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Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Ertugliflozin And Metformin In Pharmaceutical Dosage Form Using Qbd Approach

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ABSTRACT-

In this study, an improved simple, specific, rapid, sensitive, precise, accurate and stability indicating RP-HPLC method for the simultaneous estimation of ertugliflozin L-pyroglutamic acid and metformin hydrochloride in pharmaceutical dosage forms was developed and validated. The separation of ertugliflozin and metformin HCl was achieved isocratically on Agilent Poroshell C18 (150 mm × 4.6 mm, 5µm) using 0.1% TFAA in Water (40:60) ratio of mobile phase, inject with flow rate of 1 ml/min at column temperature 30 ±2°C. About 20µl standard solution of the drugs was injected, and the eluted analytes were detected at 223 nm. In developed RP-HPLC method mobile phase ratio at (45:55 % v/v) at flow rate 0.8 ml/min gives retention time for Metformin is 3.18 and Ertugliflozin is 4.43 min which was analysed by using design expert-7 software. The linearity of the developed method was confirmed over the concentration range of 266.40-399.60 µg/mL for Metformin and 4.00-6.00 µg/mL for Ertugliflozin with a correlation coefficient (r^2) of 0.99996 for Metformin and 99988 for Ertugliflozin. The percentage RSD for the precision of the method were found less than 2.0 %. The percentage recoveries were found to be in the range of 98.49-100.71 % for Metformin and 98.19-101.75 % for Ertugliflozin. The LOD and LOQ were found to be 1.546 ug/mL and 4.686 ug/mL for Metformin and 0.040 ug/mL and 0.122 ug/mL for Ertugliflozin.

This method is less time consuming; it can be performed routinely in the industry for routine quality control/analysis of bulk drug and marketed product of Ertugliflozin and Metformin.

Keywords- RP-HPLC, QBD, Metformin, Ertugliflozin, Methanol, Development, Validation.

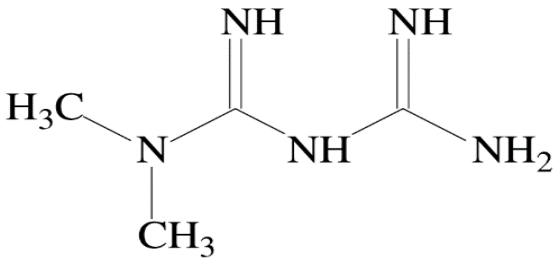
INTRODUCTION

The compound metformin having chemical Name 3-(diaminomethylidene)-1,1-dimethyl guanidine; hydrochloride. It gives antidiabetic activity. Metformin reduces blood glucose levels with minimizing hepatic glucose production also reducing the intestinal absorption of glucose, and excessing insulin sensitivity with increasing peripheral glucose uptake and utilization. Metformin inhibits mitochondrial complex I activity and above processes lead to a decrease in blood glucose, managing type II diabetes which exert positive effects on glycemic control.

The drug Ertugliflozin having chemical name (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.

Ertugliflozin is an inhibitor of SGLT2 that reduces renal reabsorption of filtered glucose and lowers the renal threshold for glucose, thereby increasing urinary glucose excretion.

As per the study of various literature review the numerous analytical methods developed for simultaneous estimation of these drugs in individual or combine form but this method has been submitted for the optimization of Ertugliflozin and Metformin in tablet dosage form. both medication shown in fig.1.



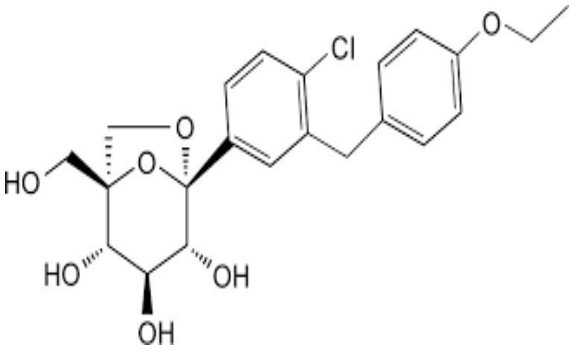


Fig.1 Metformin Ertugliflozin

MATERIALS & METHODS

Materials

The drug sample Metformin HCL & Ertugliflozin taken from Merck laboratories. The various chemicals & reagents like methanol, Acetonitrile & Trifluoracetic acid (TFAA) also taken from Merck laboratories & distil water taken from siddhi lab. The tablet sample for study purchase from local medical store.

Instrumentation

The HPLC system used for study was HPLC quaternary Gradient system model no 1260 Infinity II. Equipped with double beam UV visible spectrophotometer made up by Jasco & 20µL fixed loop for injections. Applying with Openlab EZ chrome software for chromatographic analysis. Azcet Analytical Balance & Bio-technic Ultra Sonicator with capacity upto 13.5 lit are also used during experimental analysis.

Method Development

Preparation of Stock Solutions

Abot 20 mg of metformin HCL & 25.91 mg ertugliflozin separately taken in volumetric flask with 15ml methanol and diluted it up to the mark (1000ppm). Further it diluted to make 20 ppm solution.

For analysis with HPLC The separation of ertugliflozin and metformin HCl was achieved isocratically on column Agilent Poroshell C18 (150 mm × 4.6 mm, 5µm) using 0.1% TFAA in Water (45:55) ratio of mobile phase, injected at a flow rate of 1 ml/min including column temperature at 30 ±2°C. About 20µl of standard solution of the drugs was injected, and the eluted analytes were detected at 223 nm. The isocratic elusion in reverse phase with selected mobile phase composition on c18 column gives accurate analysis of substance. The UV detector at 223nm provides necessary sensitivity to detection of concentration in substance.

The overlay spectra of both medications shown in fig.2.

Calibration Curve for Metformin & Ertugliflozin

The linearity of an analytical sample is its ability to obtain test results directly proportional to the concentration of analyte in the sample. The solution of metformin & ertugliflozin transfer into volumetric flask up to the mark with methanol. The suitable mobile phase is also added in each flask for

chromatographic analysis. Each level pumped in triplicate and mean area calculated. Calibration curve was plotted graphically as a function of analyte concentration in µg/mL on X-axis Vs mean area on y-Axis as given in results. The results shown in table 1. The calibration curves give accurate concentration of metformin & ertugliflozin base on their respective calibration curves.

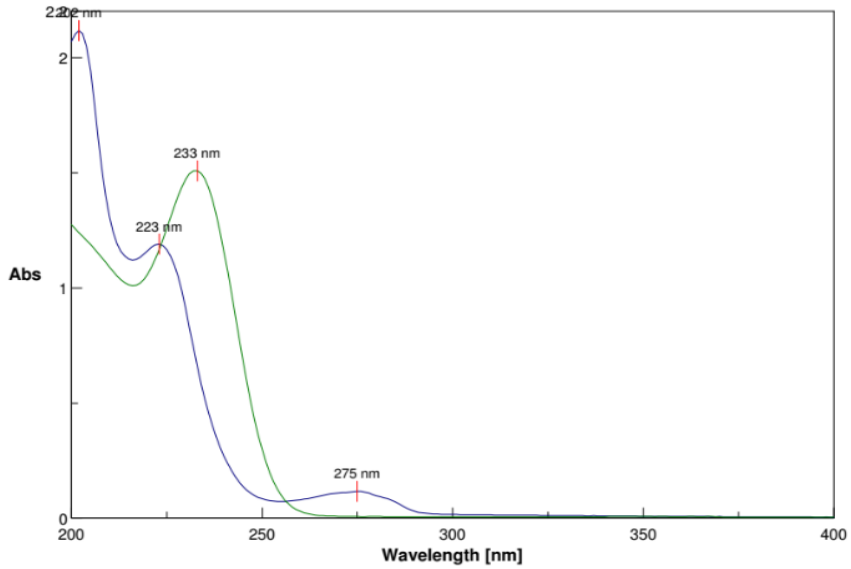


Fig 2 Overlay spectra of metformin & Ertugliflozin

Table 1 Calibration curve data for linearity

Parameter	Metformin	Ertugliflozin
Linearity range (µg/mL)	266.40-399.60	4.00-6.00
R^2	0.99996	0.99988
Slope	374671.447	420012.60
Intercept	2518911.200	49309.00

Analysis of marketed formulations

Marketed test sample having name Segluromet 7.5/500 mg having Ertugliflozin 7.5 mg and Metformin HCl 500 mg. Weighed the physical lab mixture equivalent to 333 mg of Metformin HCl and 5 mg of Ertugliflozin (499.5 mg of powder material). Transfer it into 100 mL of volumetric flask with 70 ml methanol sonicated it for 15 minutes by shaking. Made the volume up to the mark with methanol. Filter the solution by using 0.45 µ syringe filter discarding 3-5 mL of filtrate. Further diluted 1 ml of filtrate to 10 ml with mobile phase. This analytical process used for the determination of concentration of metformin and ertugliflozin in tablet sample based on their observed peak.

Validation of Methods

As per ICH guidelines an analytical method was validated. The validation process includes validation parameter outline by ICH. The % recovery of metformin & ertugliflozin calculated for evaluates accuracy.

The accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value. Accuracy will be conducted in the range from 80 % to 120 % of working concentration. The validation steps ensure that method reliably at different concentration level.

Intraday & Interday Precision

The precision study for metformin & ertugliflozin was carried out on both Interday and intraday calculate on their response three times in same day as well as different day.

LOD & LOQ

LOD and LOQ were established using the method in accordance with ICH Q2R1 recommendations. The LOD and LOQ were derived using the following formula based on the calibration curve, which was used to calculate the residual standard deviation of a regression line.

$LOD = 3.3\ \sigma / S$

$LOQ = 10\ \sigma / S$

Where,

σ = residual standard deviation of a regression line

S = Slope of regression line

Robustness

An analytical procedure's robustness indicates its ability to with stand minor but intentional changes in method parameters and shows how reliable it is under typical operating conditions.

The standard solution was pumped under various chromatographic conditions shown below.

a) Changes in flow rate by ±10%. (±0.08 ml/min)
b) Change in column oven temperature. (± 2ºC)
c) Change in wavelength (± 3 nm)

The robustness method for each drug was evaluated by change in concentration. This systematic approach provides methods ability to maintain variations in selected parameters. The overall result shown in table 2.

Table 2. Validation summary

Parameters	Metformin	Ertugliflozin
Linearity range	266.40-399.60 µg/mL	4.00-6.00µg/mL
Coefficient correlation	0.99996	0.99988
LOD	1.546 µg/mL	0.040 µg/mL
LOQ	4.686 µg/mL	0.122 µg/mL
% Recovery	99.64 %	99.93%
Precision RSD	0.768	1.248
Interday	0.573	0.461
Intraday	0.724	0.524
Robustness	Robust	Robust
Retention time ± %SD (min)	3.19	4.43
Theoretical plates	9919	13687

RESULT & DISCUSSION

The optimized mobile phase includes Methanol: 0.1% TFAA in water (45:55 % v/v) ratio with run time 0.8 ml/min which produce outstanding performance. As per the fig 2 the arrangement of metformin & ertugliflozin produce two well defined peak with low tailing factor. The design was discovered that retention time for metformin 3.19 & ertugliflozin 4.43 min. The UV wavelength absorption at 223 nm seen overlay spectra. So, this UV range finalized for the chromatographic analysis. According to table 1 the calibration curve of both drugs demonstrates linearity between 266.40-399.60 µg/mL & 4.00-6.00µg/mL respectively. The LOD 1.546 µg/mL & 0.040 µg/mL for metformin & ertugliflozin respectively as well as LOQ was 4.686 µg/mL & 0.122 µg/mL for metformin & ertugliflozin respectively.

Table no 2 shows overall validation parameter and its results. The robustness of metformin & ertugliflozin shows minor variations in peak & retention time. This developed HPLC method useful for simultaneous estimation of metformin & ertugliflozin under stated condition & verified Aas per ICH guidelines. The statistical analysis verified that this methos is accurate, precise, time consuming as well as simple, selective & sensitive.

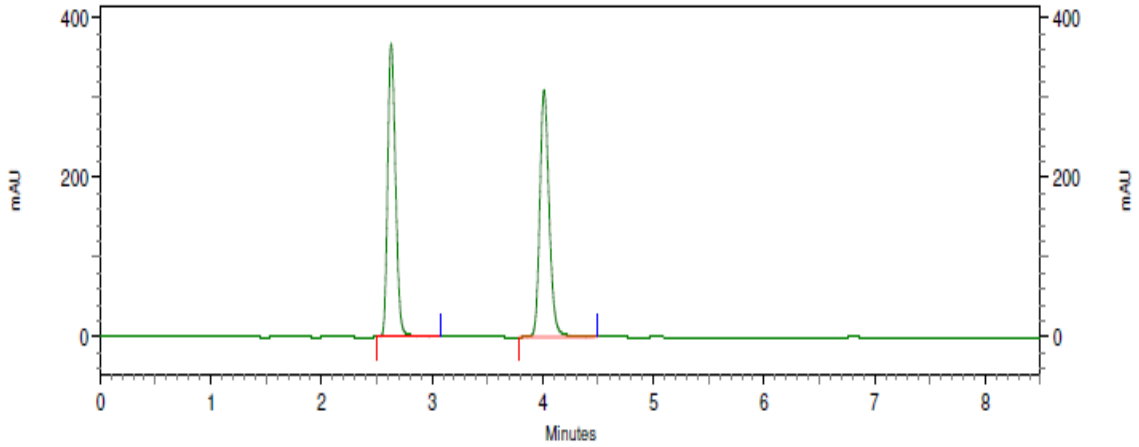
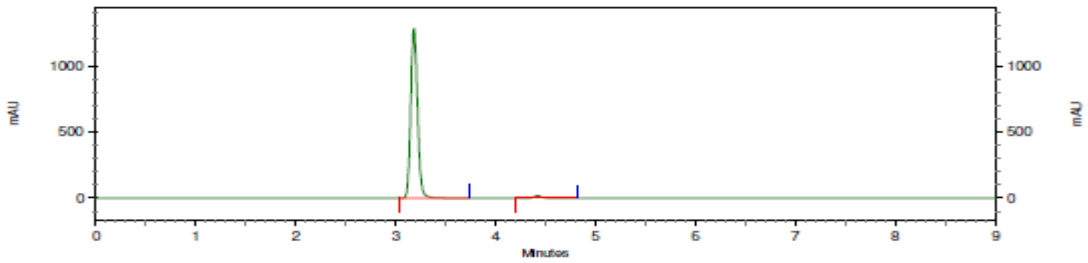


Fig. No. 3: Typical chromatogram of Mixture (Metformin and Ertugliflozin)

Sample Name: LINEARITY 80%_1

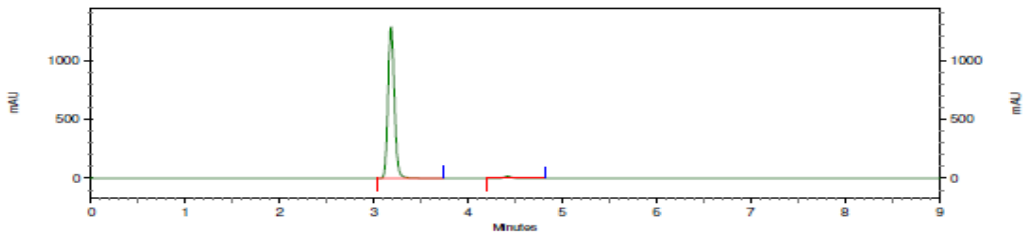


VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Metformin HCl	3.18	102510087	1.08	10472
Ertugliflozin	4.42	1729196	1.10	13898
Totals		104239283		

Fig no. 4: Typical chromatogram linearity 80%

Sample Name: ACCURACY 80%_1

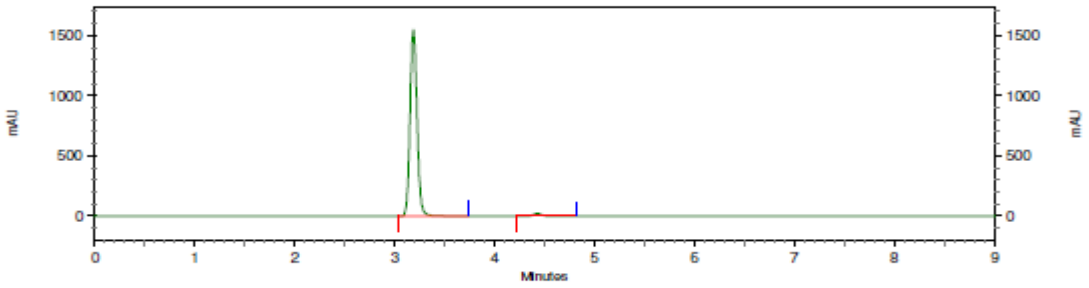


VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Metformin HCl	3.19	102458631	1.08	10479
Ertugliflozin	4.43	1719652	1.10	13883
Totals		104178283		

Fig no.5: Typical chromatogram Accuracy 80%

Sample Name: REP PRECISION TEST SAMPLE_1

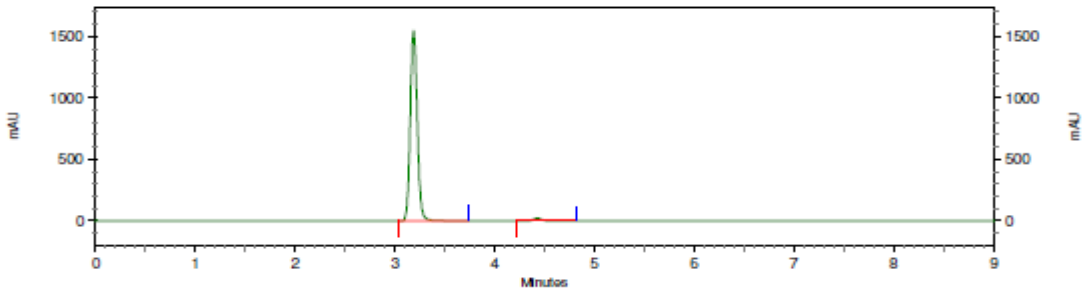


VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Metformin HCl	3.18	126025415	1.11	9830
Ertugliflozin	4.42	2093524	1.13	13569
Totals		128118939		

Fig. no. 6: Typical chromatogram of Repeatability precision

Sample Name: INT PRECISION TEST SAMPLE_1



VWD: Signal A, 223 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)
Metformin HCl	3.19	125300125	1.13	9745
Ertugliflozin	4.44	2085859	1.15	13372
Totals		127385984		

Fig. no.7: Typical chromatogram of Interday precision.

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