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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ERTUGLIFLOZIN AND METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

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Keywords:

Ertugliflozin, Metformin, UPLC, Validation

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ABSTRACT: A simple, accurate, precise method was developed for the simultaneous estimation of the Ertugliflozin and Metformin in Tablet dosage form. For the method development, Chromatogram was run through HSS C18 (100 × 2.1 mm, 1.7µ) column at a flow rate of 0.3 ml/min. and buffer used in this method was Ortho Phosphoric Acid buffer. The temperature was maintained at 30 °C. The optimized wavelength selected was 240 nm. The retention time of Ertugliflozin and Metformin was found to be 0.736 min and 1.286 min. % RSD of the Ertugliflozin and Metformin were and found to be 0.8 and 0.9 respectively. % Recovery was obtained as 100.98% and 99.81% for Ertugliflozin and Metformin, respectively. LOD, LOQ values obtained from regression equations of Ertugliflozin and Metformin were 0.02 µg/ml, 0.08 µg/ml and 1.04 µ/ml, 3016 µg/ml respectively. The regression equation of validated method for Ertugliflozin is y = 4132.x + 239.4 and y = 3921.x + 4097 of Metformin, respectively. Retention times were decreased so that run time was decreased, the developed method was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION: Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose co-transporters, more specifically type 2 which is responsible for about 90% of the glucose reabsorption from glomerulus [1-4]. Metformin is an oral antidiabetic drug in the biguanide class [5-7]. It is the first-line drug of choice for the treatment of type 2 diabetes. It is also used in the treatment of polycystic ovary syndrome and has been investigated for other diseases where insulin resistance may be an important factor.

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Metformin works by suppressing glucose production by the liver.

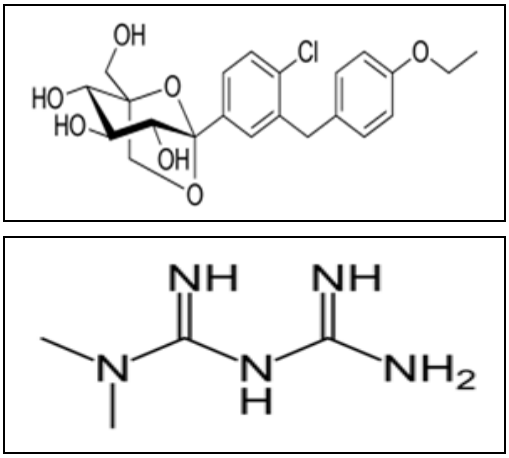

FIG. 1: STRUCTURES FOR ERTUGLIFLOZIN AND METFORMIN

Literature review [10-14] reveals that different methods RP-HPLC, UV, LCMS for its analysis in

formulations. Hence, our present plan is to develop a new, sensitive, robust & accurate method for its analysis in the formulation, after a detailed study, a new UPLC method was decided to be developed and validated as per ICH norms [15-16].

MATERIALS AND METHODS:

Instruments Used: Electronics Balance-Denver, pH meter -BVK enterprises, India, Ultra sonicator-BVK enterprises, WATERS UPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector, and Autosampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of AMT and LFT solutions.

Drug Samples: Ertugliflozin and Metformin pure drugs (API), Combination Ertugliflozin, and Metformin tablets (Segluromet).

Reagents and Solutions: Distilled water, Acetonitrile, Phosphate buffer, Methanol, Ortho-phosphoric acid [All are HPLC grade], Potassium dehydrogenate orthophosphate buffer [AR].

Analytical Methodology:

Diluent: Based upon the solubility of the drugs, diluent was selected, acetonitrile, and water taken in the ratio of 50:50.

Preparation of Standard Stock Solutions: Accurately weighed 3.75 mg of Ertugliflozin, 250 mg of Metformin, and transferred to a 50ml volumetric flask. 3/4th of diluents were added to the flask and sonicated for 10 min. Flask was made up of diluents and labeled as Standard stock solution (5000 µg/ml of Ertugliflozin and 75 µg/ml Metformin).

Preparation of Standard Working Solutions (100% Solution): 1 ml from each stock solution was pipetted out and taken into a 10 ml volumetric flask and made up with diluent (500 µg/ml of Ertugliflozin and 7.5 µg/ml of Metformin).

Preparation of Sample Stock Solutions: 10 tablets were weighed, and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (5000 µg/ml of Ertugliflozin and 75 µg/ml of Metformin).

Preparation of Sample Working Solutions (100% Solution): 1ml of filtered sample stock solution was transferred to a 10ml volumetric flask and made up with diluent (500 µg/ml of Ertugliflozin and 7.5 µg/ml of Metformin).

Preparation of Buffer:

0.1% OPA Buffer: 1 ml of orthophosphoric acid was diluted to 1000 ml with HPLC grade water.

Method Development:

Optimized Method: Trials were performed for the method development, and the best peak with least fronting factor was found to be with RT = 0.736 min for Ertugliflozin and 1.285 min for Metformin.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameter	Content
Column	HSS C18 (100 × 2.1 mm, 1.7 µ)
Mobile Phase	50% OPA (0.1%): 50% Acetonitrile
Flow Rate	0.3 ml/min
Temperature	30 ºC
Injection Volume	0.50 µL
Detection & Wavelength	Acquity TUV 240 nm

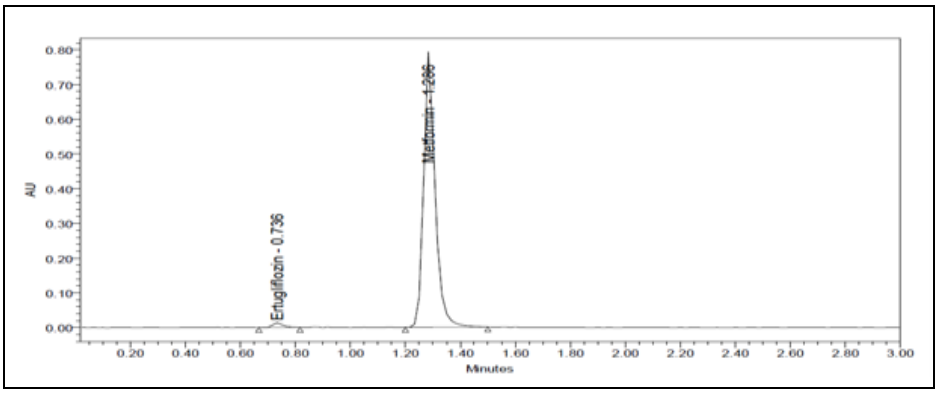

FIG. 2: OPTIMIZED CHROMATOGRAM

System suitability: According to ICH guidelines, plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the suitable system parameters were passed and were within limits.

TABLE 2: SYSTEM SUITABILITY PARAMETERS FOR ERTUGLIFLOZIN AND METFORMIN

S. no.	Ertugliflozin			Metformin			Resolution
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	
1	0.735	4339	1.89	1.284	3821	1.19	6.5
2	0.736	4376	1.83	1.284	3649	1.19	6.4
3	0.738	4297	1.89	1.288	4467	1.25	6.5
4	0.742	4987	1.77	1.292	4064	1.33	6.0
5	0.752	5022	1.55	1.299	3778	1.20	5.9
6	0.754	4938	1.39	1.301	3710	1.19	6.1

Method Validation:

Accuracy: Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy, and mean % Recovery was obtained as 100.38% and 99.81% for Ertugliflozin and Metformin, respectively.

TABLE 3: RECOVERY STUDIES FOR ERTUGLIFLOZIN AND METFORMIN

% Concentration	Ertugliflozin			Metformin		
	50%	100%	150%	50%	100%	150%
Trail-I	100.77	100.51	100.96	99.16	100.51	100.12
Trail-II	100.30	99.45	100.77	100.91	99.45	99.43
Trail-III	99.86	100.34	100.47	99.97	100.34	99.50
AVG (%Recovery)	100.3	100.10	100.73	100.01	99.73	99.69
SD	0.46	0.57	0.24	0.875	0.3913	0.3809
%RSD	0.46	0.57	0.24	0.87	0.39	0.38
Mean %Recovery		100.38			99.81	

Precision: From a single volumetric flask of working standard solution six injections were given, and the obtained areas were mentioned above. Average area, standard deviation, and % RSD were calculated for two drugs. Precision % RSD values obtained as 0.8% and 0.9% and Intermediate precision values obtained as 0.9% and 0.6% respectively for Ertugliflozin and Metformin. As the limit of Precision was less than "2" the system precision was passed in this method.

TABLE 4: SYSTEM PRECISION TABLE OF ERTUGLIFLOZIN AND METFORMIN

S. no.	Peak area of Ertugliflozin		Peak area of Metformin	
	Precision	Day_ Day Precision	Precision	Day_ Day Precision
1	32315	31331	1941365	1922518
2	33046	31198	1989881	1900063
3	32805	31594	1968740	1923950
4	32912	31949	1977705	1899308
5	33017	31723	1972480	1901364
6	32965	31653	1983584	1918731
Mean	32843	31575	1972293	1910989
S.D	272.5	272.0	16942.9	11910.6
%RSD	0.8	0.9	0.9	0.6

TABLE 5: LINEARITY TABLE FOR ERTUGLIFLOZIN AND METFORMIN

Ertugliflozin		Metformin	
Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
0	0	0	0
1.875	7985	125	464159
3.75	15670	250	993342
5.625	23563	375	1504954
7.5	31861	500	1977084
9.375	39210	625	2478785
11.25	46104	750	2903917

Linearity: Six linear concentrations of Ertugliflozin (1.875-11.25 µg/ml) and Metformin (125-750 µg/ml) were injected in a duplicate manner. Average areas were mentioned above, and linearity equations obtained for Ertugliflozin were y = 4132.x + 239.4 and of Metformin was y = 3921.x + 4097. The correlation coefficient obtained was 0.999 for the two drugs.

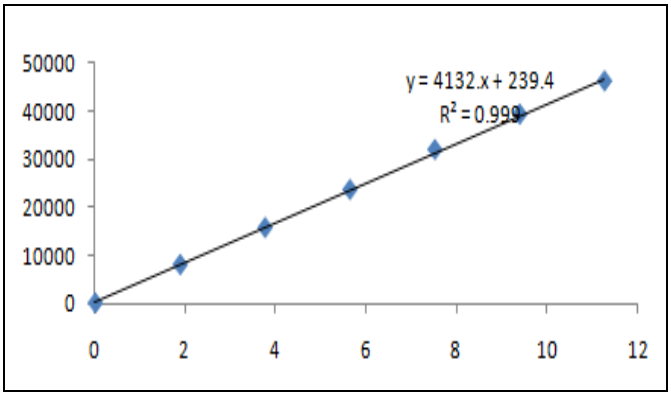

FIG. 3: CALIBRATION CURVE OF ERTUGLIFLOZIN

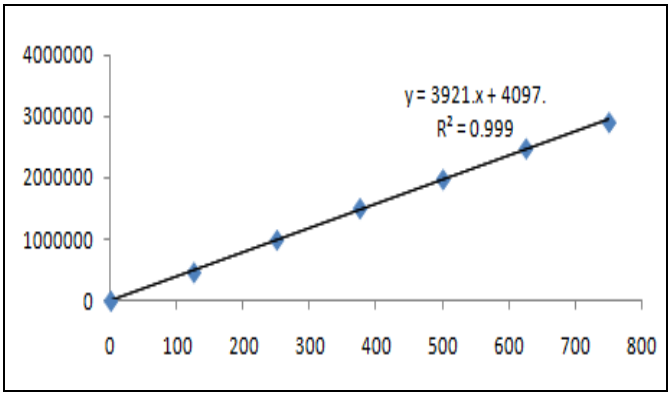

FIG. 4: CALIBRATION CURVE OF METFORMIN

Sensitivity:

TABLE 6: SENSITIVITY TABLE OF ERTUGLIFLOZIN AND METFORMIN

Molecule	LOD	LOQ
Ertugliflozin	0.02	0.18
Metformin	1.04	3.16

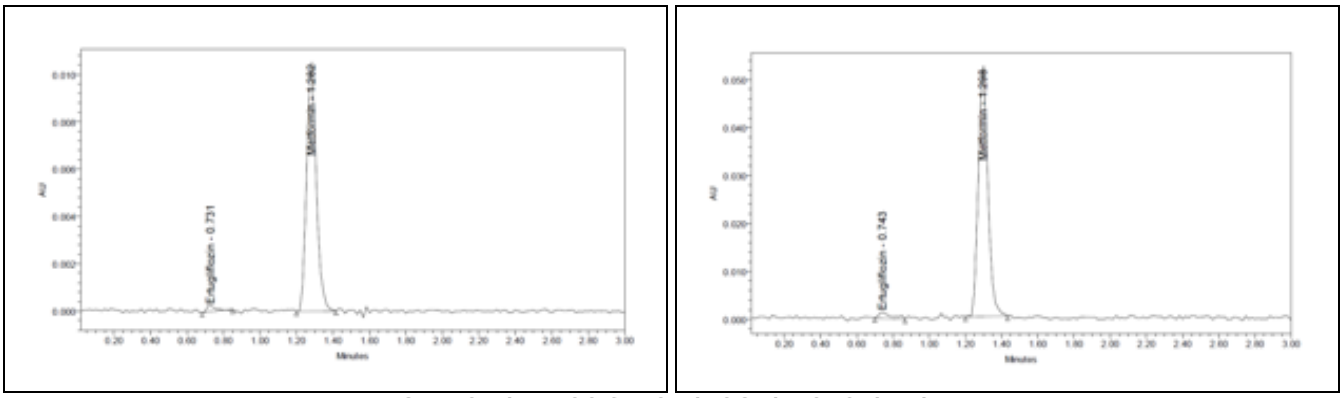

FIG. 5: LOD AND LOQ CHROMATOGRAM OF STANDARD

Robustness: Robustness conditions like Flow minus (0.20ml/min), Flow plus (0.40ml/min), mobile phase minus (55:45A), mobile phase plus (55B:45A), temperature minus (25 °C) and temperature plus (35 °C) was maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected, and all the parameters were passed. % RSD was within the limit.

TABLE 7: ROBUSTNESS DATA FOR ERTUGLIFLOZIN AND METFORMIN

S. no.	Condition	%RSD of Ertugliflozin	%RSD of Metformin
1	Flow rate (-) 0.2 ml/min	0.3	0.3
2	Flow rate (+) 0.4 ml/min	0.9	0.3
3	Mobile phase (-) 55B:45A	1.0	0.3
4	Mobile phase (+) 45B:55A	0.7	0.2
5	Temperature (-) 25 °C	0.8	0.6
6	Temperature (+) 35 °C	0.2	0.8

Degradation Studies: Degradation studies were performed with the formulation, and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

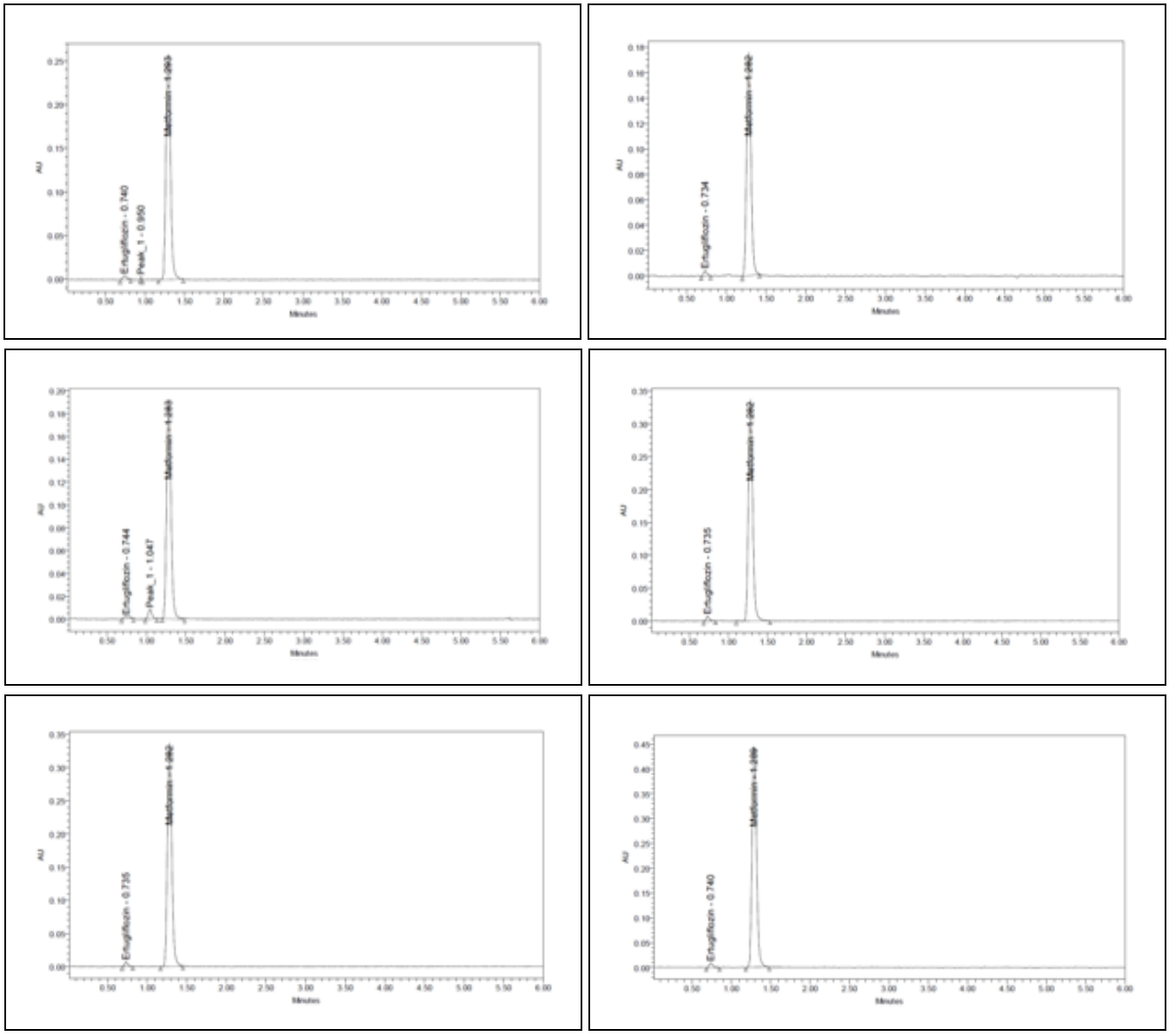

FIG. 6: DEGRADATION CHROMATOGRAMS

TABLE 8: DEGRADATION DATA OF ERTUGLIFLOZIN AND METFORMIN

S. no.	Degradation Condition	% Drug Degraded	
		Ertugliflozin	Metformin
1	Acid	8.03	7.45
2	Alkali	6.24	6.03
3	Oxidation	5.02	4.05
4	Thermal	3.54	2.27
5	UV	1.60	1.15
6	Water	1.60	0.51

CONCLUSION: A simple, Accurate, precise method was developed for the simultaneous estimation of the Ertugliflozin and Metformin in Tablet dosage form. The retention time of Ertugliflozin and Metformin was found to be 0.736 min and 1.286 min. % RSD of the Ertugliflozin and Metformin were and found to be 0.9 and 0.6 respectively. % Recovery was obtained as 100.38% and 99.81% for Ertugliflozin and Metformin, respectively. LOD, LOQ values obtained from regression equations of Ertugliflozin and Metformin were and 0.02, 0.08 & 1.04, 3.16 respectively. The regression equation of Ertugliflozin is y = 4132.x + 239.4, and y = 3921.x + 4097 of Metformin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control tests in Industries.

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CONFLICTS OF INTEREST: Nil

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