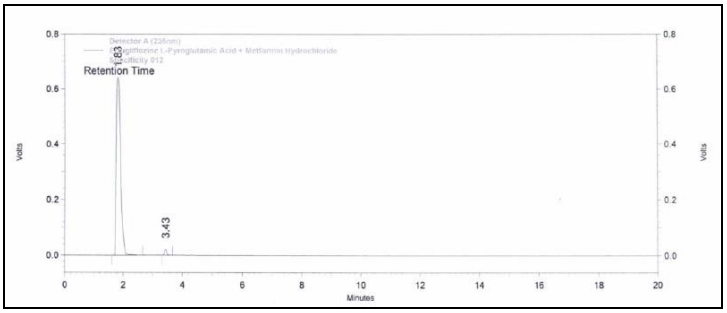

Figure 5d. Chromatogram of Sample Solution

Linearity and Range. Analytical method linearity is defined as the ability of the method to obtain test results that are directly proportional to the analyte concentration, within a specific range. The mean peak area obtained from the HPLC was plotted against corresponding concentrations to obtain the calibration graph. The results of linearity study (Figures 6 and 7) gave linear relationship over the concentration range of Metformin Hydrochloride: 350, 450, 500, 550 and 600 µg/ml, and Ertugliflozin 6.80, 8.74, 9.72, 10.69 and 11.66 µg/ml. From the regression analysis, a linear equation was obtained

and the goodness-of-fit (r2) was found to be 0.99, indicating a linear relationship between the concentration of analyte and area under the peak.

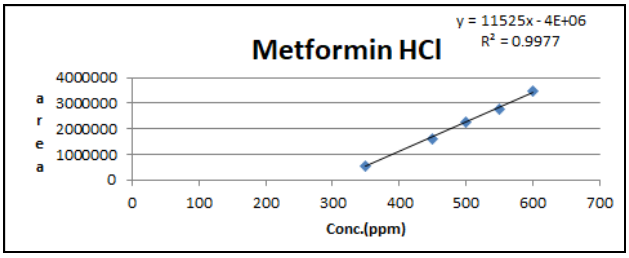

Figure 6. Standard calibration curve of Metformin Hydrochloride

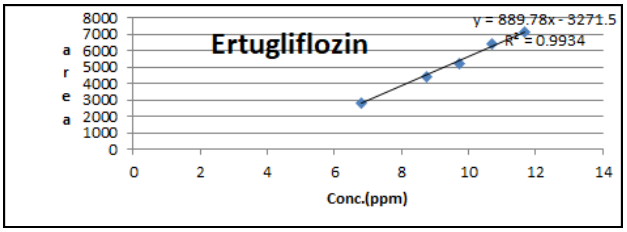

Figure 7. Standard calibration curve of Ertugliflozin

Limit of Detection and Limit of Quantification (LOD and LOQ). the limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, while the limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable sensitivity .The LOD`s were 0.0003 and 0.0009 µg/ml for Metformin Hydrochloride and Ertugliflozin respectively. The LOQ`s were 0.0037 and 0.0112 µg/ml for Metformin Hydrochloride and Ertugliflozin respectively.

Accuracy. The accuracy of an analytical procedure expresses the closeness of results obtained by that method to the true value. The results of accuracy showed percentage recovery at all three levels in the range of for Metformin Hydrochloride 98.5 % to 101.3 % and Ertugliflozin 98.2 % to 100.3%, and % RSD values were in the range of 0.84 - 0.89 % as shown in **Table 2.1 and 2.2**. The results of percentage recovery and %RSD were within the accepted limits from 98.0% to 102.0% and not more than 2.0%, respectively, which indicates the applicability of the method for routine drug analysis.

Precision. The precision of the method is deemed as "the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions," and it is normally expressed as the relative standard deviation. The results of both system and method precision showed that the method is precise within the acceptable limits. The RSD, tailing factor, and number of theoretical plats were calculated for both solutions; all the results are within limits. Acceptable precision was not more than 2.0% for the **RSD** as shown in **Tables 3, 4a and 4b.**

Table 2.1 Metformin Hydrochloride Recovery data of the proposed HPLC method

% Level	Peak Area	Mean Area	Amount found	Amount added	% recovery
70	4410893	4416223	348.80	350.20	99.6
	4412522		349.00	349.65	99.8
	4425255		342.10	349.80	100.0
100	6325252	6374559	500.23	500.20	100.0
	6412525		507.13	500.60	101.3
	6385900		505.02	499.30	101.1
120	7512522	7521681	594.12	600.60	98.9
	7562522		598.08	600.25	99.6
	7490000		592.34	600.90	98.6
Acceptance: - 98.0 to 102.0 **% RSD: - NMT 2.0 %**				**Min**	98.6
				Max	101.3
				Mean	99.9
				SD	0.89
				% RSD	**0.89**

Table 2.2. Ertugliflozin Recovery data of the proposed HPLC method

% Level	Peak Area	Mean Area	Amount found	Amount added	% recovery
	68253		5.20	5.26	99.4
	68125		5.20	5.31	98.2
70	68012	68130	5.20	5.29	98.5
	97858		7.50	7.50	99.8
	98025		7.50	7.50	99.7
100	98145	98009	7.50	7.50	100.3
	117858		9.00	9.00	100.2
	116002		8.90	9.00	98.3
120	115522	116461	8.80	8.95	98.8

Acceptance: - 98.0 to 102.0
% RSD: - NMT 2.0 %

Min	98.2
Max	100.3
Mean	99.2
SD	0.81
% RSD	**0.82**

Table 3. System Precision data from standard solution of the proposed HPLC method

Replicate No.	Metformin Hydrochloride Area	Ertugliflozin Area
1	6373163	98474
2	6330197	98506
3	6320105	98320
4	6313290	98356
5	6314865	98385
6	6282305	98359
Mean	6322320.83	98400.0
SD	29632.02	73.42
% RSD	0.47	0.07

Table 4 a. Intraday Precision data from Sample Solution of the proposed HPLC method

Replicate No.	Metformin Hydrochloride Area	Ertugliflozin Area
1	97.39	98.41
2	97.40	98.19
3	97.39	98.09
4	97.39	98.95
5	97.43	99.20
6	97.47	99.00
Mean	97.41	98.64
SD	0.03	0.47
% RSD	0.03	0.47

Robustness. The analytical method robustness was tested by evaluating the influence of minor modifications in HPLC conditions on system suitability parameters of the proposed method, as mentioned in Section 2.6.6. The results of robustness testing showed that a minor change of method conditions, such as the variation of the temperature and flow rate, is robust within the acceptable limits. The results are summarized in **Table 5**. In all modifications, good separation of Metformin Hydrochloride and Ertugliflozin was achieved, and it was observed that the percent of recovery was within acceptable limits and the %RSD is within limit of not more than 2.0 %. Acceptable limits as well. The results are shown in Table 5.

Solution Stability. The percent of recovery was within the range of 98.0% to 102.0% and RSD was not more than 2.0%, indicating a good stability of the sample and standard Solutions for 0 Hr, 12 Hr and 24 Hr at Room Temperature conditions. The peak area was as comparable to standard and percent of recovery was within acceptable limits, and the % RSD is within the limit of not more than 2.0%. The results are shown in **Table 6**.

Table 4 b. Interday precision data from sample solution of the proposed HPLC method

Replicate No.	Metformin Hydrochloride Area	Ertugliflozin Area
1	97.40	99.23
2	97.40	99.11
3	97.40	98.92
4	97.39	99.05
5	97.40	98.46
6	97.40	98.81
Mean	97.40	98.93
SD	0.00	0.27
% RSD	0.00	0.28

Table 5: Robustness data of the proposed HPLC method

Parameter	Condition	Peak Area		% of Assay		% RSD	
		Met	Ertu	Met	Ertu	Met	Ertu
Column Temperature ±2°C	**23 °C**	6474763	97278	98.45	98.83	0.22	0.23
	25 °C	6376932	98381	100.9	100.1	0.03	0.46
	27 °C	6474773	98278	98.39	99.62	0.41	0.20
Flow Rate ±0.2 ml/min	**0.8 ml/min**	6260056	95278	98.58	98.95	0.11	0.14
	1.0 ml/min	6376932	98381	100.9	100.1	0.03	0.46
	1.2 ml/min	6574773	99278	97.15	96.81	0.30	0.07

Table 6. Solution stability data of the proposed HPLC method

Parameter	Time Point	Peak Area			
		Met		Ertu	
Standard Solution	**0 Hr (Initial) at RT**	6376932		98381	
	After 12 Hr at RT	6313290		98056	
	After 24 Hr at RT	6313290		97856	
Parameter	**Time Point**	**Peak Area**		**% Assay**	
		Met	**Ertu**	**Met**	**Ertu**
Sample Solution	**0 Hr (Initial) at RT**	6376932	98381	100.90	100.10
	After 12 Hr at RT	6374932	97981	100.98	99.96
	After 24 Hr at RT	6354928	97569	100.66	99.75

CONCLUSION

In the present research, a fast, simple, accurate, precise, and linear HPLC method has been developed and validated for quantitative analysis of Metformin Hydrochloride and Ertugliflozin in combined dose and formulation, and hence it can be employed for routine quality control analysis for finished and stability sample analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Metformin Hydrochloride and Ertugliflozin. In addition, the main features of the developed method are short run time and retention time around 1.8 min for Metformin Hydrochloride (Met) and 3.8 min for Ertugliflozin (Ertu). The method was validated in accordance with ICH/FDA guidelines. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions.

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CONFLICT OF INTEREST

Author has no conflict of Interest.

DATA AVAILABILITY

Data available through correspondence if required.

FUNDING SOURCE

Not Applicable

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