Development and Validation of HPLC Method for Simultaneous Estimation of Metformin HCl with Ertugliflozin L-Pyroglutamic Acid in Tailored Formulation

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Summary: The present study introduced an innovative and quick RP-HPLC approach for simultaneous determination of Metformin HCl (MET) and Ertugliflozin L-pyroglutamamic acid (ERT). This new method is simple, accurate, precise and highly sensitive. The separation of both drugs was optimized at 40°C using HPLC column (C8, 4.6 x 150mm 5 microns) and mobile phase comprising of triethylamine in sodium octane sulfonate (pH 4) : MeOH: ACN in a ratio of 45:45:10 respectively, with a flow rate of 1.0 ml /min. The specificity of method showed that there was no interference from placebo or diluent during the drug's retention period. Accuracy and linearity studies conducted at different concentrations displayed good precision and the calibration curves exhibited high correlation i.e. R^2= 0.9982 and 0.9996 for ERT and MET, respectively. Precision was assessed for repeatability and intermediate precision, both delivering satisfactory results. Robustness was evaluated under different conditions, including wavelength and flow rate variations, showing acceptable results. Limits of detection (LOD) and quantification (LOQ) demonstrated good sensitivity. The accuracy and reliability of the suggested approach for the simultaneous measurement of MET and ERT are guaranteed by the analytical method validation. Comparative analysis of the bespoke new formulation's complete dissolution profile (CDP), Ertozin-M (7.5/500mg) with innovator tablet and Segluromet (7.5/500mg) was also observed at three different pH mediums (0.1 N HCl and buffer solutions of pH 4.5 and pH 6.8). This study was conducted according to International Council for Harmonization (ICH) guideline Q2(R2) on validation of analytical procedures and Q4B annex 7(R2) for Dissolution Test. The developed HPLC method was found highly suitable for combined estimation of Metformin HCl and Ertugliflozin L-pyroglutamic acid in quality control routine analysis for development of customized pharmaceutical formulations.

Keywords: RP-HPLC, Metformin HCl, Analytical method validation, Dissolution profile, Ertugliflozin L-pyroglutamic acid

Introduction

Diabetes is a chronic illness that can be cured. It is mostly caused by insufficient production of pancreatic insulin or by an inefficient metabolism that affects the body's capacity to use insulin. Elevated blood glucose levels associated with diabetes can affect the kidneys, eyes, nerves, heart, and kidneys [1]. Type 1 diabetes, also known as insulin-dependent diabetes, is characterized by insufficient insulin production in the body. People with Type 1 diabetes require daily injections of synthetic insulin to manage their blood glucose levels. The prevalence of Type 2 diabetes is increasing around the world. Environmental risk factors, genetic predispositions, and behavioral factors all interact to affect this illness. A family history of the condition, ethnicity, prior gestational diabetes, obesity, a poor diet, physical inactivity, increased age, and smoking are all risk factors for Type 2 diabetes [2]. As an oral biguanide anti-diabetic medication, metformin HCl (Fig.1) is frequently used as the first line of treatment for Type 2 diabetes, particularly in overweight or obese people with normal renal function [3]. It has also been studied for other conditions where insulin resistance is a factor, and it is advised for the treatment of polycystic ovarian syndrome. The way that metformin works is by blocking

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the hepatic process of gluconeogenesis, which suppresses the production of glucose. The medication functions by inhibiting the synthesis of glucose [4].

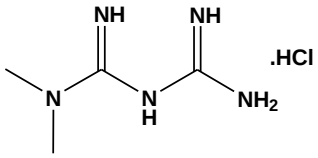

Fig. 1: Structure of Metformin. HCl

The pharmacological properties and therapeutic uses of MET make it a commonly prescribed and effective drug for the treatment of Type 2 diabetic [5]. However, its widespread uses, MET monotherapy may not be sufficient to maintain adequate glycemic control in all patients, as Type 2 diabetic is a progressive disease. In such cases, additional antihyperglycemic therapy is required. Additional therapies such as SGLT2 inhibitors like ERT is combined with MET. Ertugliflozin L-pyroglutamic acid (ERT) (Fig. 2), is a more recent class of antidiabetic medications that lower renal glucose reabsorption and increase urine glucose excretion, which has positive effects on blood pressure, body weight, and glycemic control. The European Union, Canada, Australia, and the United States have approved the use of ERT, a selective SGLT2 inhibitor, in addition to diet and exercise to help persons with Type 2 diabetes improve their glycemic control [5,6]. ERT is a sodium-glucose cotransporter-2 (SGLT2) inhibitor that lowers blood glucose levels by preventing the kidneys from reabsorbing glucose Despite having distinct modes of action, these two medications are frequently taken in tandem to treat Type 2 diabetes [7,8]. According to Biopharmaceutics Classification System (BCS), Metformin being highly soluble and having low permeability in the gut was classified as BCS Class III drug, whereas Ertugliflozin L-pyroglutamic acid based on its high solubility and high permeability categorized as BCS Class I drug [9,10].

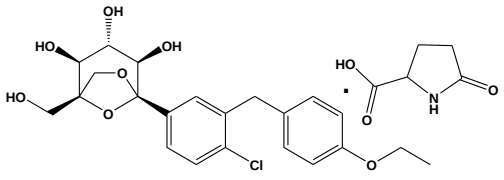

Fig. 2: Structure of Ertugliflozin L-pyroglutamic Acid.

When MET monotherapy is insufficient to produce sufficient glycemic control in individuals with Type 2 diabetes, ERT is a viable alternative for combination therapy due to its effectiveness in lowering HbA1c, FPG, body weight, and blood pressure. Additionally, clinical research has demonstrated that ERT is typically well tolerated, with little chance of hypoglycemia and few side effects [11,12].

In the determination of Metformin HCl (MET) and Ertugliflozin L-pyroglutamic acid (ERT) in pharmaceutical dosage forms, various analytical techniques have been employed, each offering distinct advantages in terms of sensitivity, specificity, and reliability. Among these techniques, UV spectrophotometry, voltammetry, and high-performance liquid chromatography (HPLC) are commonly utilized. In addition to these techniques, the simultaneous determination of Metformin HCl (MET) and Ertugliflozin L-pyroglutamic acid (ERT) through reversed-phase high-performance liquid chromatography (RP-HPLC) offers several advantages and challenges. RP-HPLC methods are widely employed due to their excellent sensitivity, selectivity, reproducibility, and cost-effectiveness. However, variations exist in the reported methods, each with its strengths and weaknesses i.e. lengthy, matrix effects and limited robustness [12].

By addressing these gaps, new RP-HPLC methods can offer superior performance, increased sustainability, and broader applicability for the simultaneous determination of MET and ERT in pharmaceutical dosage forms, further advancing pharmaceutical analysis and quality control practices. By adhering to ICH guidelines for validation, pharmaceutical companies can enhance the credibility of their analytical results, support regulatory submissions, and ultimately contribute to the safety and efficacy of pharmaceutical products for patient use.

Earlier research studies and developed projects have provided important information about similar combinations in the field of anti-diabetic medications. Research on the combined formulations of distinct antidiabetic drugs offers a basis for comprehending possible obstacles, honing formulation tactics, and defining the extent of therapeutic benefits. The current study trajectory is driven by the ideas developed from these prior investigations, which direct the scientific community to utilize the synergistic potential of Metformin HCl and Ertugliflozin L-pyroglutamic acid for optimal diabetes control [13–15].

Experimental

Materials

The standard samples of Metformin HCl (MET) and (Ertugliflozin L-pyroglutamic acid) ERT were provided by Genix Pharmaceutical Private Limited. Samples of the innovative pharmaceutical drugs, including acetonitrile (HPLC grade), methanol (HPLC grade), purified water (HPLC grade), sodium salt of octane sulfonic acid (analytical grade), triethylamine (TEM), and segluromet (7.5 mg ERT/500 MET mg), were acquired from Merck and Honeywell private limited.

Instruments

The Shimadzu HPLC system employed for analysis comprised of a modular setup with LC-20A pump, aided by the DGU-20A degasser. The SIL-20AHT auto sampler, while the CTO-20AC column oven ensured precise temperature control for optimal separations, and the detection were made on SPD-20A. HPLC software version Lab Solution 6.72 sp1 was used for controlling instrument functions and data acquisition. Separation was achieved on an Agilent Zorbax C8, 4.6 x 150mm 5 micron or equivalent.

Other instruments included Mettler Toledo M105 DU Electronic balance and Mettler Toledo S220K pH meter was used for measuring weight and pH respectively and Ultrasonic GT sonic E120H Dis sonicator was used for sonication.

Prior to injection onto the HPLC system, all samples and mobile phase underwent filtration through Durapore Membrane 0.22 and 0.45µm membrane filters respectively. The initial eluent (a few milliliters) was discarded to waste to ensure equilibration of the filter with the sample solution. This initial waste collection minimizes potential contamination of the HPLC column by any filter-adsorbed material.

HPLC Method Optimization

Diluent solution optimization

The solubility of both drugs (MET & ERT) was determined in different ratios of water, methanol, and acetonitrile and the diluent ratio H_2O: MeOH: ACN 45:45:10 v:v was found suitable and provide solubility up to 10 mg /ml for both drugs.

Analytical method optimization

Various mobile phase compositions were explored to achieve ideal chromatographic separation by employing C8 stationary phase (150 x 4.6mm, 5µm) with a mobile phase composed of a few percent sodium octane sulfonate, acidic buffer, methanol, and acetonitrile. The C18 column exhibited excessive retention for ERT, resulting in an asymmetrical peak. To minimize this interaction and achieve earlier elution of ERT, a C8 column was chosen. However, this led to premature elution of MET with minimal retention. Buffer solutions were prepared using triethylamine in sodium octane sulfonate at a specific pH (adjusted with phosphoric acid) and mixed with varying ratios of methanol and acetonitrile. Table-1 summarizes the tested mobile phase compositions, temperatures, and their corresponding chromatographic outcomes.

After several attempts of optimization, pH 4 buffer: MeOH: ACN (45:45:10) ratio was selected as optimized mobile phase. The Agilent Zorbax (C8, 4.6 x 150mm 5 micron) or equivalent column was used during analysis with flow rate of 1.0 ml /min at 40 ºC elution temperature and detection wavelength 263 nm and 225 nm for MET and ERT, respectively.

Analytical method validation

System suitability

For the determination of system suitability, 5 runs of standard solution of MET and ERT were injected. The optimized mobile phase was used as an eluent.

Preparation of stock standard solutions

Stock standard solution of MET was prepared by transferring 25 mg of MET into a 50ml volumetric flask, initially around 20ml of diluent was added and solubility was obtained via sonication and labeled as flask A. Stock standard solution of ERT was prepared by transferring 32.4 mg of ERT into a 200ml volumetric flask, initially around 50ml of diluent was added and solubility was obtained via sonication then volume of flask was made up to the mark with diluent and labeled as flask B.

Preparation of working standard solution

3.0 ml ERT solution was transferred from flask B into flask A and diluted the volume up to the mark with diluent and filtered through a 0.45µm filter before use.

Table-1: Optimization of mobile phase with dependent (X) and Independent (Y) variable.

Run	Dependent variables (X)					Independent variable (Y)			
	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4
	Buffer Solution (%)	Methanol (%)	Acetonitrile (%)	Column temperature (°C)	pH	Rt.1	Rt.2	Tp.1	Tp.2
1	60	30	10	35	3.0	3.071	10.851	2883	3930
2	55	35	10	35	3.0	2.845	10.624	2348	2686
3	55	35	10	35	3.5	2.941	10.721	3070	4148
4	50	40	10	35	3.5	2.721	10.623	3459	3929
5	50	40	10	40	4.0	2.521	10.465	5386	4859
6	55	40	5	40	4.0	2.461	10.236	3208	4946
7	50	30	20	40	4.0	2.321	10.250	3088	3997
8	45	45	10	40	4.0	2.217	9.129	4361	2995
9	50	45	05	40	4.0	2.513	9.425	3426	4117
10	50	45	05	40	4.5	2.112	9.102	4148	4830
11	50	45	05	40	4.5	2.313	9.562	3929	2883
12	45	45	10	40	4.5	2.344	9.621	4859	2519
13	60	35	5	45	4.5	2.642	9.372	4946	3788
14	55	35	10	45	4.5	2.591	9.222	3675	2348

Rt.1- Retention time of MET; Rt.2- Retention time of ERT; Tp.1- Theoretical plates of MET per column; Tp2.- Theoretical plates of ERT per column, whereas green color showed the optimized condition.

Acceptance criteria

For optimal chromatographic performance, stringent criteria were set for peak characteristics. The relative standard deviation (RSD) of peak areas for both MET and ERT should be less than 2.0%, ensuring consistency in quantification. The peak tailing factor, a measure of peak symmetry, for both analytes must also be below 2.0, indicating minimal peak distortion. Additionally, a minimum theoretical plate number of 2000 is mandated for both MET and ERT peaks. This parameter reflects column efficiency, with higher values signifying sharper peaks and improved resolution between analytes. Finally, the resolution factor between MET and ERT peaks must exceed 1.5, guaranteeing their complete chromatographic separation.

Specificity

To evaluate the method's specificity, a series of injections were performed. This involved injecting blank samples (diluent/mobile phase), a placebo solution (matrix without analytes), standard solutions containing known concentrations of MET and ERT, and finally, the actual sample solutions. By analyzing the chromatograms from each injection, any potential interferences from the sample matrix or background noise could be identified, ensuring the method can accurately distinguish and quantify the target analytes (MET and ERT) in the presence of other sample components.

Preparation of placebo solution

Stock placebo solution was prepared by transferring 417.5 mg of placebo in 200 ml volumetric flask, initially, 50 ml of diluent was added followed by sonication for 15 min then volume was made up to the mark with diluent. After filtration of this solution, 5ml of filtrate was transferred into 25ml volumetric flask and diluted the volume to the mark with diluent and mixed well. The solution was filtered through 0.45µm filter paper before injection.

Preparation of sample solution

For the sample solution the average weight of 20 tablets was determined then all tablets were crushed into powder. The weight, equivalent to 1 tablet was transferred into a 200ml volumetric flask, initially 50ml of diluent was added followed by sonication for 15 min then volume was made up to the mark with diluent. After filtration of this solution, 5ml of filtrate was transferred into 25ml volumetric flask and diluted the volume up to the mark with diluent and mixed well. The working sample solution was filtered through 0.45µm filter paper before injection.

Acceptance criteria

No interference was observed at retention time of principle peaks of MET and ERT in diluent/mobile phase, placebo solutions.

Accuracy

Accuracy, a measure of how closely the analytical method reflects the true value of the analyte in the sample, was assessed using a bracketing approach. Three concentration levels: the expected sample concentration (50%, 100%, and 150%). were prepared in triplicate for the target analytes (MET and ERT) with placebo by employing described sample solution. Each of these nine solutions (25 µL) were then injected separately under the optimized chromatographic conditions. The obtained results were analyzed to ensure the measured concentrations

accurately represent the actual amount of analytes present in the samples.

Preparation of accuracy solutions

All stock solutions were prepared in triplicate as per Table 1S and sonicated it 10 min for dissolution and further diluted 5 ml of filtrate in 25 ml separately.

Acceptance criteria

All accuracy samples should have an assay result percentage of 98–102%. Every accuracy sample at each level should have a percentage RSD of less than 2.0%.

Precision

Repeatability

The repeatability was determined by injecting 6 different samples of same (100% accuracy level) concentration. The samples were prepared and run by the same procedures as mentioned in previous sections with optimized chromatographic conditions.

Acceptance criteria

Six sample solutions' %RSD for the MET and ERT assay findings should be less than 2.0.

Intermediate precision

The intermediate precision was determined by injecting 6 different samples of the same (100% accuracy level) concentration. The samples were prepared and run by the same procedures as mentioned in repeatability sections using same optimized chromatographic conditions, but analyst and day was changed.

Acceptance criteria

Six sample solutions' %RSD for the MET and ERT assay findings should be less than 2.0. Less than 2.0 should also be the overall percentage RSD for results in repeatability and moderate precision.

Linearity

Five distinct solutions were prepared to ascertain the linearity. Concerning the standard solution, the concentrations of the solutions were 50%, 75%, 100%, 125%, and 150%. The same optimal chromatographic conditions were used for analysis. Standard protocols were used to prepare all five solutions, and Table 2S summarizes the quantity of samples.

Preparation of stock solutions for linearity of Ertugliflozin L-Pyroglutamic Acid and Metformin HCl

Weigh and transfer the working standard amounts of MET into a 50 ml volumetric flask. This is flask A. Add 20ml of diluent and sonicate to dissolve. Weigh and transfer the working standard amounts of ERT, into a 200 ml volumetric flask. After adding 50ml of diluent and sonicating it to dissolve, dilute the volume to the appropriate amount and thoroughly mix. This is flask B. Pour 3ml from flask B into flask A, use diluent to adjust the volume, and thoroughly mix. This solution was filtered through nylon syringe filter 0.22 µm filter paper into an HPLC vial. Table 3S demonstrates the preparation scheme of solutions having different concentrations. Single run of each concentration (25 µL) was taken to check the linearity of HPLC.

Acceptance criteria

The regions of MET and ERT linearity solutions should have coefficients of correlation ("r") greater than 0.997.

Range

The results of linearity, accuracy, and precision were used to determine the analytical method's range.

Limit of Detection and Quantification

The detection and quantification limits are determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected and quantified. This method is limited to analytical processes that display baseline noise.

The formulas below can also be used to compute LOD and LOQ based on the slope of the calibration curve (S) at values that approximate the LOD and LOQ and the standard deviation of the response (Sy) of the curve:

LOD = 3.3 X (Sy/S)

LOQ = 10 X (Sy/S)

Procedure

For the determination, five distinct concentrations of the solutions were prepared. In comparison to the reference solution, the concentrations of the solutions were 50%, 75%, 100%, 125%, and 150%. Method of analysis used the same chromatographic conditions as described under system suitability.

Table-2: Variations in chromatographic conditions from optimized conditions.

Changes in optimized method	New condition
Decrease in flow rate (– 0.2mL)	0.8 mL / min
Increase in flow rate (+ 0.2mL)	1.2 mL / min
Decrease in wavelength (– 2nm)	261nm for MET and 223nm for ERT
Increase in wavelength (+ 2nm)	265nm for MET and 227nm for ERT

Calculation

Calculate the standard error and slope of regression line, then limit of detection was calculated by using LOD formula as mentioned above.

Robustness

The robustness was performed by injecting 5 separate runs of standard solution of MET and ERT with 4 sets of samples in duplicate using slight variations in chromatographic conditions. The variations in wavelengths and flowrate from optimized chromatographic conditions are summarized in Table-2.

Acceptance criteria

The system suitability parameters, including resolution, tailing factor, theoretical plates, and percentage RSD of areas, should fulfill the requirements. Additionally, the assay findings for MET and ERT produced under various circumstances should be within 2%.

Dissolution profile

To conduct the dissolution profile, a Teledyne Hanson CD 14 outfitted with an auto sampler 850 DS and a 14-position dissolution unit was utilized. This system is specifically made for such tests to conduct comparative dissolution under the same conditions. The comparative dissolution profiles of customized formulation Ertozin-M (7.5/1000mg) with innovator tablet Segluromet (7.5/500mg) were assessed in 3 different solutions that is 0.1 N HCl, buffer solution pH 4.5, and buffer solution pH 6.8 as per ICH guidelines for dissolution test. The dissolution percentages of MET and ERT in both formulations were determined at 10, 15, 20, 30, 45, and 60 min.

Results and Discussion

Analytical method validation

System suitability

The optimized chromatographic conditions successfully separated the target analytes, metformin (MET) and ertugliflozin (ERT), with respective retention times of 2.17 and 9.13 minutes (Fig. 3). Validation data presented in Table (4S & 5S) confirmed satisfactory performance for both analytes. The relative standard deviation (% RSD) of peak areas indicated excellent precision, while tailing factors demonstrated minimal peak distortion. Additionally, the number of theoretical plates ensured efficient separation, and the resolution factor guaranteed complete chromatographic distinction between MET and ERT.

Specificity

A specificity test was performed to check the selectivity of the method. The chromatograms of single run of each solution such as diluent/mobile phase, placebo solution, separate standard solution of MET and ERT, combine solution of MET and ERT standard, and drug formulation sample are shown in Fig. 4. The retention times of the ERT and MET peaks were not affected by the diluent or placebo solution.

Accuracy

To evaluate the method's accuracy, triplicate samples were prepared at three concentration levels encompassing 50%, 100%, and 150% of the expected sample concentration for both MET and ERT. These solutions were then injected (25 µL), and the obtained data showcased satisfactory accuracy across all concentration levels, demonstrating the method's ability to faithfully reflect the true concentrations of MET and ERT in the samples. Additionally, good precision was observed, as further detailed in the summarized results presented in Table 6S and Table (3 & 4). The amount of standard of MET and ERT takes was 25.0 mg and 32.4 mg, respectively.

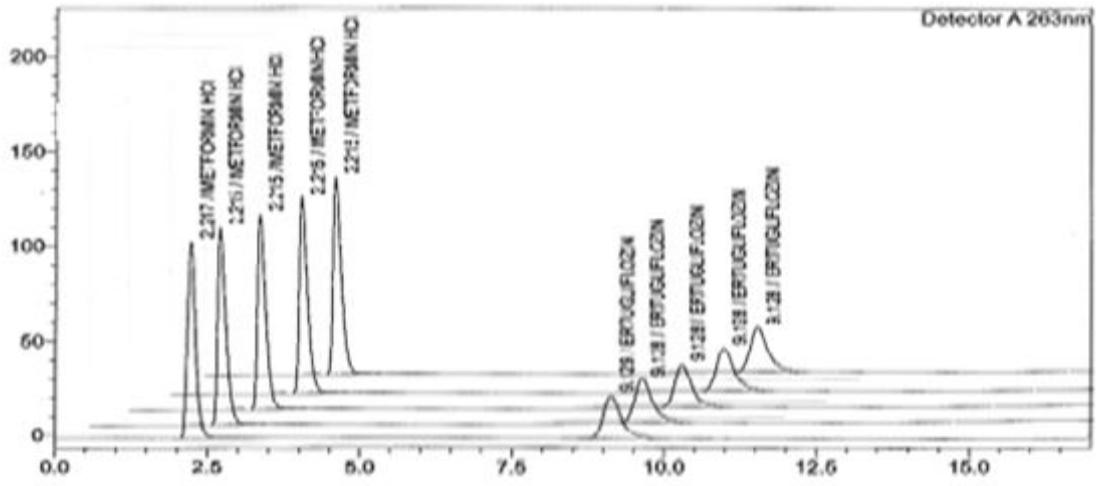

Fig. 3: Overlay standard solution chromatogram of MET and ERT for system suitability.

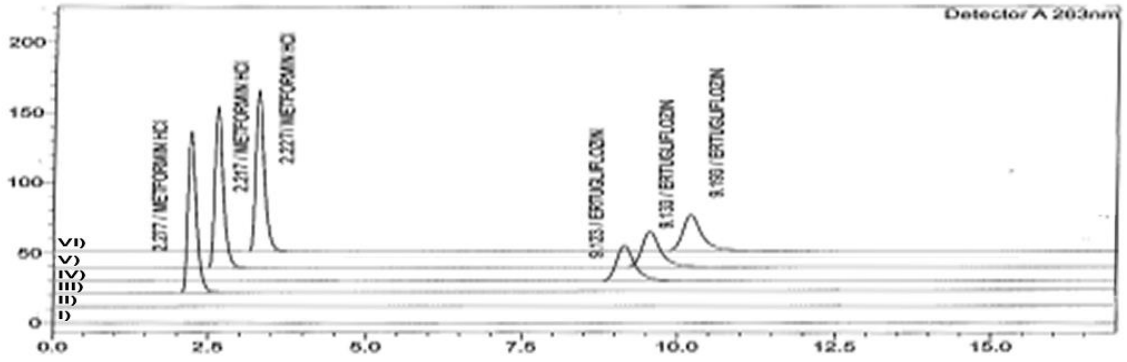

Fig. 4: Specificity chromatogram (I) diluent/mobile phase, (II) placebo, (III) standard MET, (IV) standard ERT, (V) combine standard solution of ERT and MET, (VI) drug formulation.

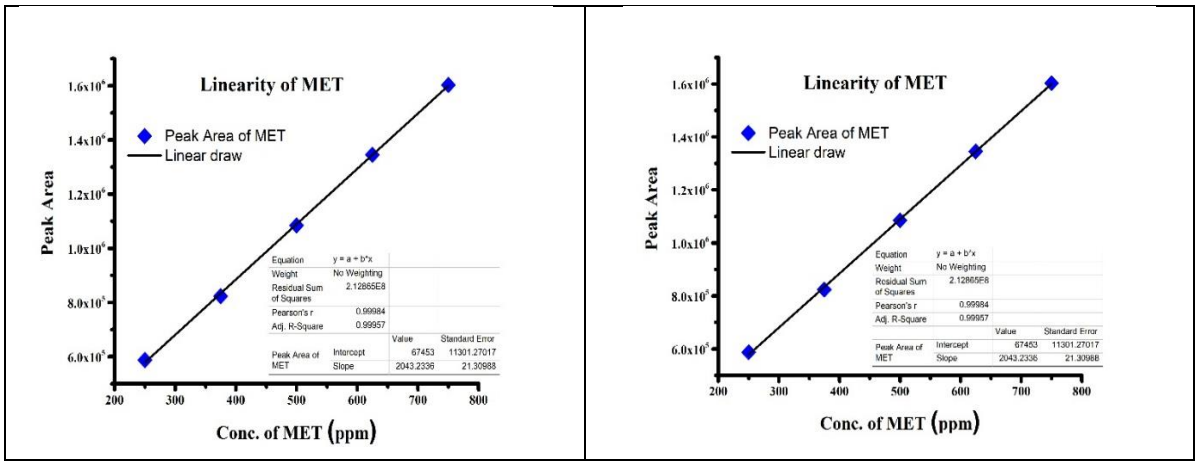

Fig. 5: Linearity curve between concentration of MET and ERT vs Peak Area.

Table-3: Recovery of MET at three different concentration level.

S.No.	Recovery level	Amount of standard MET (mg)	Amount of placebo (mg)	Actual Conc. of MET (ppm)	Peak area of sample	Recover Conc. of MET (ppm)	% Recovery	Mean % Recovery
1	50	258.63	417.75	250.0	577987	252.81	101.13	101.47
	50	259.28	417.75	250.0	582454	254.13	101.65	
	50	262.78	417.75	250.0	590186	254.07	101.63	
3	100	491.33	417.75	500.0	1083385	498.89	99.78	100.12
	100	472.92	417.75	500.0	1048501	501.62	100.32	
	100	485.13	417.75	500.0	1074762	501.24	100.25	
5	150	749.05	417.75	750.0	1664554	754.17	100.56	100.15
	150	746.71	417.75	750.0	1650347	750.08	100.01	
	150	744.6	417.75	750.0	1643880	749.22	99.90	

Table-4: Recovery of ERT at three different concentration level.

S.No.	Recovery level	Amount of standard ERT (mg)	Amount of placebo (mg)	Actual Conc of ERT (ppm)	Peak area of sample	Recover Conc. of ERT (ppm)	% Recovery	Mean % Recovery
1	50	3.76	417.75	3.75	261693	3.78	100.67	101.61
	50	3.77	417.75	3.75	260700	3.76	100.29	
	50	3.77	417.75	3.75	264808	3.82	101.87	
3	100	7.5	417.75	7.5	521534	7.52	100.31	100.35
	100	7.5	417.75	7.5	521619	7.52	100.33	
	100	7.5	417.75	7.5	521999	7.53	100.40	
5	150	11.25	417.75	11.25	769998	11.11	98.74	99.72
	150	11.25	417.75	11.25	782414	11.29	100.33	
	150	11.25	417.75	11.25	780568	11.26	100.09	

Repeatability

Intra-assay precision, also known as repeatability, was assessed using six replicate samples prepared at a 100% concentration level of the sample solution. This evaluation ensures the method's consistency in producing precise results within a single analytical run. The typical solution preparation process remained unchanged, as did the system appropriateness. Table 7S displays the results, which were determined to have adequate precision across all six repetitions.

1. The tablet's average weight is 928 mg.
2. 7.5 mg is the usual weight of ERT.
3. Standard MET weight: 500 mg
4. Standard ERT average area: 537475
5. Standard MET average area: 1066559.6
6. Standard ERT potency: 98.93%
7. Standard MET potency: 99.41%
8. Tablet label claims: ERT (7.5 mg) and MET (500 mg).

Intermediate precision

Six sample solutions were tested for intermediate precision at a 100% concentration level using two different analysts (A & B) on two separate days. Findings are displayed in Tables (8S to 10S). Analysts A and B determined that the intermediate precision of all six replicates was sufficient.

1. The tablet's average weight is 929.2 mg.
2. 7.5 mg is the usual weight of ERT.
3. MET standard weight: 500 mg
4. Standard ERT potency: 98.93%
5. Standard MET potency: 99.41%
6. Tablet label claim: MET (500 mg) and ERT (7.5 mg).

Linearity

As mentioned in the previous section, the linearity of MET and ERT was tested at five different concentrations. The linearity runs' chromatograms are displayed in Fig.5. Table 11S demonstrates that all the ERT and MET's linearity parameters were determined to be good.

Limit of Detection and Quantification

The Sensitivity of the given method was determined by the values of LOD and LOQ. These values were calculated using the formulae based on the standard deviation of the y-intercept of regression lines and the slope of the calibration curve. The LOD of ERT and MET were found to be 0.47 and 15.10 µg/ml respectively, whereas LOQ of ERT and MET were found to be 1.43 and 45.75 respectively as shown in Table 12S (a & b) and Table 13S (a & b).

Table-5: Robustness study at variable wavelength and flow rate.

S.No.	Wavelength variation of ± 2 nm from optimized 263/225 nm			Flow rate variation of ± 0.2 mL/min from optimized 1.0 mL/min		
1	Conditions	% Metformin	% Ertugliflozin	Conditions	% Metformin	% Ertugliflozin
2	At –2 nm	100.01 %	100.34 %	At –0.2 ml/min	100.12 %	100.45 %
3	At +2 nm	100.17 %	100.15 %	At +0.2 ml/min	100.08 %	100.00 %
4	At 263 nm	100.39 %	100.72 %	At 1.0 ml/min	100.39 %	100.72 %

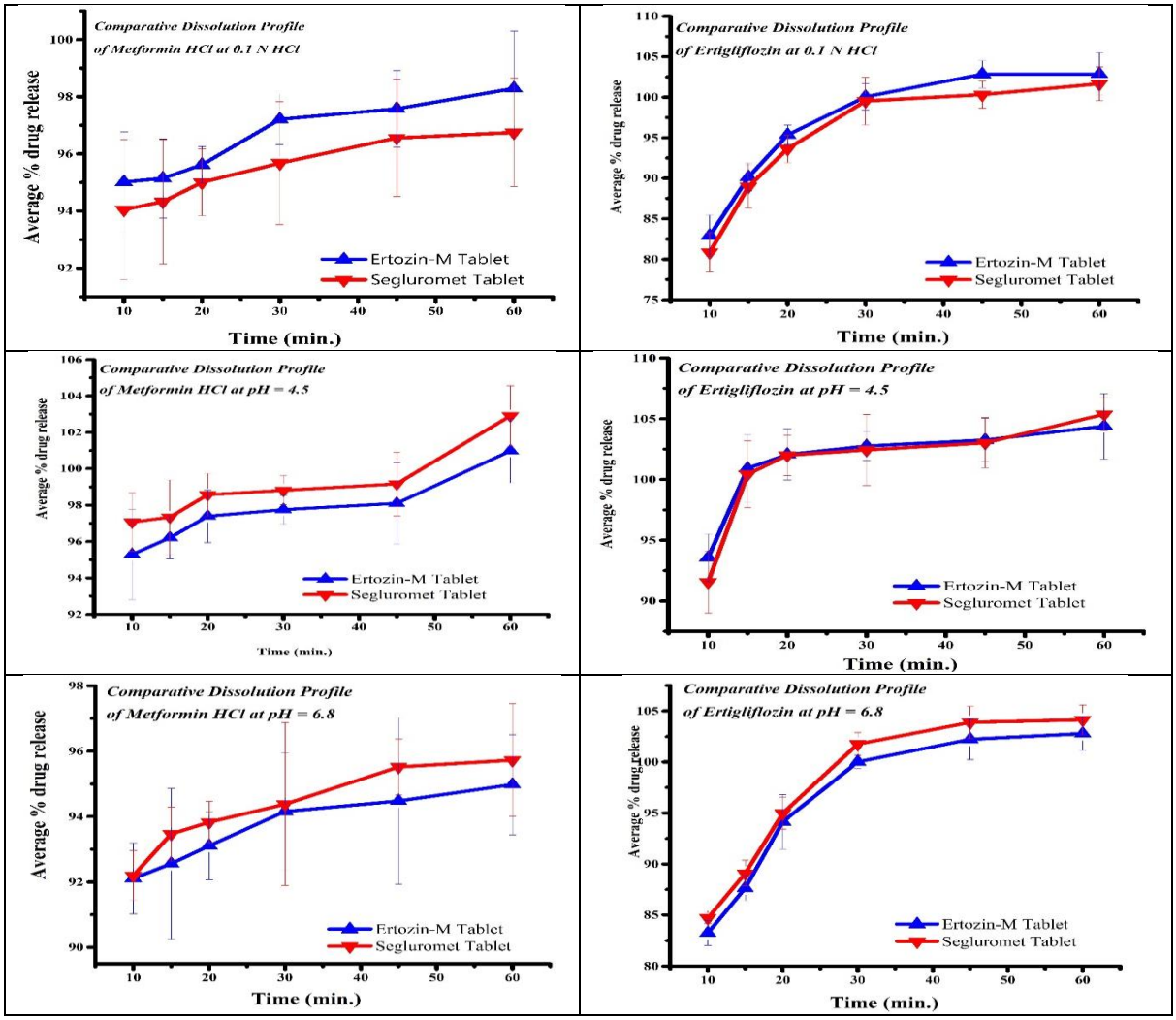

Fig. 6: Comparative dissolution profiles of MET and ERT at different pH.

Robustness

The robustness of this new method at four different conditions was performed. Amount of standard ERT: 32.4mg, Amount of standard MET: 25 mg, Concentration of ERT: 7.5 ppm, Concentration of MET:500.0 ppm. Robustness studies at variable wavelength and flow rate are displayed in Table 5.

Dissolution profile

The comparative dissolution profiles of customized tablet Ertozin-M (7.5/500mg) and innovator tablet Seglurometr (7.5/500mg) were assessed in three different mediums (0.1 N HCl, buffer solution of pH 4.5. and 6.8) and the dissolution percentages of MET and ERT from both formulations were determined at 10, 15, 20, 30, 45, and 60 min. At 0.1 N HCl after 10 min it was observed that more than 80% of ERT and more than 90% MET dissolved in medium. As the time progressed, both formulations demonstrated an increase in dissolution percentages and after 60 min more than 100% ERT and more than 95 % MET dissolution were achieved in both formulations. At pH 4.5 after 10 min, it was observed that more than 90% of ERT and more than 95% MET dissolved in medium. As the time progressed, both formulations demonstrated an increase in dissolution percentages and after 60 min both pharmaceutical dosage forms (customized and innovator) showed more than 100% dissolution of MET and ERT. At pH 6.8 after 10 min, it was observed that more than 80% of ERT and more than 90% MET dissolved in medium. As time progressed, both formulations demonstrated an increase in dissolution percentages and after 60 min both pharmaceutical dosage forms (customized and innovator) showed more than 100% dissolution of ERT and about 95% dissolution of MET.

The dissolution profiles are presented in Fig. 6. These dissolution profile results are well with the limit of dissolution test according to ICH guidelines.

Conclusion

In the present study, an analytical method was developed and validated as per ICH guidelines. The new HPLC method is simple, fast, precise, robust, linear and accurate for the quantitative analysis in the combined dosage formulation of Metformin hydrochloride (MET) and Ertugliflozin L-pyroglutamamic acid (ERT). In addition, the developed HPLC method also achieved high resolution, low tailing factors, and high theoretical plates, indicating optimal separation. The accuracy and recovery at three different concentration levels (50%, 100 and 150%) were found 101.47, 100.12 and 100.15 % for MET and 100.61, 100.35 and 99.72 % for ERT. The Linearity was also excellent with R-squared values of 0.9982 and 0.9996 for ERT and MET, respectively. Limits of detection (LOD) and quantification (LOQ) demonstrated good sensitivity. Finally, a comparative dissolution profile study suggested good bioavailability in various pH media. Therefore, this rapid method is suitable for quality control analysis of ERT and MET in bulk and pharmaceutical formulations.

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Supplementary Information

For the information link: https://1drv.ms/b/c/938cbeb9852b31c2/Ed_wPJXRonVAnO_XX-WMuj8B7e_glsrRN-ylwGTs4wzmzQ?e=rhREPV

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