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Research Article

DEVELOPMENT AND VALIDATION OF NEW STABILITY INDICATING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN HYDROCHLORIDE AND ERTUGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The present study deals with the development, validation, and application of simple, precise, and accurate high-performance liquid chromatography (HPLC) method for the simultaneous estimation of metformin hydrochloride and ertugliflozin in pharmaceutical formulation and to validate.

Methods: The analytical conditions were optimized on BDS C8 column (150 mm × 4.6 mm, 5 µm) at room temperature. The mobile phase consists of buffer: acetonitrile in 55:45 v/v ratio. Injection volume was 10 µl. The flow rate was maintained at 1.0 ml/min, and the analysis was carried out at 224 nm.

Results: The method was found to be linear in the concentration range of 125–750 µg/ml and 1.875–11.25 µg/ml for metformin hydrochloride and ertugliflozin with regression coefficient r^2 = 0.999. The method was found to be precise with percentage relative standard deviation below 2%. The limit of detection and limit of quantification were found to be within the limits. The percentage recovery of the developed method was 100.15%. All the validation parameters such as robustness, recovery, and precision were found to be within the limits. Degradation parameters such as acid, base, thermal and peroxide, light, temperature, and humidity were performed and found that the drugs are stable in all the extreme conditions.

Conclusions: A simple, accurate, precise, and less time-consuming reversed-phase HPLC method for the simultaneous estimation of metformin hydrochloride and ertugliflozin has been developed and validated in accordance with the ICH guidelines.

Keywords: Metformin hydrochloride, Ertugliflozin, Reversed-phase - high-performance liquid chromatography, Validation, Simultaneous, Degradation.

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INTRODUCTION

Type 2 diabetes is a disease in which the body does not make enough insulin to control the level of glucose in the blood or when the body is unable to use insulin effectively. The result is a high level of glucose in the blood. The two active substances such as metformin hydrochloride and ertugliflozin work in different ways to lower glucose levels. Metformin hydrochloride is an antihyperglycemic agent (AHA) that improves glucose tolerance in patients with type 2 diabetes mellitus by lowering both basal and post-prandial plasma glucose. It is not chemically or pharmacologically related to any other class of oral AHA. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake, and utilization ertugliflozin helps to lower blood glucose by making the patient pass out glucose in the urine. It does this by blocking a protein in the kidneys (called sodium-glucose cotransporter-2 [SGLT2]) that normally take glucose back into the blood from the kidneys [1,2].

Metformin hydrochloride is chemically called as N, N-dimethylimido-dicarbonimidic diamide hydrochloride (Fig. 1) and does not related to any other classes of oral antihyperglycemic agents. It is a white to off-white crystalline compound with a molecular formula of $C_4H_{11}N_5$•HCl [3].

Ertugliflozin is the fourth SGLT2 inhibitor approved by the FDA. The chemical name of ertugliflozin L-pyroglutamic acid is (1*S*,2*S*,3*S*,4*R*,5*S*)-5-(4-chloro-3-(4-ethoxy benzyl)phenyl)-1-(hydroxymethyl)-6,8-dioxabicyclo octane-2,3,4-triol, compound with (2*S*)-5oxopyrrolidine- 2-carboxylic acid (Fig. 2), and its molecular formula is $C_8H_{15}NO_2$ [4,5].

Even though numerous methods are available for the estimation of metformin hydrochloride individually and in combination with other drugs, no method has been reported for the estimation of metformin hydrochloride and ertugliflozin simultaneously [6-16].

METHODS

The reference sample of metformin hydrochloride and ertugliflozin was obtained as a gift samples, and the tablet containing metformin hydrochloride 500 mg and ertugliflozin 7.5 mg was procured from the local market. Water (high-performance liquid chromatography [HPLC] grade) from Rankem; acetonitrile (HPLC grade), orthophosphoric acid (AR grade), sodium hydroxide (pure), and hydrogen peroxide (pure) from Merck Ltd., and 0.45 µm Nylon filter was from Zodiac life sciences were used.

Instrumentation

Waters HPLC 2695 system equipped with quaternary pumps, photodiode array detector, and autosampler integrated with Empower 2 Software. UV-VIS spectrophotometer, PG Instruments T60 with special bandwidth of 2 mm and 10 mm, and matched quartz cells integrated with UV win 6 software were used for measuring absorbances of metformin hydrochloride and ertugliflozin solutions, Electronic Balance, Denver pH meter, Ultrasonicator - BVK Enterprises India.

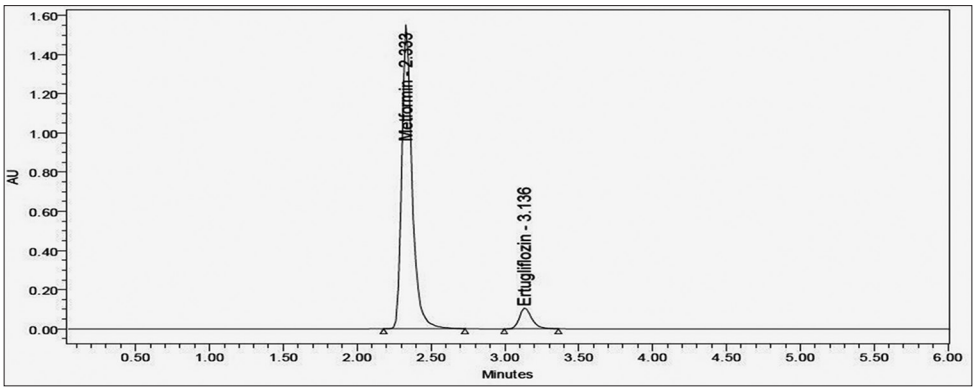

Fig. 1: Typical chromatogram of metformin hydrochloride and ertugliflozin

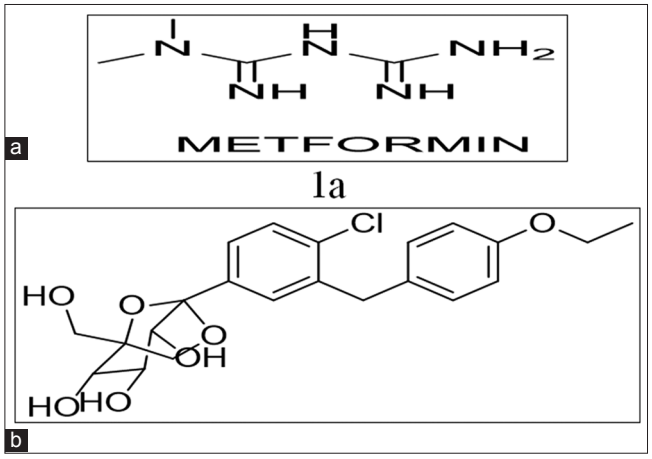

Fig. 2: Chemical structures of (a) metformin HCL, (b) ertugliflozin

Chromatographic conditions

Optimized chromatographic conditions for the separation were used as Standard BDS C_8 (150 mm × 4.6 mm, 5 µm particle size) column. Temperature was maintained ambient, mobile phase used was buffer: acetonitrile (55:45 v/v), and the flow rate was maintained at 1 ml/min. The diluent used throughout the method was water: acetonitrile (50:50 v/v) and the runtime was 6 min. All the samples and mobile phase were degassed for 30 min and filtered by ultrasonic filtration using 0.45-µm nylon (N66) 47-mm membrane filter. Detection was carried out at 224 nm using PDA detector with an injection volume of 10 µl. Using the above-optimized conditions, method was developed.

Preparation of buffer

Buffer: ($0.01\%KH_2PO_4$)

About 1.36 g of potassium dihydrogen phosphate was weighed and transferred into a 1000 ml volumetric flask added about 100 ml of milli-Q water and finally made the volume up to 1000 ml with milli-Q water.

Orthophosphoric acid buffer

1 ml of orthophosphoric acid was diluted to 1000 ml with HPLC grade water.

Preparation of mobile phase

Buffer (55%) and acetonitrile (45%) were mixed and degassed in an ultrasonicate water bath for 10 min and then filtered through 0.45 µ filter under vacuum filtration.

Diluent

Diluent used throughout the method was water: acetonitrile (50:50 v/v) chosen purely based on the solubility of the drugs.

Preparation of standard solutions

Accurately weighed and transferred 500 mg of metformin hydrochloride and 7.5 mg of ertugliflozin working standards into a 100 ml clean dry volumetric flasks; 10 ml of diluent was added and sonicated for 10 min and made up to the final volume with diluent.

Preparation of sample solution

A total of 10 tablets were weighed, and their mean weight was determined and crushed in mortar. An amount of powder weight equivalent to 500 mg of metformin hydrochloride and 7.5 mg of ertugliflozin was taken and transfered to 100 ml volumetric flask. The powder obtained was dissolved in mobile phase and sonicated for 20 min for complete extraction. The solution was made up to the volume with mobile phase. The solution was filtered through membrane filter. The stock solution was further diluted with diluent to get a concentration of 500 µg/ml of metformin hydrochloride and 7.5 µg/ml of ertugliflozin.

Quantitative estimation

$$\%\text{Assay}\frac{AT}{AS}\times\frac{WS}{DS}\times\frac{DT}{WT}\times\frac{P}{100}\times100$$

Where

AT = Average area of each main peak obtained from chromatogram of the sample solution

AS = Average area of each main peak obtained from chromatograms of the standard solution

WS = Weight of each metformin hydrochloride and ertugliflozin in standard solution (mg/ml)

WT = Weight of each metformin hydrochloride and ertugliflozin in sample (mg/ml)

DS = Dilution of standard solution

DT =Dilution of test solution

P = Potency of each standard

Method validation [17,18]

System suitability

System suitability tests are a fundamental part of liquid chromatographic method. It ensures that system is working correctly. System suitability parameters such as number of theoretical plates, retention time, and tailing factor were evaluated. This was performed by injecting mixture of standard in six replicates.

Linearity

The linearity of the proposed method was determined by quantitative dilution of the standard solution of metformin hydrochloride and ertugliflozin to obtain a solution in concentration range of 125–750 µg/ml and 1.875–11.25 µg/ml for metformin hydrochloride and ertugliflozin, respectively. A graph of peak area versus concentration in µg/ml was plotted for all three drugs in triplicate. The slope, intercept, and correlation coefficient of the regression line were determined.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ represent the concentration of analyte that would yield signal-to-noise ratio of 3 for LOD and 10 for LOQ. LOD and LOQ were calculated using the following formula,

LOD = 3.3 σ/S
LOQ = 10 σ/S

Where σ = standard deviation of response (peak area) and S = average slope of the calibration curve.

Method precision
The method precision of the proposed method was determined by injecting six replicates of sample and standard on the same day to ensure that the analytical method is repeatable.

System precision
The system precision is checked by injecting six replicates of standard solution to ensure that the analytical system is working properly.

Accuracy
The accuracy of this method was performed at three different levels (50%, 100%, and 150%), by the addition of a known amount of standard to the sample at each level. Each level was repeated 3 times (n=3).

Robustness
Robustness is the measure of optimized method capacity to remain unaffected by small but deliberate variations in method parameters such as mobile phase flow rate (±0.2 ml/min), wavelength nm (±1 nm), and column oven temperature (±1°C).

Degradation studies [19]

Oxidative degradation

To 1 ml of stock solution of metformin hydrochloride and ertugliflozin, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min. For HPLC study, the resultant solution was diluted to obtain (500 µg/ml and 7.5 µg/ml) solution and 10 µl were injected into the system, and the chromatograms were recorded to assess the stability of sample.

Acid degradation

As described in the oxidative degradation, the same method was followed using 2N hydrochloric acid and refluxed for 30 min.

Alkali degradation

The same procedure was followed as the above, in which 1 ml of 2N sodium hydroxide was used and refluxed for 30 min.

Thermal degradation

The standard drug solution was placed in an oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 500 µg/ml and 7.5 µg/ml of solution and 10 µl was injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Photo stability
The photochemical stability of the drug was also studied by exposing the solution to UV light by keeping the beaker in UV Chamber for 7 days in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 500 µg/ml and 7.5 µg/ml of solutions and 10 µl was injected into the system, and the chromatograms were recorded to assess the stability of sample.

Water degradation

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60°C. For HPLC study, the resultant solution was diluted to 500 µg/ml and 7.5 µg/ml of solution and 10 µL was injected into the system, and the chromatograms were recorded to assess the stability of the sample.

RESULTS

System suitability
The system suitability was performed by injecting mixed standard solution containing 500 µg/ml of metformin hydrochloride and 7.5 µg/ml of ertugliflozin in six replicates. The acceptance criteria for evaluating system suitability are percentage relative standard deviation (% RSD) <2, tailing factor <2, resolution >2, and theoretical plate >2000.

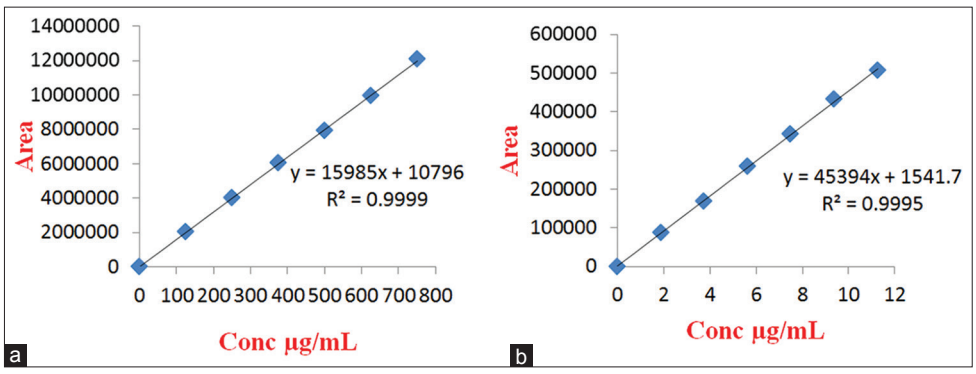

Fig. 3: Graphs representing calibration curve of (a) metformin HCl and (b) ertugliflozin

Table 1: Results of system suitability studies

Replicates (n=6)	Metformin hydrochloride			Ertugliflozin			Resolution
	RT (min)	USP plate count	USP tailing	RT (min)	USP plate count	USP tailing	
1	2.334	5356	1.34	3.136	7732	1.25	5.7
2	2.335	5640	1.33	3.138	7487	1.25	5.6
3	2.336	5406	1.32	3.142	7428	1.24	5.7
4	2.336	5544	1.34	3.142	7225	1.27	5.7
5	2.336	5241	1.36	3.142	6981	1.25	5.7
6	2.338	5359	1.32	3.143	6985	1.26	5.7

The result indicates that the system suitability parameters are within the acceptable limits, hence ideal for the chromatographic samples. The results are summarized in Table 1.

Linearity
Linearity of the proposed method was determined by constructing calibration graph between the tested concentration level and corresponding peak areas for metformin hydrochloride and ertugliflozin in triplicate. The results showed an excellent correlation between peak areas and concentrations level within the tested concentration range of 125–750 µg/ml for metformin hydrochloride and 1.875–11.25 µg/ml for ertugliflozin. The correlation coefficients were >0.999 for all two drugs, which meet the method validation acceptance criteria, and hence, the method is said to be linear for the drugs [Fig. 3a and b].

LOD and LOQ
The LOD and LOQ were found to be 1.70 µg/ml and 5.16 µg/ml for metformin hydrochloride and 0.07 µg/ml and 0.21 µg/ml for ertugliflozin which indicates that the method is sensitive. The results are summarized in Table 2.

Method precision
The %RSD value for six replicates injection of sample and standard carried out on the same day was found to be <2%, which indicates that the method is repeatable. The results for method precision are given in Table 3.

System precision
System precision was determined by measuring the peak area of six replicate injections of standard solution. The value of %RSD was found to be <2, which ensures that the analytical system is working properly. The results of system precision are tabulated in Table 3.

Table 2: LOD and LOQ results

Drug	LOD	LOQ
Metformin HCl (µg/ml)	1.70	5.16
Ertugliflozin (µg/ml)	0.07	0.21

LOD: Limit of detection LOQ: Limit of quantitation

Accuracy
The accuracy of this method was determined by calculating percent recovery of metformin hydrochloride and ertugliflozin in formulation at three different levels (50%, 100%, and 150%). The percentage recovery obtained was found to be in the range of 99.13–101.63% for metformin hydrochloride and 99.05–101.10% for ertugliflozin. The accepted limits of mean recovery are 98–102% and the obtained results were within the acceptable range, which indicates that recovery values were good, affirming the accuracy of the developed method. The results are summarized in Tables 4 and 5.

Robustness
The method was found to be robust when minor changes were made in optimized chromatographic conditions such as oven temperature (±5°C), mobile phase flow rate (±0.1 ml/min), and ratio of mobile phase (±5 ml). It was observed that there was no marked change in the analytical data of the drugs which indicate good reliability during normal usage. The results are shown in Table 6.

Force degradation studies
Force degradation of metformin hydrochloride and ertugliflozin under the conditions of hydrolysis acidic, basic, oxidation, photolysis, thermal, and humidity was carried out.

In acidic conditions (2N hydrochloride for 3 h), it was found that 5.97% of metformin hydrochloride and 5.37% of ertugliflozin content were degraded, respectively.

In basic condition (2N sodium hydroxide for 3 h), it was found that 4.66% of metformin hydrochloride and 4.19% of ertugliflozin content were degraded, respectively.

The drug sample when subjected to oxidation (20% of hydrogen peroxide), it was found that 3.64% of metformin hydrochloride and 3.86% of ertugliflozin content were degraded, respectively.

In thermal condition (at 105°C for 6 h), it was found that 3.81% of metformin hydrochloride and 2.63% of ertugliflozin content were degraded, respectively.

Table 3: Precision data of metformin and ertugliflozin

Replicates (n=6)	Intraday		Interday	
	Area of metformin HCl	Area of ertugliflozin	Area of metformin HCl	Area of ertugliflozin
1	8061896	345429	8083648	342481
2	8093162	346408	8025772	347098
3	8054073	343925	8045907	340545
4	8045184	343865	8066204	347348
5	8053730	344007	8095717	347348
6	8070321	345636	8022367	344224
Mean	8063061	344878	8056603	344841
S.D	17015.1	1087.4	30300.4	2900.7
%RSD	0.2	0.3	0.4	0.8

N: Number of injections, SD: Standard deviation, % RSD: Percentage relative standard deviation

Table 4: Accuracy data for metformin hydrochloride

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %recovery
50%	250	253.73	101.49	100.15%
	250	254.06	101.63	
	250	252.96	101.18	
100%	500	498.89	99.78	
	500	495.99	99.20	
	500	495.67	99.13	
150%	750	747.76	99.70	
	750	746.09	99.48	
	750	748.11	99.75	

Table 5: Accuracy data for ertugliflozin

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery
50%	3.75	3.73	99.47	99.98%
	3.75	3.72	99.28	
	3.75	3.71	99.05	
100%	7.5	7.52	100.24	
	7.5	7.56	100.80	
	7.5	7.46	99.42	
150%	11.25	11.22	99.72	
	11.25	11.37	101.10	
	11.25	11.33	100.75	

Table 6: Robustness evaluation of method

Change in the chromatographic conditions	%RSD of metformin HCl	%RSD of ertugliflozin
Flow rate (-) 0.9 ml/min	0.3	0.6
Flow rate (+) 1.1 ml/min	0.1	0.9
Mobile phase (-) 60B: 40A	0.4	0.9
Mobile phase (+) 50B: 50A	0.5	0.8
Temperature (-) 25°C	0.6	0.4
Temperature (+) 35°C	0.5	0.4

RSD: Relative standard deviation

Table 7: Force degradation studies of metformin hydrochloride and ertugliflozin

Drug	Stress conditions	Area	% Recovered	% Degraded
Metformin HCl	Acid	7618829	94.05	5.95
	Alkali	7723637	95.34	4.66
	Oxidation	7806085	96.36	3.64
	Thermal	7792853	96.19	3.81
	UV	7868537	97.13	2.87
	Water	8001805	98.77	1.23
Ertugliflozin	Acid	328541	94.63	5.37
	Alkali	332643	95.81	4.19
	Oxidation	333787	96.14	3.86
	Thermal	338068	97.37	2.63
	UV	339944	97.91	2.09
	Water	343322	98.89	1.11

The drug sample when exposed to UV light for 7 days, it was found that 2.87% of metformin hydrochloride and 2.09% of ertugliflozin content were degraded, respectively.

The drug sample when placed under neutral conditions (refluxing the drug in water for 6 h at temperature 60°C), it was found that 1.23% of metformin hydrochloride and 1.11% of ertugliflozin content were degraded, respectively.

No interference was observed due to excipients and other components that are present in the pharmaceutical dosage form and as well in the degraded products, so it can be concluded that the developed method is stability indicating reversed-phase HPLC (RP-HPLC) method for simultaneous estimation of metformin hydrochloride and ertugliflozin in the formulation combination. The results for forced degradation studies are shown in Table 7.

CONCLUSIONS

No method is available for the estimation of metformin hydrochloride and ertugliflozin combination, so a new simple, precise, accurate, and repeatable RP-HPLC method for the estimation of these simultaneously have been developed and validated according to the ICH guidelines. All the validation parameters including system suitability, linearity, accuracy, precision, LOD, LOQ, and robustness were within the recommended limits of the ICH. The developed method has a total run time of 6 min, which permits the analysis of a large number of samples in a short period of time, thereby reducing solvent cost. Exposure of the drugs to different stress conditions such as acid, base, UV, thermal, photolytic, and water showed the drugs are stable in all the conditions. Developed method can be used for route analysis of these compounds simultaneously.

AUTHOR'S CONTRIBUTION

Author P. Venkateswara Rao done the literature survey, analysis of the study, and wrote the first draft of the paper. Author A. Lakshmana Rao designed the study plan, corrected the first draft of the paper, and prepared the final manuscript for submission.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in the present investigation.

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