ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND ERTUGLIFLOZIN IN PURE AND TABLET DOSAGE FORM BY RP-HPLC

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Abstract:
A simple, accurate, precise HPLC method was developed for the simultaneous estimation of the Metformin and Ertugliflozin in Tablet dosage form. Retention time of Metformin and Ertugliflozin were found to be 2.336 min and 3.142 min. %RSD of the Metformin and Ertugliflozin were and found to be 0.3 and 0.3 respectively. %Recovery was obtained as 100.30% and 100.49% for Metformin and Ertugliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin and Ertugliflozin were 0.72, 0.01 and 2.18, 0.04 respectively. Regression equation of Metformin is y = 5799x + 2367, and y = 44530x + 320.6 of Ertugliflozin. 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 test in Industries.
Keywords: RP-HPLC, Method Development, Validation, Metformin and Ertugliflozin

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1. INTRODUCTION:

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Liquid chromatography is an analytical chromatographic technique that is useful for separating ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase to differing degrees due to differences in adsorption, ion exchange, partitioning or size. These differences will allow the mixture components to be separated from each other by using these differences to determine the time of the solutes through a column. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography and thin layer chromatography (TLC). However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time pressure liquid chromatography began to be used to decreased flow through time, thus reducing separation time of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure debated. High pressure liquidchromatography quickly improved with the development of column packing materials. Additional convenience of on-line detectors became rapidly a powerful separation technique and is today called as High Performance Liquid Chromatography(HPLC)[1-10].

Reversed Phase-High Performance Liquid Chromatography (RP-HPLC)

RP-HPLC employs mainly dispersive forces (hydrophobic or vanderwal's interactions). The polarities of mobile and stationary phases are reversed, such that the surface of the stationary phase in RP-HPLC is hydrophobicand mobile phase is polar, where mainly water-based solutions are employed. RP- HPLC is by far the most popular mode of chromatography. Almost 90% of allanalysis of low molecular-weight samples are carried out using RP-HPLC. Dispersive forces employed in this separation mode are the weakest intermolecular forces, thereby making the overall background interaction energy in the chromatographic system very low compared to other separation techniques. This low backgroundenergy allows for distinguishing very small differences in molecular interactions of closely related analytes. Adsorbents employed in this mode of chromatography are porous rigid materials with hydrophobic surfaces. The majority of packing materials used in RP-HPLC are chemically modified porous silica[10-19].

2. MATERIALS AND METHODS:

2.1. Materials Used

Metformin and Ertugliflozin pure drugs (API), combination Metformin and Ertugliflozin tablets (Segluromet), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho- phosphoric acid. All the above chemicals and solvents are from Rankem.

2.2. Methods Used

Preparation of Standard stock solutions: Accurately weighed 3.75 mg of Ertugliflozin 250 mg of Metformin 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 (100% solution): 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 diluents 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 (100% solution): 2.5 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluents (250 µg/mL of Metformin and 3.75 µg/mL of Ertugliflozin).
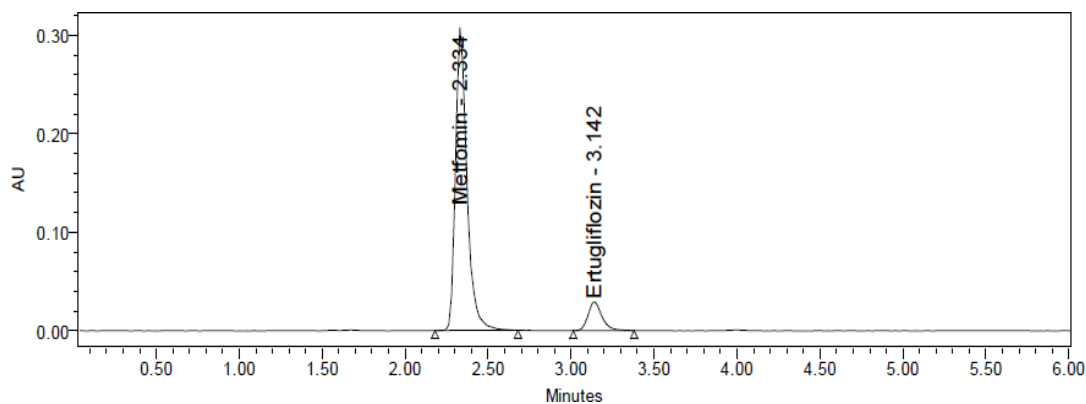
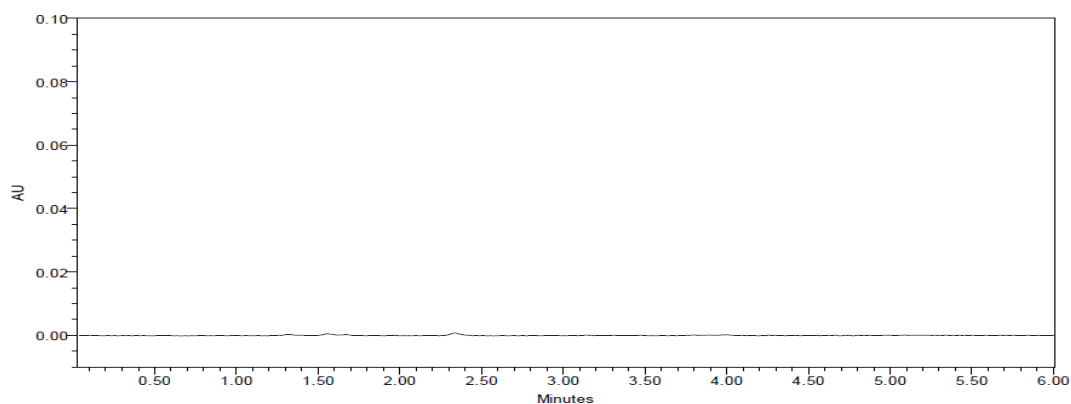## 3. RESULTS:

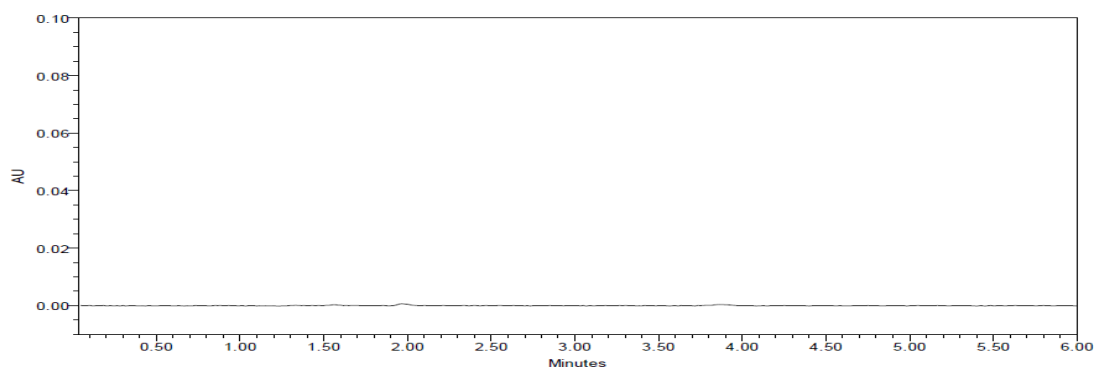**Fig-1: System suitability Chromatogram**

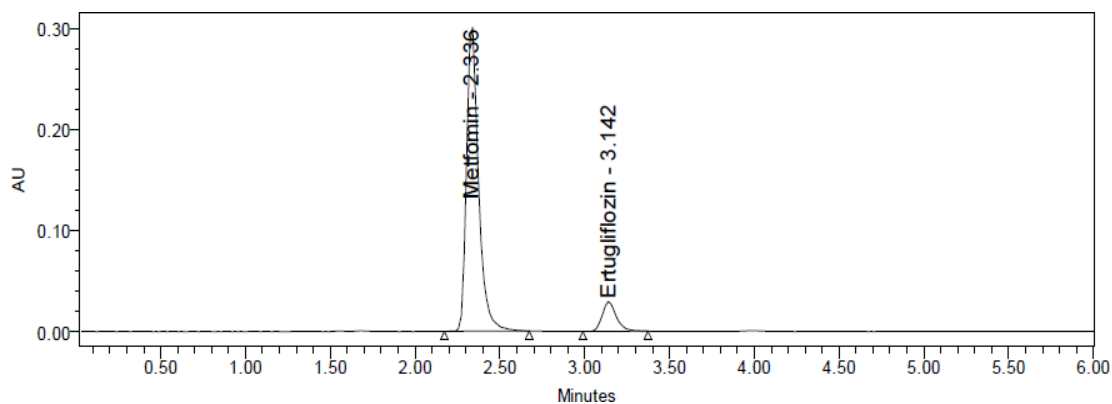**Fig-2: Chromatogram of blank**

Fig-3: Chromatogram of placebo

Fig-4: Optimized chromatogram

Table-1: Linearity table for Metformin and Ertugliflozin.

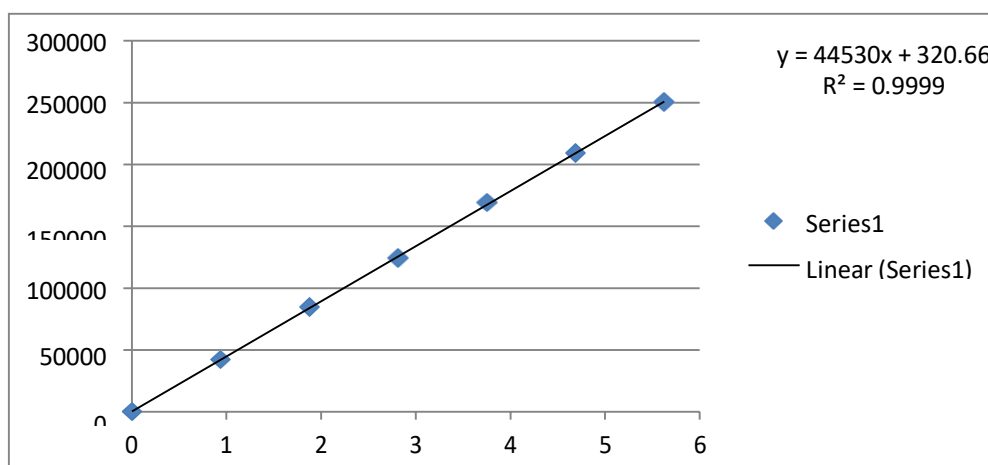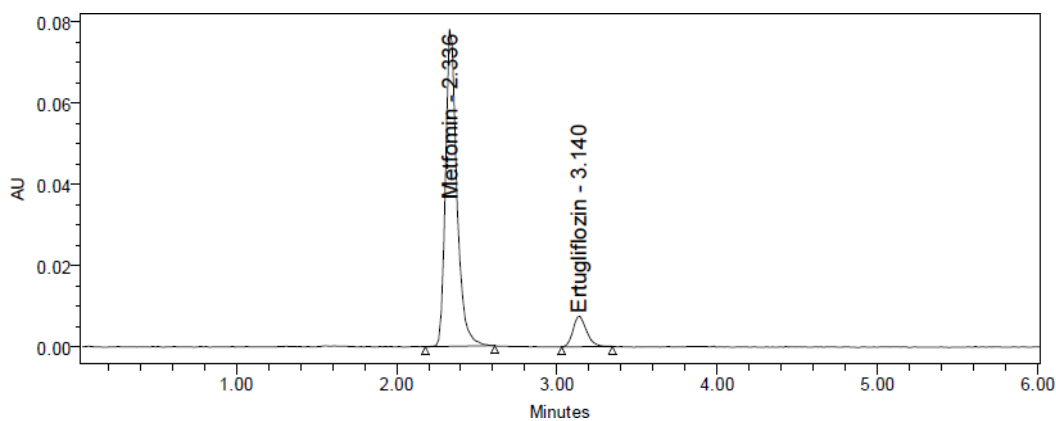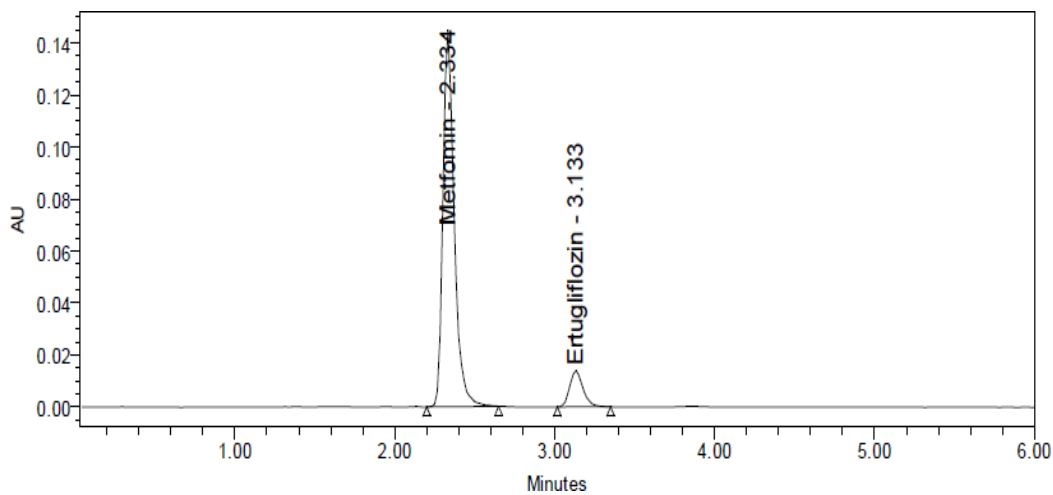| METFORMIN		ERTUGLIFLOZIN	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
62.5	359928	0.9375	42108
125	729371	1.8750	84536
187.5	1086779	2.8125	124092
250	1466140	3.7500	169023
312.5	1827149	4.6875	208889
375	2159508	5.6250	250292

$y = 5799.8x + 2367.4$
$R^2 = 0.9998$
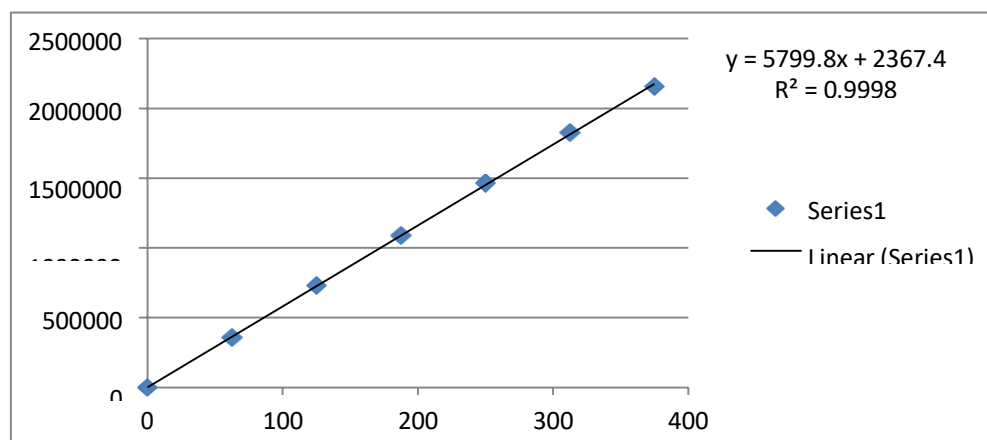
Fig-5: Calibration curve of Metformin

Fig-6: Calibration curve of Ertugliflozin

Fig-7: Linearity 25% Chromatogram of Metformin and Ertugliflozin

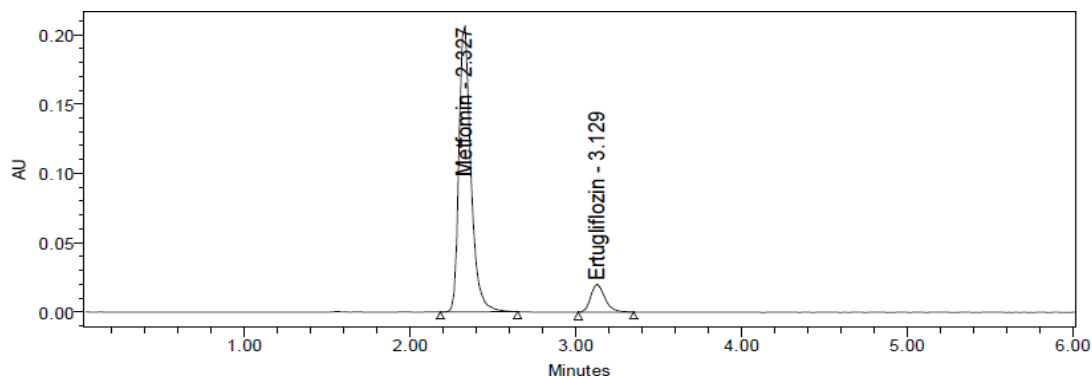**Fig-8: Linearity 50% Chromatogram of Metformin and Ertugliflozin**

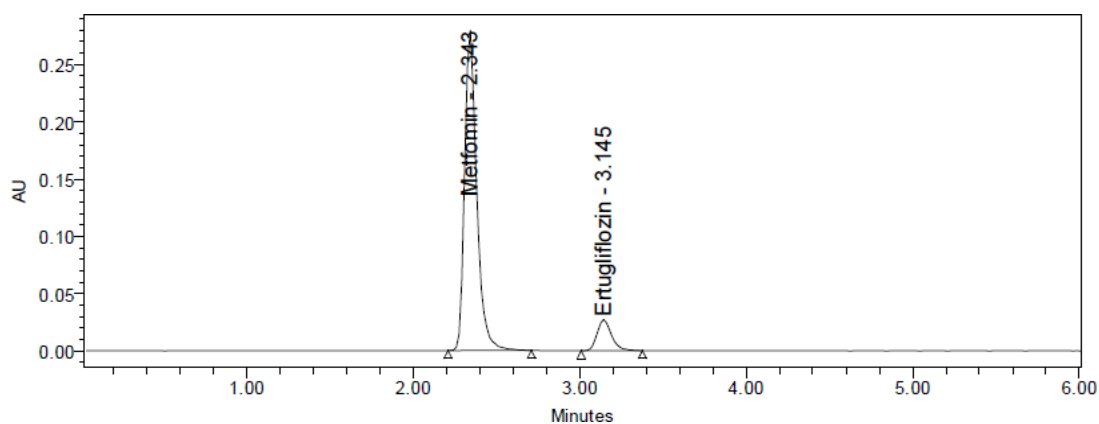**Fig-9: Linearity 75% Chromatogram of Metformin and Ertugliflozin**

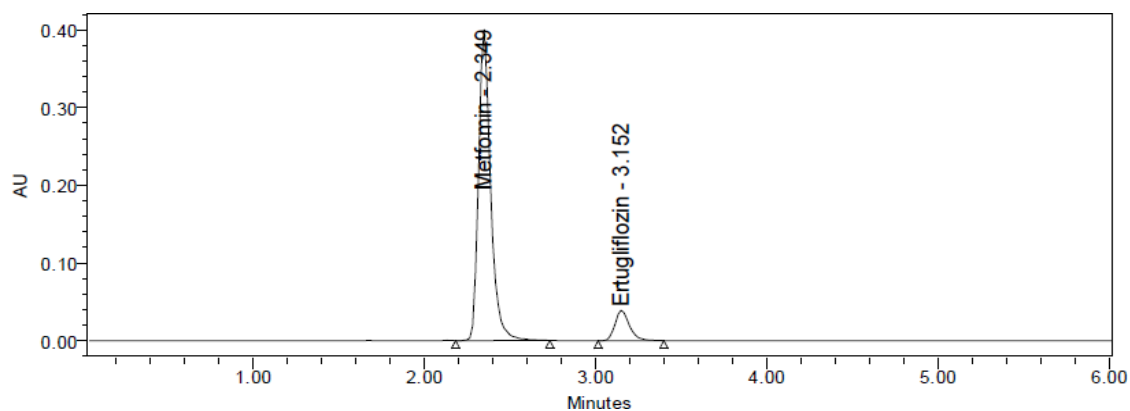**Fig.10: Linearity 100% Chromatogram of Metformin and Ertugliflozin**

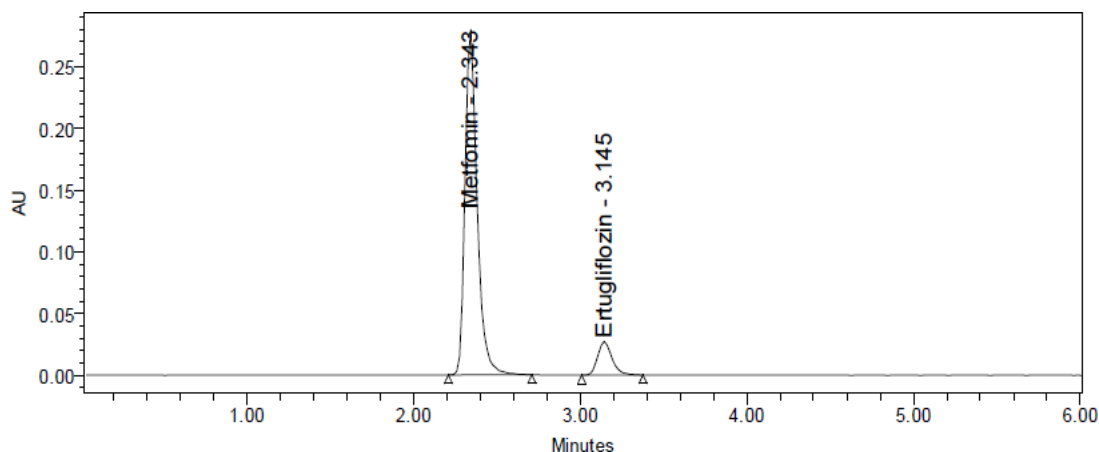**Fig-11: Linearity 125% Chromatogram of Metformin and Ertugliflozin**

Fig-12: Linearity 150% Chromatogram of Metformin and Ertugliflozi

Table-2: System precision table 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
S.D	10054.7	1045.1
%RSD	0.7	0.6

Table-3: Repeatability table of Metformin and Ertugliflozin

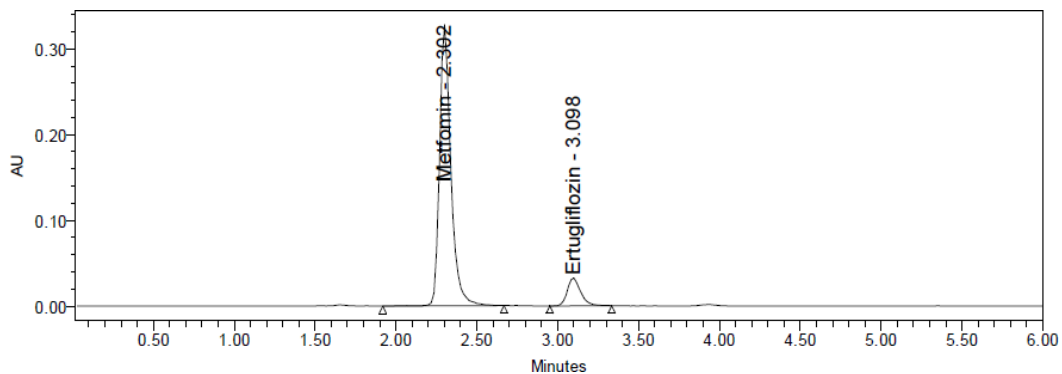| S. No.	Area of Metformin	Area of Ertugliflozin
1.	1414760	161685
2.	1408575	162978
3.	1398103	160958
4.	1418917	160198
5.	1400044	159335
6.	1411347	160984
Mean	1408624	161023
S.D	8189.3	1248.7
%RSD	0.6	0.8

Fig-13: Inter Day precision Chromatogram

Table-4: Accuracy table of Ertugliflozin

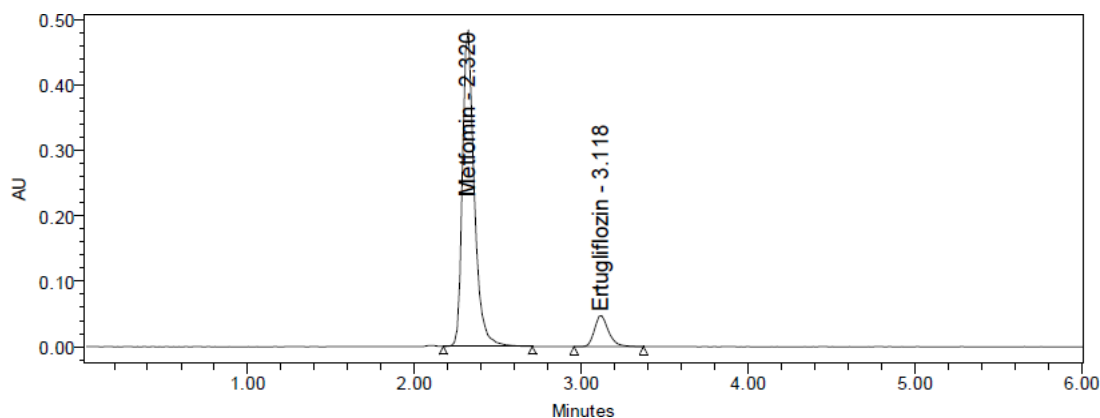| % Level	Amount Spiked (μg/mL)	Amount Recovered (μg/mL)	% Recovery	Mean %Recovery
50%	1.875	1.87	99.80	
	1.875	1.84	98.35	
	1.875	1.87	99.80	
100%	3.75	3.79	100.95	100.49%
	3.75	3.79	101.19	
	3.75	3.82	101.88	
150%	5.625	3.79	100.95	
	5.625	3.79	101.19	
	5.625	3.82	101.88	

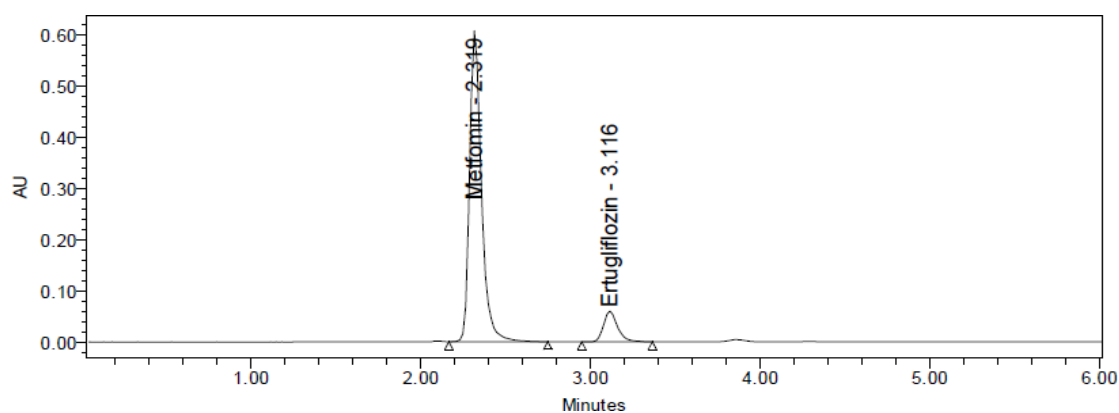**Fig-14: Accuracy 50% Chromatogram of Metformin and Ertugliflozin**

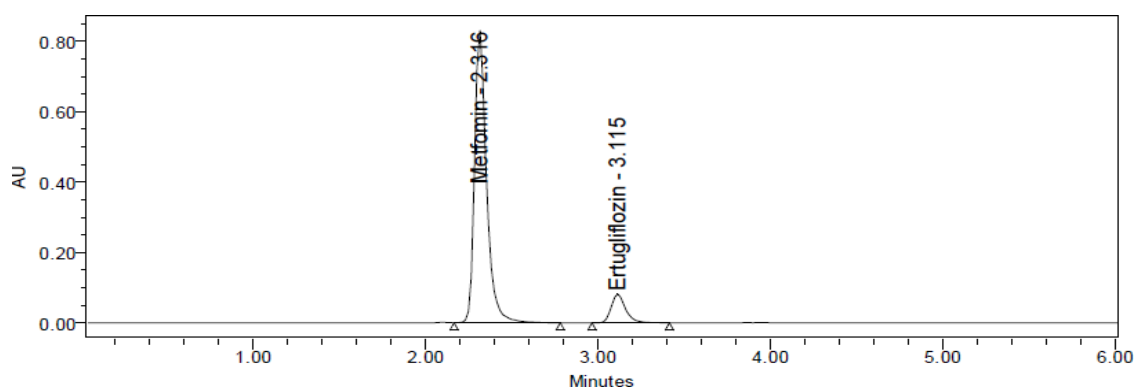**Fig-15: Accuracy 100% Chromatogram of Metformin and Ertugliflozin**
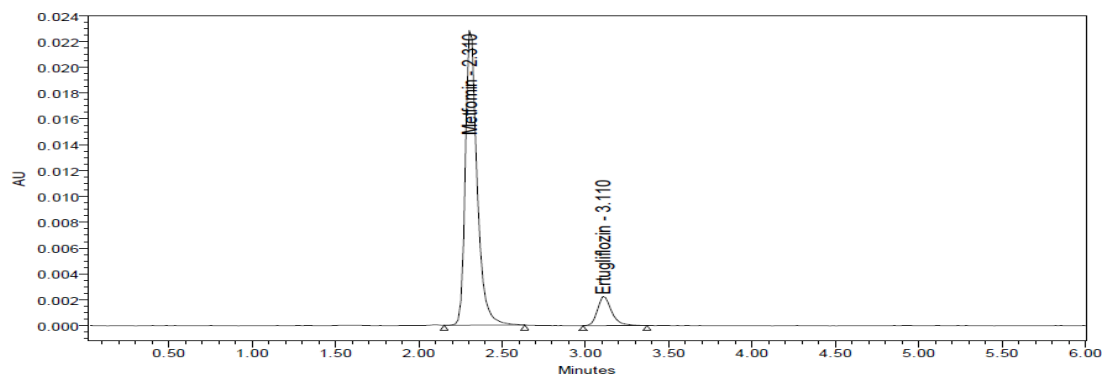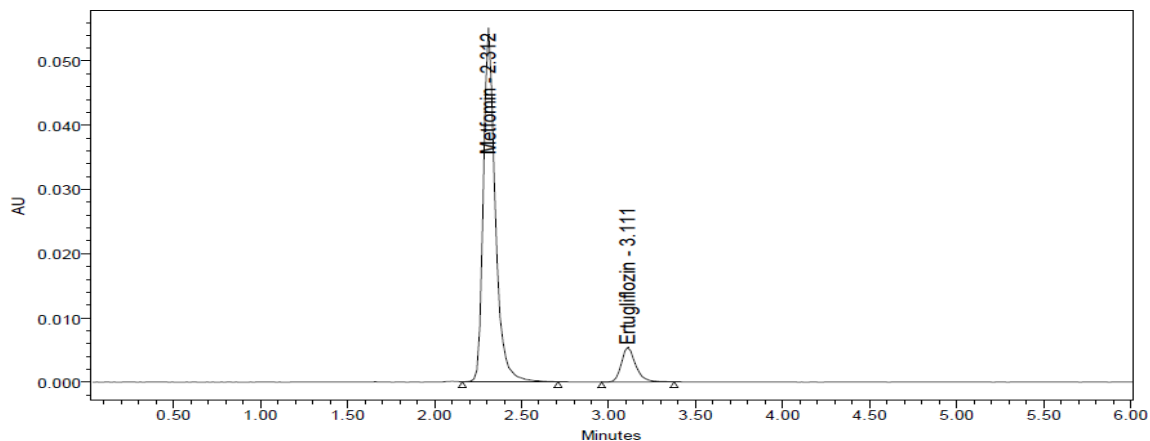

Fig-16: Accuracy 150% Chromatogram of Metformin and Ertugliflozin

Table-5: Sensitivity table of Metformin and Ertugliflozin

Molecule	LOD	LOQ
Metformin	0.72	2.18
Ertugliflozin	0.01	0.04

Fig-17: LOD Chromatogram of Standarad

Fig-18: LOQ Chromatogram of of Standard

Table -6: Robustness data for Metformin and Ertugliflozin

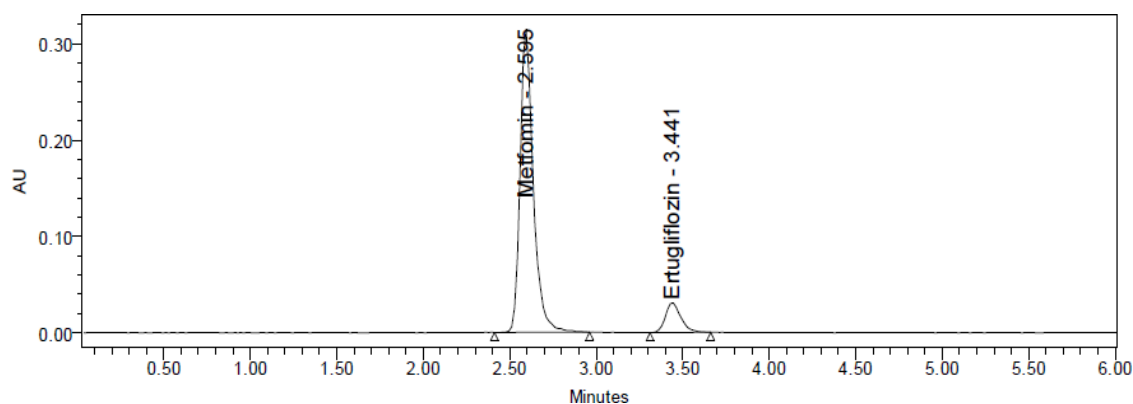| S. No.	Condition	%RSD of Metformin	%RSD of Ertugliflozin
1	Flow rate (-) 0.9mL/min	0.1	0.6
2	Flow rate (+) 1.1mL/min	0.2	0.6
3	Mobile phase (-) 65B:35A	0.4	0.7
4	Mobile phase (+) 55B:45A	0.7	0.5
5	Temperature (-) 25°C	0.4	0.7
6	Temperature (+) 35°C	0.6	0.8

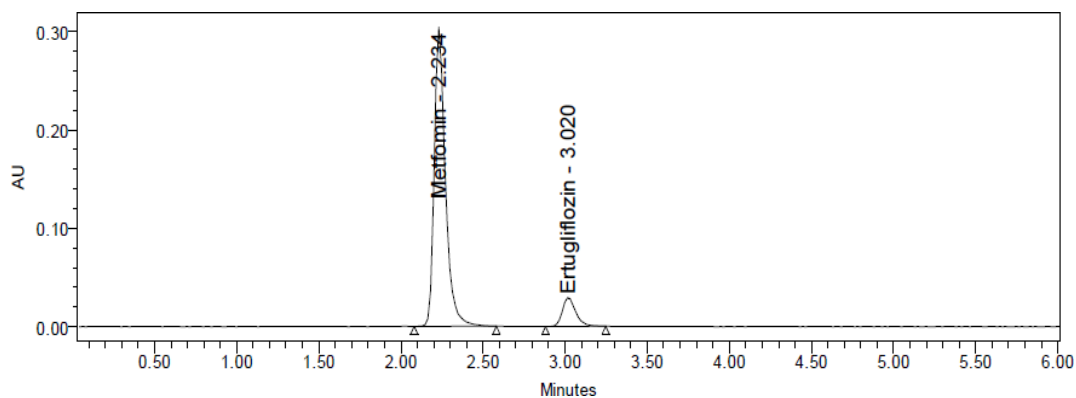**Fig-19: Flow minus Chromatogram of Metformin and Ertugliflozin**

Fig-20: Flow plus Chromatogram of Metformin and Ertugliflozin
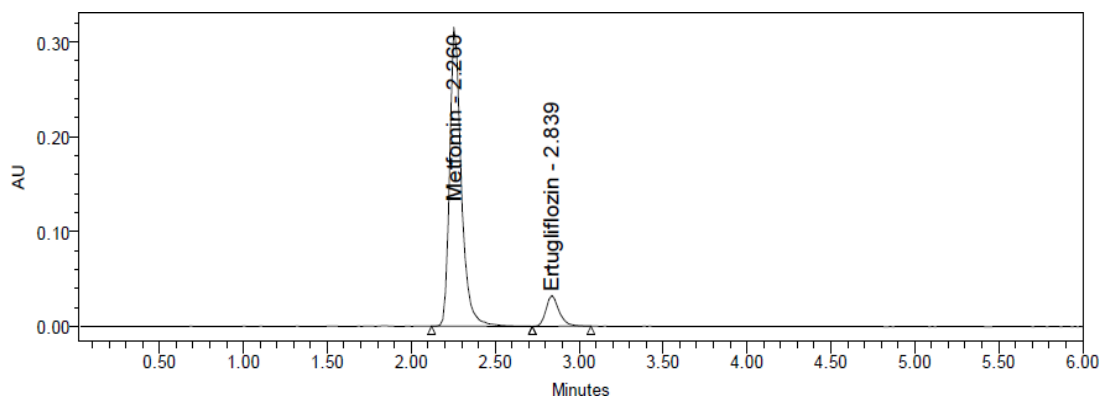

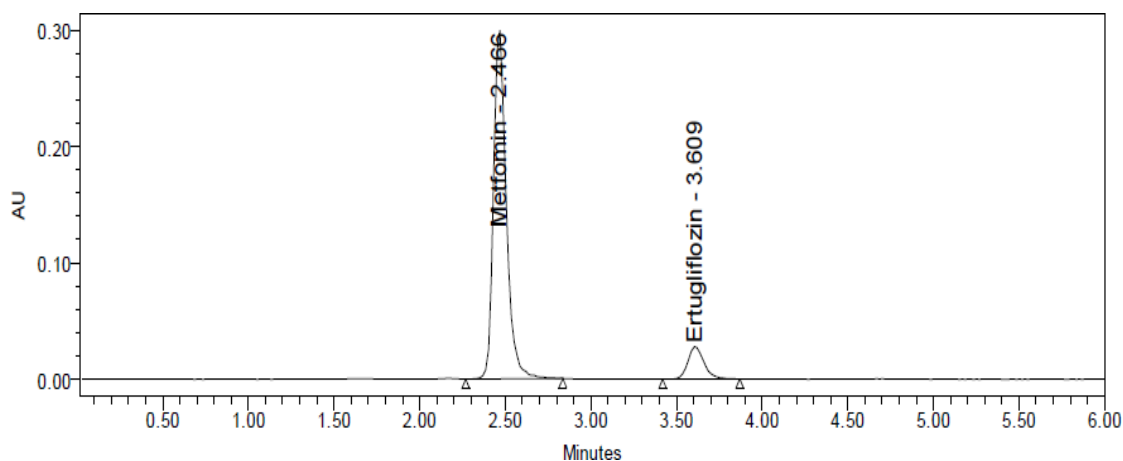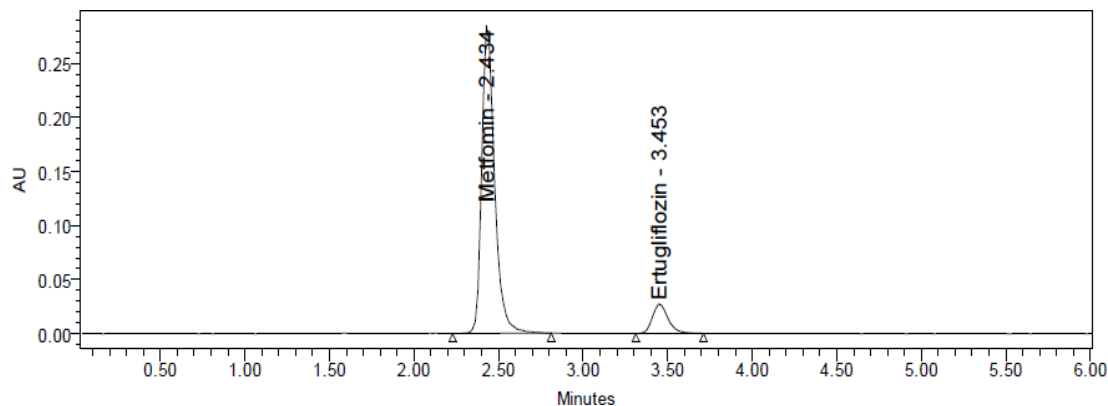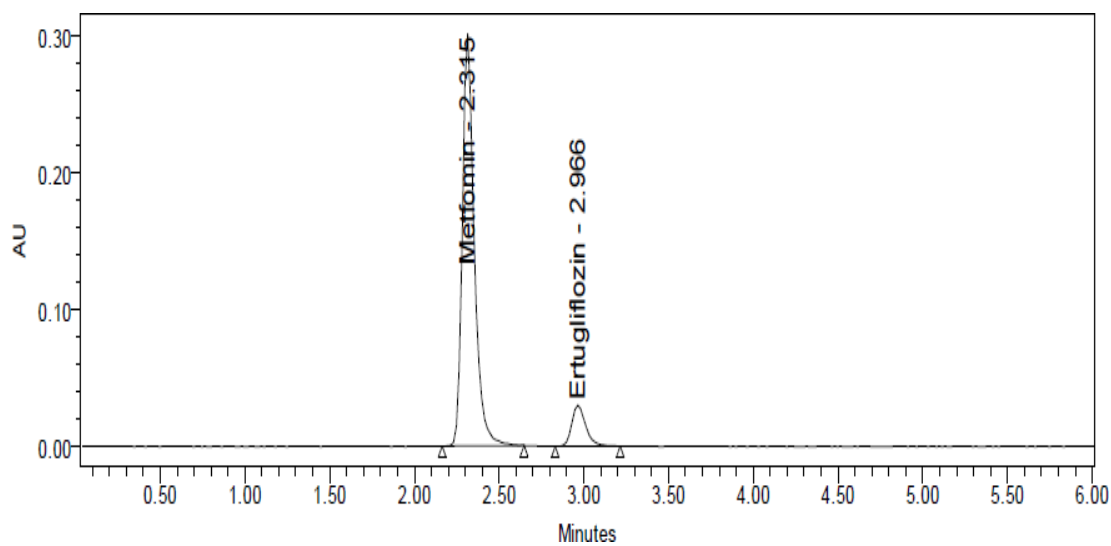**Fig-21: Mobile phase minus Chromatogram of Metformin and Ertugliflozin**

Fig-22: Mobile phase Plus Chromatogram of Metformin and Ertugliflozin

Fig-23: Temperature minus Chromatogram of Metformin and Ertugliflozin

Fig-24: Temperature plus Chromatogram of Metformin and Ertugliflozin

Table-7: Assay Data of Metformin

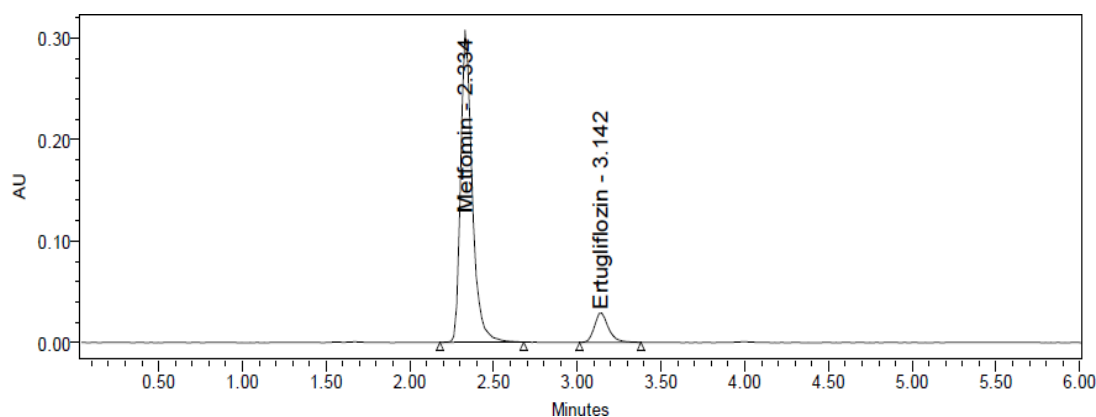| S. No.	Standard Area	Sample area	% Assay
1	1470127	1479729	100.98
2	1457186	1488454	101.57
3	1449577	1489567	101.65
4	1460165	1481190	101.08
5	1476850	1482777	101.18
6	1469803	1487053	101.48
Avg	1463951	1484795	101.32
Stdev	10054.7	4098.6	0.28
%RSD	0.7	0.3	0.28

Fig-25: Chromatogram of working standard solution

Table-8: Degradation Data of Metformin

S.No.	Degradation Condition	% Drug Degraded
1	Acid	3.31
2	Alkali	2.37
3	Oxidation	3.43
4	Thermal	0.99
5	UV	0.55
6	Water	0.15

Table-9: Degradation Data of Ertugliflozin

S.No.	Degradation Condition	% Drug Degraded
1	Acid	4.25
2	Alkali	3.58
3	Oxidation	4.06
4	Thermal	2.91
5	UV	1.31
6	Neutral	0.19
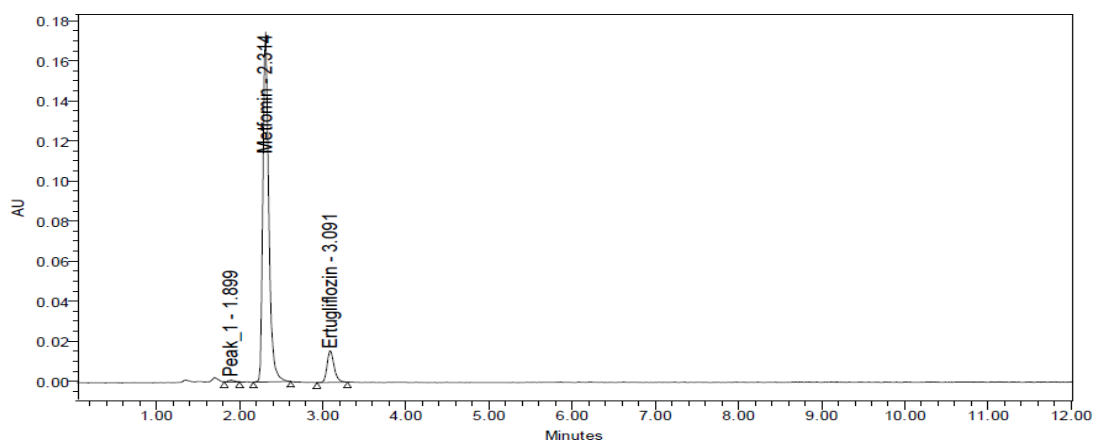
Fig-26: Acid degration chromatogram of Metformin and Ertugliflozin
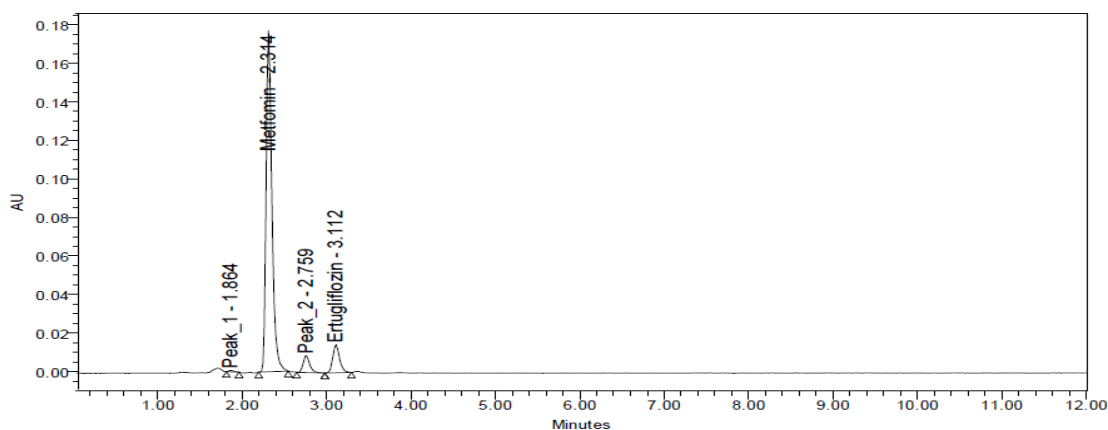
Fig-27: Alkali degradation chromatogram of Metformin and Ertugliflozin

Fig-28: Oxidative degradation chromatogram of Metformin and Ertugliflozin

Fig-29: Thermal degration chromatogram of Metformin and Ertugliflozin
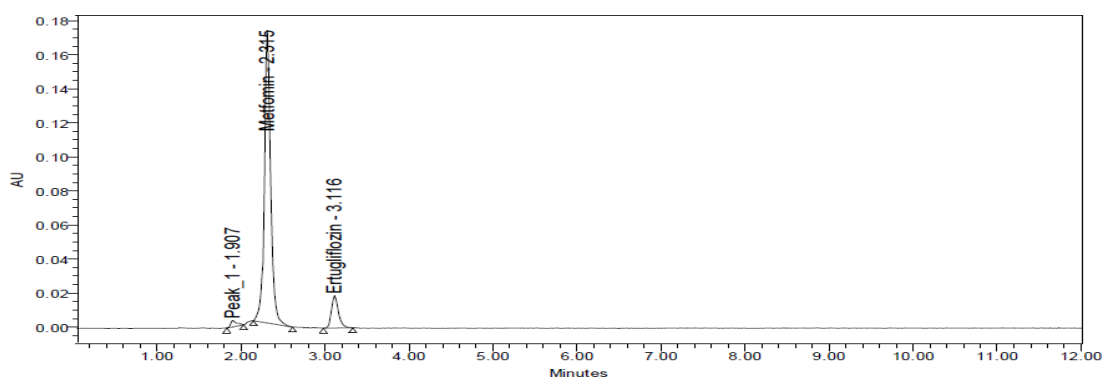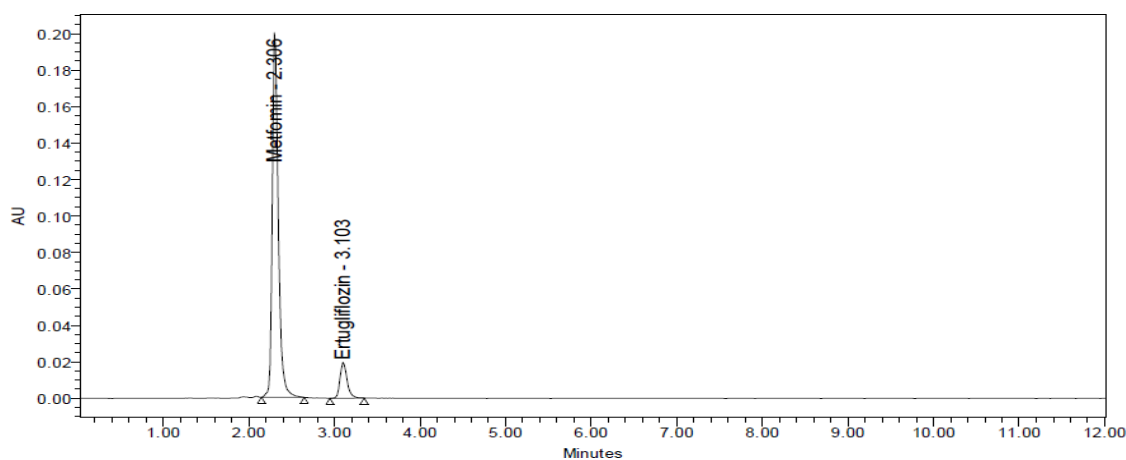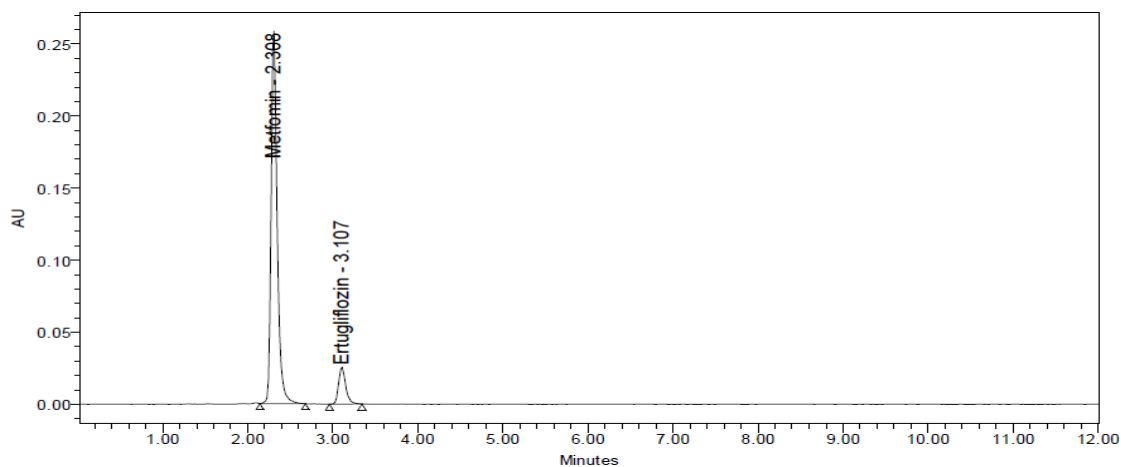

Fig-30: UV degration chromatogram of Metformin and Ertugliflozin
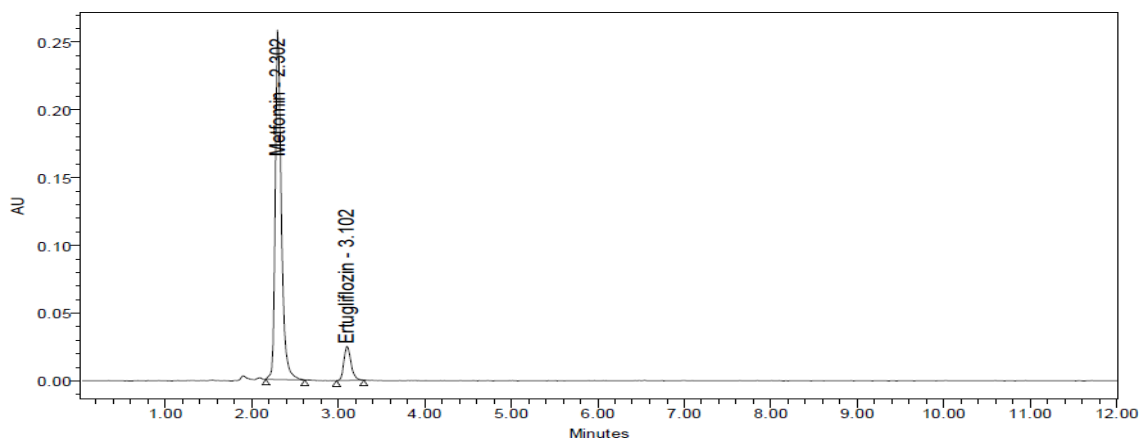
Fig-31: Neutral degration chromatogram of Metformin and Ertugliflozin

4. SUMMARY

Parameters		Metformin	Ertugliflozin	Limit
Linearity Range (µg/mL)		62.5-375µg/Ml	0.9375-5.625 µg/mL	
Regression coefficient		0.999	0.999	R< 1
Slope (m)		5799	44530	
Intercept (c)		2367	320.6	
Regression equation (y = mx+c)		y = 5799x + 2367	y = 44530.x + 320.6	
Assay (% mean assay)		101.32%	99.46%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.7	0.6	NMT 2.0%
Method precision %RSD		0.3	0.3	NMT 2.0%
Accuracy %recovery		100.30%	100.49%	98-102%
LOD		0.72	0.01	NMT 3
LOQ		2.18	0.04	NMT 10
Robustness	FM	0.1	0.6	%RSD NMT2.0
	FP	0.2	0.6	
	MM	0.4	0.7	
	MP	0.7	0.5	
	TM	0.4	0.7	
	TP	0.6	0.8	

5. CONCLUSION:

A simple, accurate, precise HPLC method was developed for the simultaneous estimation of the Metformin and Ertugliflozin in Tablet dosage form. Retention time of Metformin and Ertugliflozin were found to be 2.336 min and 3.142 min. %RSD of the Metformin and Ertugliflozin were and found to be 0.3 and 0.3 respectively.

%Recovery was obtained as 100.30% and 100.49% for Metformin and Ertugliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin andErtugliflozin were 0.72, 0.01 and 2.18, 0.04 respectively. Regression equation of Metformin is $y = 5799x + 2367$, and $y = 44530x + 320.6$ of Ertugliflozin. 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 test in Industries.

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