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Stability Indicating RP-HPLC Method for Simultaneous Estimation of Metformin and Ertugliflozin

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Abstract

A simple, rapid, precise and accurate stability indicating reverse phase high performance liquid chromatography (HPLC) method was developed for the simultaneous estimation of Metformin and Ertugliflozin. Isocratic separation was achieved on Denali C18 (150 x 4.6 mm, 5 µm) column with mobile phase comprising of 0.01 N KH_2PO_4: acetonitrile (60:40 V/V), pH adjusted 5.4 with 0.01% ortho phosphoric acid. The flow rate was maintained at 1 mL/min and analytes were screened with UV detector at 224 nm. The method was validated according to ICH guidelines with respect to linearity, accuracy, precision and specificity. The drugs were exposed to various stress conditions like, acid, alkali, oxidation, thermal, UV and neutral and the stressed samples were analysed by the proposed method. No co-eluting, interfering peaks from excipients, impurities were observed during stress conditions and all the degraded peaks are well resolved from parent peaks.

Keywords: Metformin; Ertugliflozin; Validation; HPLC.

Introduction

Metformin is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes mellitus[1]. Chemically it is 1,1-dimethylbiguanide (Fig. 1).[2] Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.[3]

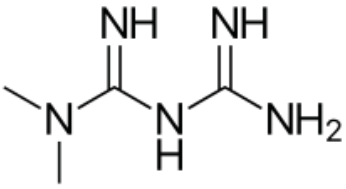

Fig. 1: Chemical structure of Metformin

Ertugliflozin is potent and selective inhibitors of the sodium-dependent glucose co transporters (SGLT).[4] Chemically it is (1S, 2S, 3S, 4R, 5S)-5-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-1-(hydroxymethyl)-6,8-dioxabicyclo [3.2.1] octane-2,3,4-triol (Fig. 2).[5] SGLT2 is the predominant transporter responsible for the resorption of glucose back into circulation from glomerular filtrate. Ertugliflozin inhibits the reabsorption of glucose mediated by this specific transporter, which increases the renal excretion of glucose and helps decrease glucose levels in circulation.[6] Ertugliflozin, in combination with Metformin hydrochloride, is indicated to improve glycemic control in patients with diabetes type 2 diabetes mellitus.[7]

Literature survey revealed that few HPLC methods were reported for simultaneous estimation of Metformin and Ertugliflozin in combined pharmaceutical dosage form.[8] The

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objective of the present work was to develop a simple, accurate, precise, sensitive and fast high-performance liquid chromatographic analytical method for simultaneous estimation of Metformin and Ertugliflozin and its degradation products formed under various stress conditions and validated the method as per ICH guidelines.[9,10]

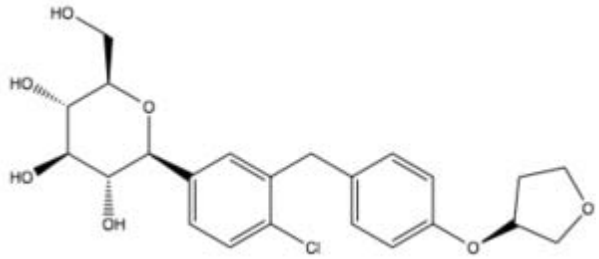

Fig. 2: Chemical structure of Ertugliflozin

Materials and Methods

Materials

Metformin and Ertugliflozin pure drugs were obtained from Shree Icon Pharmaceutical Laboratories, Vijayawada, India. Commercial formulations of Metformin and Ertugliflozin (Segluromet) tablets were procured from local pharmacy store. Acetonitrile, potassium dihydrogen ortho phosphate, ortho phosphoric acid and distilled water were obtained from Rankem Chemicals Ltd., Mumbai, India.

Instrumentation

The analysis of drugs was carried out on Waters HPLC 2695 system equipped with quaternary pumps, photodiode array detector and auto sampler integrated with Empower 2 software was used for data acquisition and recording chromatograms. A 20 µL hamilton syringe was used for injecting the samples. A double-beam PG Instruments T60 UV-Visible spectrophotometer was used for measuring absorbances of Metformin and Ertugliflozin solutions. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

Chromatographic conditions

The developed mobile phase consisted of 0.01 N KH_2PO_4: acetonitrile (60:40 V/V), pH adjusted 5.4 with 0.01% ortho phosphoric acid. The diluent selected was acetonitrile: water (50:50 V/V). For stationary phase, Denali C18 (150 x 4.6 mm ID, 5 µm particle size) column was selected as it showed the good resolution and sharp peaks of both the analytes within relatively shorter run times. The flow rate was kept at 1 mL/min and run time kept was 6 min. The sample injector volume was 20 µL with analysis being carried out ambient temperatures.

Preparation of standard stock solutions

Accurately weighed 250 mg of Metformin, 3.75 mg of Ertugliflozin and transferred to 100 mL volumetric flask. 3/4th of diluent was added to the flask and sonicated for 10 minutes. Flask was made up with diluent and labeled as standard stock solution (2500 µg/mL of Metformin and 37.5 µg/mL Ertugliflozin).

Preparation of standard working solutions

1 mL from each stock solution was pipetted out and taken into a 10 mL volumetric flask and made up with diluent (250 µg/mL of Metformin and 3.75 µg/mL of Ertugliflozin).

Preparation of sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 500 mL volumetric flask, 50 mL of diluent was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000 µg/mL of Metformin and 15 µg/mL of Ertugliflozin).

Preparation of sample working solutions

2.5 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent (250 µg/mL of Metformin and 3.75 µg/mL of Ertugliflozin).

Method development

The present method was developed by performing various trails with different mobile phases in different compositions and with different columns. Finally the mobile phase consisting of 0.01 N KH_2PO_4: acetonitrile (60:40 V/V), pH adjusted 5.4 with 0.01% ortho phosphoric acid and Denali C18 (150 x 4.6 mm ID, 5 µm particle size**)** column was selected for analysis. The flow rate was maintained at 1.0 mL/min and the response was detected at 224 nm which gave good resolution, sharp peaks, high theoretical plates, minimum tailing factor with short run time for the drugs Metformin and

Ertugliflozin. The optimized chromatographic conditions were shown in Table 1. The optimized chromatogram was shown in Fig. 3.

Method validation

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Metformin and Ertugliflozin and the solutions were injected six times and the parameters like USP plate count, peak tailing and resolution were determined. All the system suitability parameters were within the range and satisfactory as per ICH guidelines and the results are furnished in Table 2.

Specificity

Specificity is the parameter used to check the interference in the optimized method. We should not find interfering peaks in blank, placebo, standard and sample at retention times of these drugs in this method. So this method was said to be specific.

Table 1: Optimized chromatographic conditions

Parameter	Condition
Mobile phase	0.01 M KH_2PO_4: acetonitrile (60:40 V/V)
pH	5.4
Diluent	Acetonitrile: water (50:50 V/V)
Column	Denali C18 (150 x 4.6 mm, 5 µm)
Column temperature	30°C
Wave length	224 nm
Injection volume	10 mL
Flow rate	1.0 mL/min
Run time	6 min

Table 2: System suitability parameters

Parameter	Metformin	Ertugliflozin
Retention time (tR)	2.357 min	3.209 min
Theoretical plates (N)	5675	7593
Tailing factor (T)	1.33	1.26
Resolution (Rs)	0.0	5.7

Table 3: Linearity results for Meformin and Ertugliflozin

S. No.	Metformin		Ertugliflozin	
	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
1	62.5	359928	0.9375	42108
2	125	729371	1.8750	84536
3	187.5	1086779	2.8125	124092
4	250	1466140	3.7500	169023
5	312.5	1827149	4.6875	208889
6	375	2159508	5.6250	250292

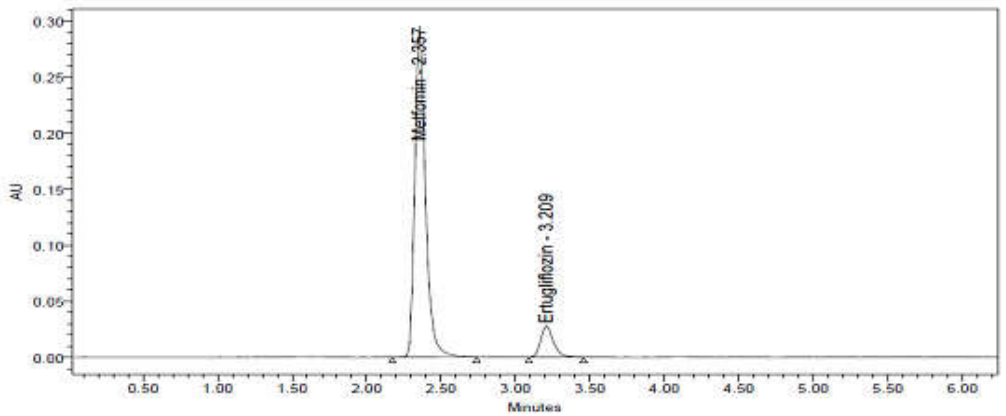

Fig. 3: Optimized chromatogram of Metformin and Ertugliflozin

Linearity

Six linear concentrations of Metformin (62.5-375 µg/mL) and Ertugliflozin (0.9375-5.625 µg/mL) were injected in a duplicate manner. The method was found to be linear and the results were furnished in Table 3 and linearity curves were shown in Fig. 4 and 5.

Precision

Precision of method was studied by performing system precision, repeatability and intermediate precision by injecting the 6 replicates of standard solution in the same day and six different days. The %RSD values were tabulated in Table 4 to 6.

Table 4: System precision results of Metformin and Ertugliflozin

S. No.	Area of Metformin	Area of Ertugliflozin
1	1470127	168813
2	1457186	167312
3	1449577	168896
4	1460165	169996
5	1476850	167320
6	1469803	168919
Mean	1463951	168543
SD	10054.7	1045.1
%RSD	0.7	0.6

Table 5: Repeatability values of Metformin and Ertugliflozin

S. No.	Area of Metformin	Area of Ertugliflozin
1	1414760	161685
2	1408575	162978
3	1398103	160958
4	1418917	160198
5	1400044	159335
6	1411347	160984
Mean	1408624	161023
SD	8189.3	1248.7
%RSD	0.6	0.8

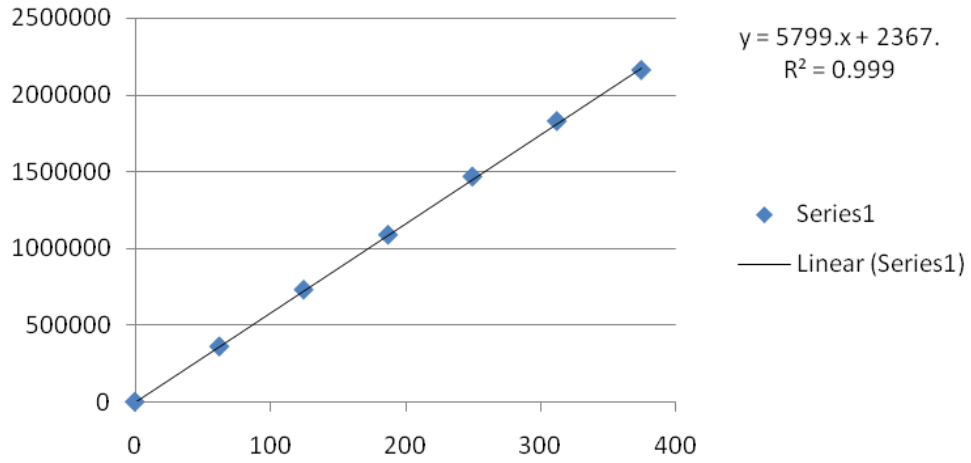

Fig. 4: Linearity curve of Metformin

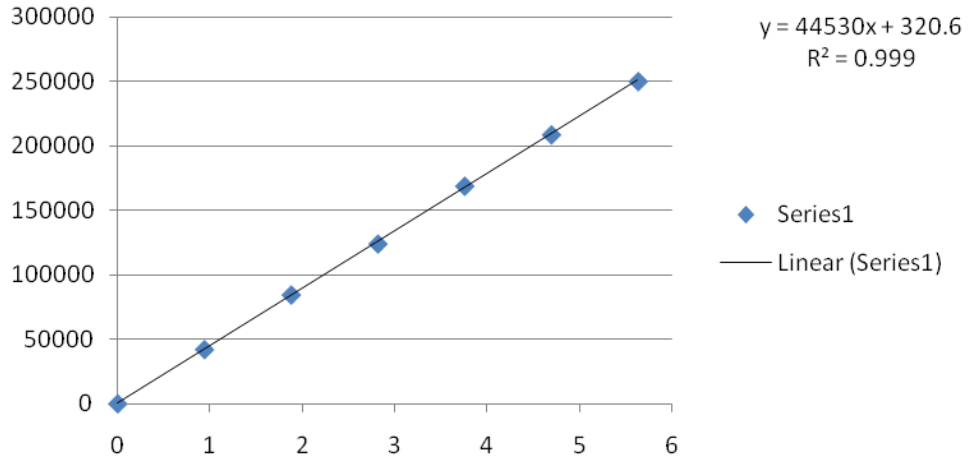

Fig. 5: Linearity curve of Ertugliflozin

Accuracy

The accuracy of the method was established by calculating percentage recovery of Metformin and Ertugliflozin by the method of addition. Known amount of Metformin and Ertugliflozin at 50%, 100% and 150% was added to a prequantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery at each level should be not less than 98% and not more than 102% and the results are tabulated in Table 7 & 8.

Table 6: Intermediate precision values of Metformin and Ertugliflozin

S. No.	Area of Metformin	Area of Ertugliflozin
1	1479729	167139
2	1488454	167420
3	1489567	168787
4	1481190	168030
5	1482777	167749
6	1487053	167689
Mean	1484795	167802
SD	4098.6	569.8
%RSD	0.3	0.3

Table 7: Accuracy results of Metformin

% Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery
50%	125	126.12	100.89	
	125	125.02	100.01	
	125	125.86	100.69	
100%	250	254.24	101.69	
	250	250.29	100.11	100.30%
	250	253.41	101.37	
150%	375	372.55	99.35	
	375	372.36	99.30	
	375	372.30	99.28	

Table 8: Accuracy results of Ertugliflozin

% Level	Amount Spiked (µg/mL)	Amount Recovered (µg/mL)	% Recovery	% Mean Recovery
50%	1.875	1.87	99.80	
	1.875	1.84	98.35	
	1.875	1.87	99.80	
100%	3.75	3.79	100.95	
	3.75	3.79	101.19	100.49%
	3.75	3.82	101.88	
150%	5.625	3.79	100.95	
	5.625	3.79	101.19	
	5.625	3.82	101.88	

Ruggedness

Ruggedness of the method was confirmed by the analysis of samples was done by different analysts. Samples of Metformin and Ertugliflozin were analyzed by different analysts. It was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature.

Robustness

To demonstrate the robustness of the method, samples of Metformin and Ertugliflozin were injected at different variable conditions like using different conditions like changes in flow rate, mobile phase composition and temperature. The robustness results were furnished in Table 9.

Table 9: Robustness results of Metformin and Ertugliflozin

S. No.	Condition	%RSD of Metformin	%RSD of Ertugliflozin
1	Flow rate (-) 0.9 mL/min	0.1	0.6
2	Flow rate (+) 1.1 mL/min	0.2	0.6
3	Mobile phase (-) 65B:35A	0.4	0.7
4	Mobile phase (+) 55B:45A	0.7	0.5
5	Temperature (-) 25°C	0.4	0.7
6	Temperature (+) 35°C	0.6	0.8

Limit of detection (LOD)

0.25 mL each from two standard stock solutions was pipetted out and transferred to two separate 10 mL volumetric flasks and made up with diluent. From the above solutions 0.1 mL each of Metformin and Ertugliflozin solutions respectively were transferred to 10 mL volumetric flasks and made up with the same diluent.

Limit of quantitation (LOQ)

0.25 mL each from two standard stock solutions was pipetted out and transferred to two separate 10 mL volumetric flasks and made up with diluent. From the above solutions 0.3 mL each of Metformin and Ertugliflozin solutions respectively were transferred to 10 mL volumetric flasks and made up with the same diluent.

LOD and LOQ values are calculated from calibration curve method.

Estimation of Metformin and Ertugliflozin in tablet dosage form

About 20 tablets (Segluromet) containing Metformin 500 mg and Ertugliflozin 7.5 mg were weighed

and average weight was determined. The tablets were powdered and an accurate equivalent weight containing 250 mg of Metformin and 3.75 mg of Ertugliflozin was transferred to 100 mL volumetric flask and dissolved in diluent. The contents were sonicated for 15 min and filtered through 0.45µ membrane filter. An aliquot was taken and further diluted with diluent to get final concentration of 2500 µg/mL of Metformin and 37.5 µg/mL Ertugliflozin. The solutions were analysed using optimized chromatographic conditions. The data of analysis of marketed formulation was given in Table 10 & 11.

Table 10: Assay results of Metformin

S. No.	Standard area	Sample area	%Assay
1	1470127	1479729	100.98
2	1457186	1488454	101.57
3	1449577	1489567	101.65
4	1460165	1481190	101.08
5	1476850	1482777	101.18
6	1469803	1487053	101.48
Mean	1463951	1484795	101.32
SD	10054.7	4098.6	0.28
%RSD	0.7	0.3	0.28

Table 11: Assay results of Ertugliflozin

S. No.	Standard area	Sample area	%Assay
1	168813	167139	99.07
2	167312	167420	99.23
3	168896	168787	100.04
4	169996	168030	99.60
5	167320	167749	99.43
6	168919	167689	99.39
Mean	168543	167802	99.46
SD	1045.1	569.8	0.34
%RSD	0.6	0.3	0.34

Degradation studies

Acid degradation studies

To 1 mL of stock solution Metformin and Ertugliflozin, 1 mL of 2 N hydrochloric acid was added and refluxed for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 250 µg/mL Metformin solution & 3.75 µg/mL Ertugliflozin solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies

To 1 mL of stock solution Metformin and Ertugliflozin, 1 mL of 2N sodium hydroxide was added and refluxed for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 250 µg/mL Metformin solution & 3.75 µg/mL Ertugliflozin solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies

To 1 mL of stock solution of Metformin and Ertugliflozin, 1 mL of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^{0}C. The resultant solution was diluted to obtain 250 µg/mL Metformin solution & 3.75 µg/mL Ertugliflozin solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation studies

To 1mL of stock solution of Metformin and Ertugliflozin was placed in oven at 105°C for 6 hrs. The resultant solution was diluted to obtain 250 µg/mL Metformin solution & 3.75 µg/mL Ertugliflozin solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo stability studies

To 1mL of stock solution of Metformin and Ertugliflozin was exposed to UV light by keeping the beaker in UV Chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber. The resultant solution was diluted to obtain 250 µg/mL Metformin solution & 3.75 µg/mL Ertugliflozin solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral degradation studies

To 1 mL of stock solution Metformin and Ertugliflozin was refluxed in water for 6 hrs at a temperature of 60^{o}C. The resultant solution was diluted to obtain 250 µg/mL Metformin solution & 3.75 µg/mL Ertugliflozin solution and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

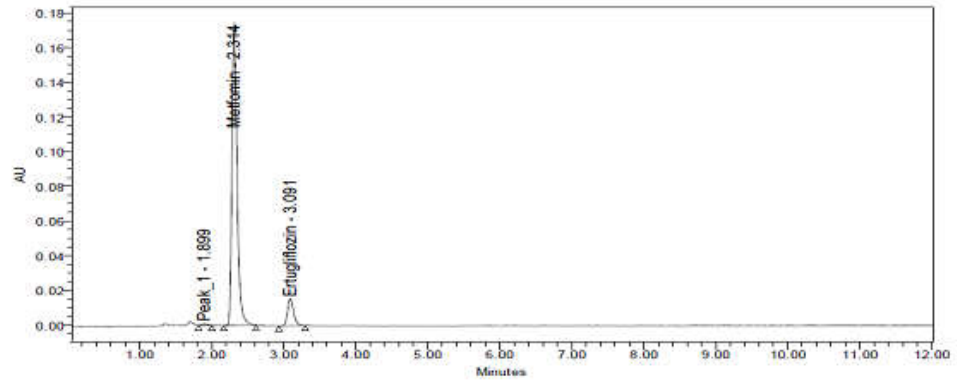

Fig. 6: Acid degradation chromatogram of Metformin and Ertugliflozin

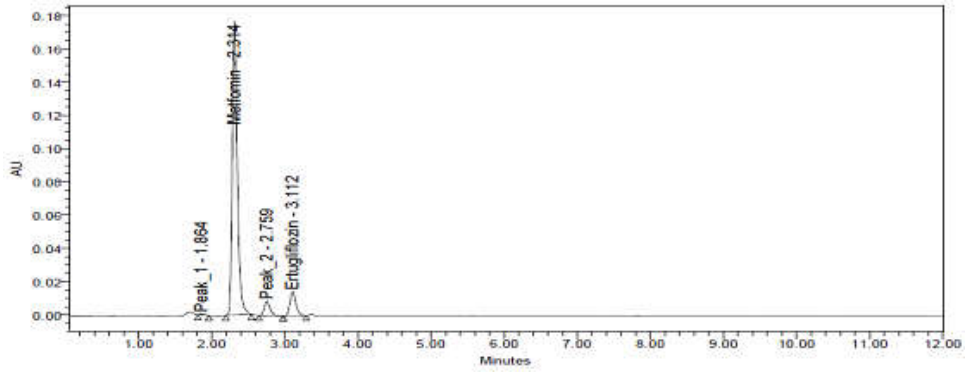

Fig. 7: Alkali degradation chromatogram of Metformin and Ertugliflozin

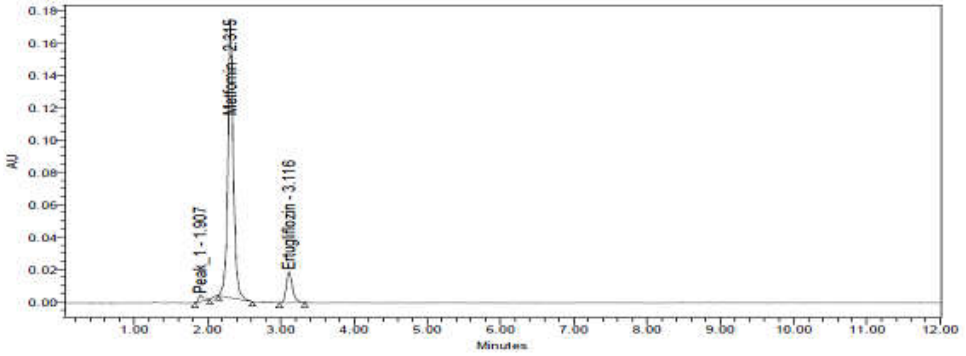

Fig. 8: Oxidative degradation chromatogram of Metformin and Ertugliflozin

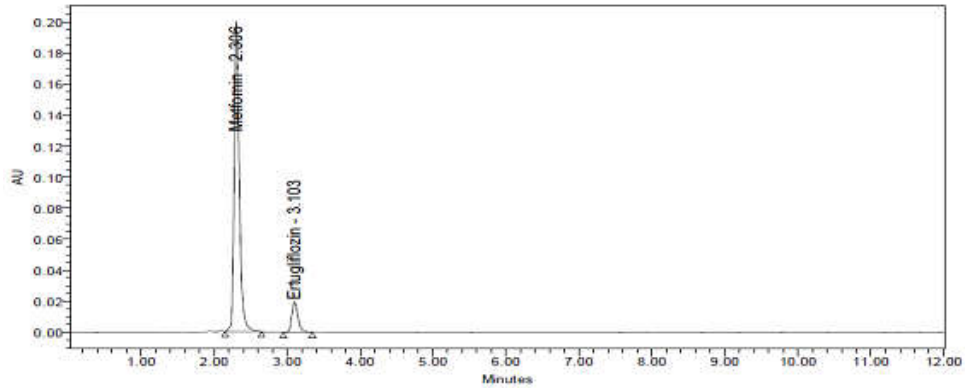

Fig. 9: Thermal degradation chromatogram of Metformin and Ertugliflozin

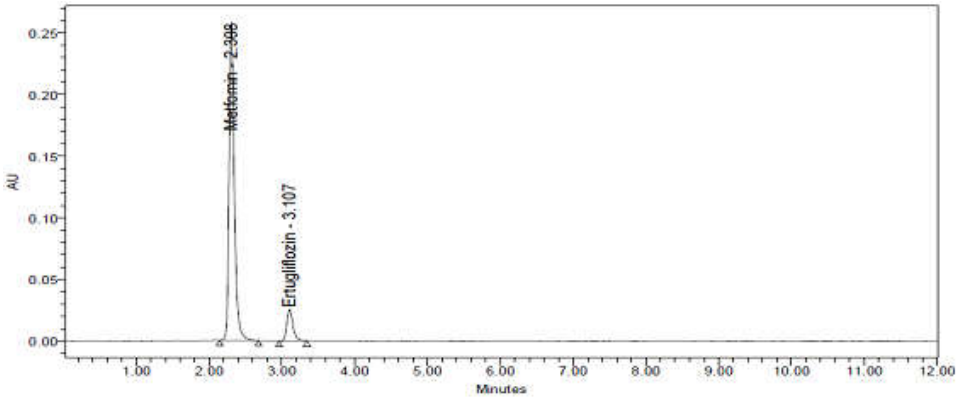

Fig. 10: UV degradation chromatogram of Metformin and Ertugliflozin

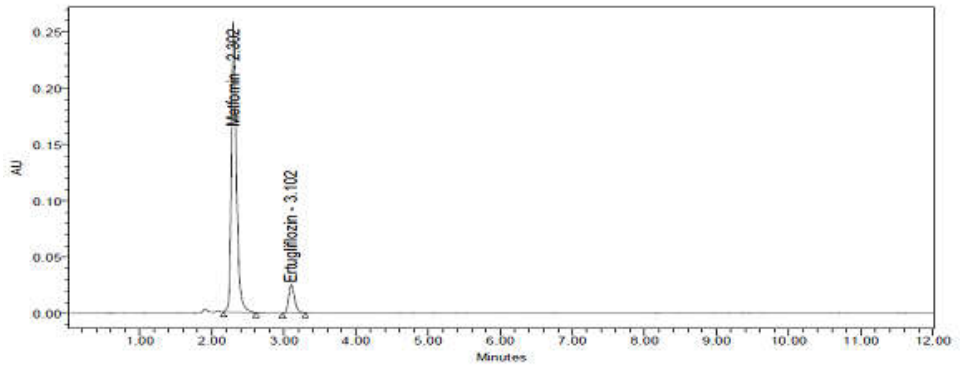

Fig. 11: Neutral degradation chromatogram of Metformin and Ertugliflozin

Degradation studies results of Metformin and Ertugliflozin was tabulated in Table 12. The typical chromatograms of degradation behavior of Metformin and Ertugliflozin in different stress conditions were shown in Figs. 6 to 11.

Table 12: Degradation data of Metformin and Ertugliflozin

Stress condition	% Metformin degraded	% Ertugliflozin degraded
Acid	3.31	4.25
Alkali	2.37	3.58
Oxidation	3.43	4.06
Thermal	0.99	2.91
Photo stability	0.55	1.31
Neutral	0.15	0.19

Results and Discussion

A stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Metformin and Ertugliflozin by using mobile phase consisting of 0.01 N KH_2PO_4 and acetonitrile in the ratio of 60:40 V/V. The retention times for Metformin and Ertugliflozin were found to be 2.357 min and 3.209 min respectively. The proposed method was validated as per ICH guidelines. The theoretical plates for Metformin and Ertugliflozin were found to be 5675 and 7593 respectively, which indicates the efficient performance of the column. Linearity range was found to be 62.5-375 µg/mL for Metformin and 0.9375-5.6250 µg/mL for Ertugliflozin. The %RSD values for system precision of Metformin and Ertugliflozin were found to be 0.7 and 0.6 respectively. The %RSD values for repeatability of Metformin and Ertugliflozin were found to be 0.6 and 0.8 respectively. The %RSD values for intermediate precision of Metformin and Ertugliflozin were found to be 0.3 and 0.3 respectively and hence the proposed method is precise. The mean percentage recoveries of Metformin and Ertugliflozin were found to be 100.30% and 100.49% respectively and the method is found to be accurate. LOD for Metformin and Ertugliflozin were found to be 0.72 µg/mL and 0.01 µg/mL respectively. LOQ for Metformin and Ertugliflozin were found to be 2.18 µg/mL and 0.04 µg/mL respectively. Degradation studies were carried out in acid, alkali, oxidative, thermal, photo stability and neutral stressed conditions. The results revealed that both the drugs are stable

in described conditions. Thus it is evident that the described method can be adopted for routine estimation of Metformin and Ertugliflozin in combined pharmaceutical formulation.

Conclusion

The newly developed RP-HPLC method for the simultaneous estimation of Metformin and Ertugliflozin is found to be simple, economical, precise, accurate and robust. There was no interference from placebo and diluents, hence the method was specific. Hence the proposed method can be applied for the simultaneous estimation of Metformin and Ertugliflozin in pharmaceutical formulation.

References

1. Nasri H and Kopaei MR. Metformin: current knowledge. Journal of Research in Medical Sciences. 2014;19(7):658–64.
2. Rena G, Hardie DG and Pearson ER. The mechanisms of action of Metformin. Diabetologia. 2017;60(9):1577–85.
3. Rena G, Pearson ER and Sakamoto K. Molecular mechanism of action of Metformin. Diabetologia. 2013;56(9):1898–1906.
4. Scott LJ. Ertugliflozin in type 2 diabetes; a profile of its use. Drugs and Therapy Perspectives. 2019; 35(8):351–62.
5. Cinti F, Moffa S, Impronta F, et al. Spotlight on Ertugliflozin and its potential in the treatment of type 2 diabetes: evidence to date. Drug Design, Development and Therapy. 2017;11:2905–19.
6. Miao Z, Nucci G, Amin N, et al. Pharmacokinetics, metabolism and excretion of the antidiabetic agent Ertugliflozin in healthy male subjects. Drug Metabolism & Disposition. 2013;41(2):445–56.
7. Frias JP. Fixed-dose combination of Ertugliflozin and Metformin hydrochloride for the treatment of type 2 diabetes. Expert Review of Endocrinology and Metabolism. 2019;14(2):75–83.
8. Jagadeesh K and Annapurna N. Stability indicating method development and validation of Metformin and Ertugliflozin by HPLC with PDA detection and its application to tablet dosage form. Asian Journal of Pharmaceutical and Clinical Research. 2019; 12(3);353–58.
9. ICH Harmonised Tripartite Guideline, Validation of analytical procedures: Text and methodology, Q2 (R1), International Conference on Harmonization, Geneva, 2005, pp. 1–13.
10. ICH Harmonised Tripartite Guideline, Stability Testing of New Drug Substances and Products, Q1A (R2), International Conference on Harmonization, Geneva, 2003, pp. 1–18.