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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF ERTUGLIFLOZIN AND METFORMIN IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Ertugliflozin in Tablet dosage form. Chromatogram was run through Std BDS C18 150 x 4.6 mm, 5⌠. Mobile phase containing Buffer 0.1% OPA (3.0ph): Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA. Temperature was maintained at 30°C. Optimized wavelength selected was 230 nm. Retention time of Metformin and Ertugliflozin were found to be 2.565min and 3.221min. %RSD of the Metformin and Ertugliflozin were and found to be 0.1 and 0.5 respectively. %Recovery was obtained as 100.52% and 99.80% for Metformin and Ertugliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin and Ertugliflozin were 0.36, 1.08 and 0.01, 0.03 respectively. Regression equation of Metformin is y = 21782x + 8044.9., and y = 18601x + 127.71 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.

Key Words: Metformin, Ertugliflozin , RP-HPLC

1. INTRODUCTION [1-24]

Metformin: Metformin is an oral antidiabetic drug in thebiguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function. Its use in gestational diabetes has been limited by safety concerns although at least one study has been conducted which showed no concerns for children prenatally exposed to Metformin up to 2 years of age. 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. Metformin works by suppressing glucose production from three-carbon molecules (like propionic acid, a byproduct of dietary fibre fermentation in the large intestine and pyruvate, a byproduct of glucose breakdown in the muscles) by the liver.

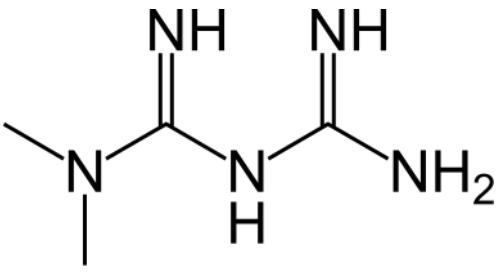

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Fig 2.1 Metformin

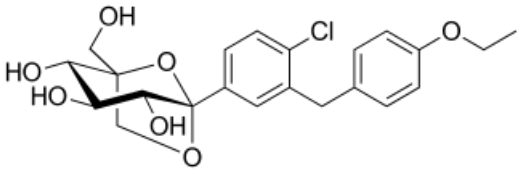
Synonym:	N,N-Dimethylimidodicarbonimidic Diamide-d6, Hydrochloride; Diabetosan-d6; Diabex-d6, Metformin-d6; Metiguanide-d6
Application:	A deuterium labeled oral hypoglycemic agent
Molecular Weight:	171.66
Molecular Formula:	$C_4H_6D_6ClN_5$
Appearance:	Crystalline
Physical State:	Solid
Solubility:	Soluble in DMSO, and methanol.
Storage:	Store at -20° C
Melting Point:	215-218°C (lit.)

Clinical Uses: Metformin is primarily used for type 2 diabetes, but is increasingly being used in polycystic ovary syndrome (PCOS),[14] and in prediabetes.

Ertugliflozin

Description: Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters (SGLT), more specifically the type 2 which is responsible for about 90% of the glucose reabsorption from glomerulus.[1] This drug was developed under the collaboration of Merck and Pfizer. It was FDA approved as monotherapy and in combination with sitagliptin or metformin hydrochloride on December 22, 2017.[6].

Structure



CAS number: 1210344-57-2

Weight Average: 436.89 Monoisotopic: 436.1288808

Chemical Formula: C22H25ClO7

IUPAC Name:(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

Indication: Ertugliflozin as a monotherapy is indicated to improve the glycemic control in adult patients with type 2 diabetes.[FDA Label] Ertugliflozin, in combination with metformin hydrochloride, is indicated to

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improve glycemic control in patients with diabetes type 2 who are not controlled on a regimen of ertugliflozin or metformin or in patients who are already treated with both ertugliflozin and metformin.[7] The administration of ertugliflozin in combination with sitagliptin is indicated to improve glycemic control in adult patients with type 2 diabetes when treatment with ertugliflozin and sitagliptin is appropriate.[8] It is pointed out that the use of ertugliflozin has to be an adjunct therapy to the use of diet and exercise. The type 2 diabetes mellitus is characterized by insulin resistance in muscle and liver, which results in the elevation of glucose levels in blood, or by presence of insulin deficiency. The insulin resistance is related to genetic factors, obesity, sedentary lifestyle or/and aging. This increase in the blood glucose can cause severe damage to kidney, eyes and vascular system.[2]

Uses: Ertugliflozin is used with a proper diet and exercise program to control high blood sugar in people with type 2 diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems. Proper control of diabetes may also lessen your risk of a heart attack or stroke. Ertugliflozin works by increasing the removal of sugar by your kidneys.

2. AIM, OBJECTIVE AND PLAN OF WORK

Aim

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Metformin, Ertugliflozin in bulk and tablet dosage form.

Objective and Plan:

Following are the objectives of the present work:

- To develop a new stability indicating HPLC method for simultaneous estimation of Metformin and Ertugliflozin and to develop the validated method according to ICH guidelines.
- To apply the validated method for the simultaneous estimation of Metformin and Ertugliflozin in pharmaceutical formulation

5. MATERIALS AND METHODS

Materials:

- 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.

Instruments:

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler 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 Metformin and Ertugliflozin solutions.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

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Preparation of Standard stock solutions: Accurately weighed 3.75mg of Ertugliflozin 250mg of Metformin and transferred to 100ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (2500µg/ml of METFORMIN and 37.5µg/ml ERTUGLIFLOZIN)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (250µg/ml of METFORMIN and 3.75µg/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, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of METFORMIN and 15µg/ml of ERTUGLIFLOZIN)

Preparation of Sample working solutions (100% solution): 2.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (250µg/ml of METFORMIN and 3.75µg/ml of ERTUGLIFLOZIN)

Preparation of buffer:

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Orthophosphoric acid solution.

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

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Ertugliflozin (3.75ppm) and Metformin (250ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method.We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions: Accurately weighed 3.75mg of Ertugliflozin 250mg of Metformin and transferred to 100ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (2500µg/ml of METFORMIN and 37.5µg/ml ERTUGLIFLOZIN)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (250µg/ml of METFORMIN and 3.75µg/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, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of METFORMIN and 15µg/ml of ERTUGLIFLOZIN)

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Preparation of Sample working solutions (100% solution): 2.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(250µg/ml of METFORMIN and 3.75µg/ml of ERTUGLIFLOZIN)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 3.75mg of Ertugliflozin 250mg of Metformin and transferred to 100ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (2500µg/ml of METFORMIN and 37.5µg/ml ERTUGLIFLOZIN).

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (62.5µg/ml of METFORMIN and 0.9375µg/ml of ERTUGLIFLOZIN)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (125µg/ml of METFORMIN and 1.875µg/ml of ERTUGLIFLOZIN)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (187.5µg/ml of METFORMIN and 2.8125µg/ml of ERTUGLIFLOZIN)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (250µg/ml of METFORMIN and 3.75µg/ml of ERTUGLIFLOZIN)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (312.5µg/ml of METFORMIN and 4.6875µg/ml of ERTUGLIFLOZIN)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (375µg/ml of METFORMIN and 5.625µg/ml of ERTUGLIFLOZIN)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 3.75mg of Ertugliflozin 250mg of Metformin and transferred to 100ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (2500µg/ml of METFORMIN and 37.5µg/ml ERTUGLIFLOZIN)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102
Robustness: Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

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LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Ertugliflozine, Metformin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Ertugliflozine, Metformin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation:

To 1 ml of stock solution ofErtugliflozine and Metformin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 3.75µg/ml&250µg/ml solution and 10 µl wereinjected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock s solution Ertugliflozine and Metformin, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 3.75µg/ml&250µg/ml 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 Ertugliflozine and Metformin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 3.75µg/ml&250µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 3.75µg/ml&250µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 37.5µg/ml&2500µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 3.75µg/ml&250µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 3.75µg/ml&250µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

6. RESULTS AND DISCUSSION

Optimized method:

Chromatographic conditions:

Mobile phase	55% 0.1% OPA buffer: 45% Acetonitrile
Flow rate	1ml/min
Column	BDS C18 (4.6 x 150mm, 5µm)
Detector wave length	230.0 nm
Column temperature	30°C
Injection volume	10⌈L
Run time	**6**min
Diluent	Water and Acetonitrile in the ratio 50:50
Results	Both peaks have good resolution, tailing factor,Theoretical plate count and resolution

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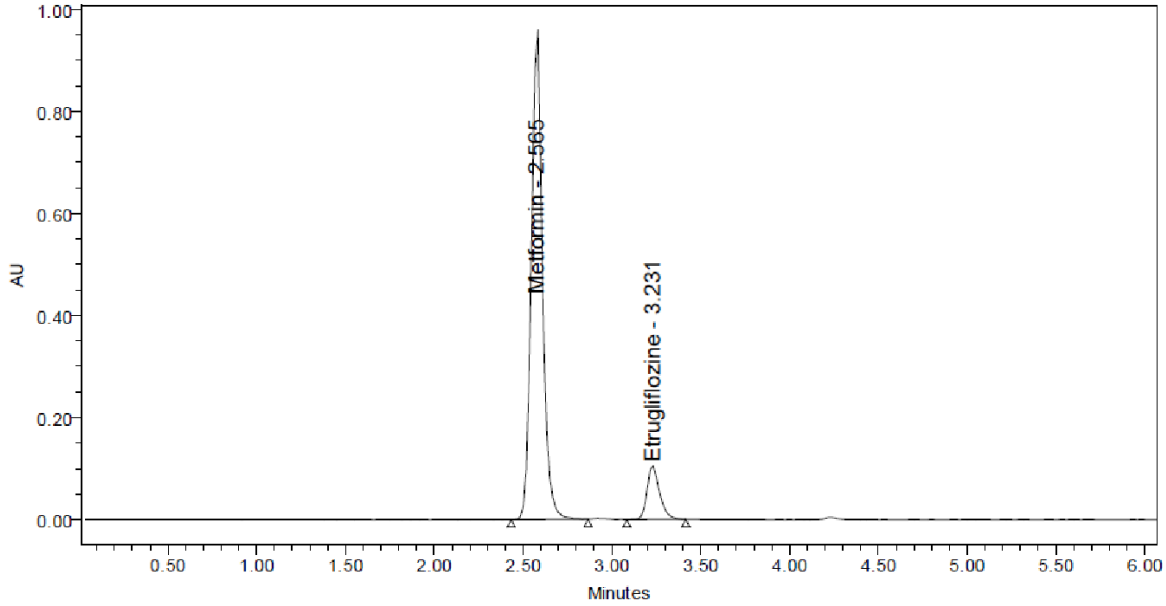

Optimized Chromatogram

Observation: Metformin and Ertugliflozin were eluted at 2.565 min and 3.231 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

System suitability parameters for Metformin and Ertugliflozin

S no	**Metformin**			**Ertugliflozin**			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.547	6243	1.08	3.179	7000	1.10	4.3
2	2.549	6321	1.08	3.180	7113	1.10	4.3
3	2.554	6133	1.08	3.186	6790	1.11	4.4
4	2.554	6263	1.09	3.205	7051	1.14	4.5
5	2.565	7882	1.08	3.206	7732	1.14	4.5
6	2.566	6728	1.08	3.221	9457	1.20	5.2

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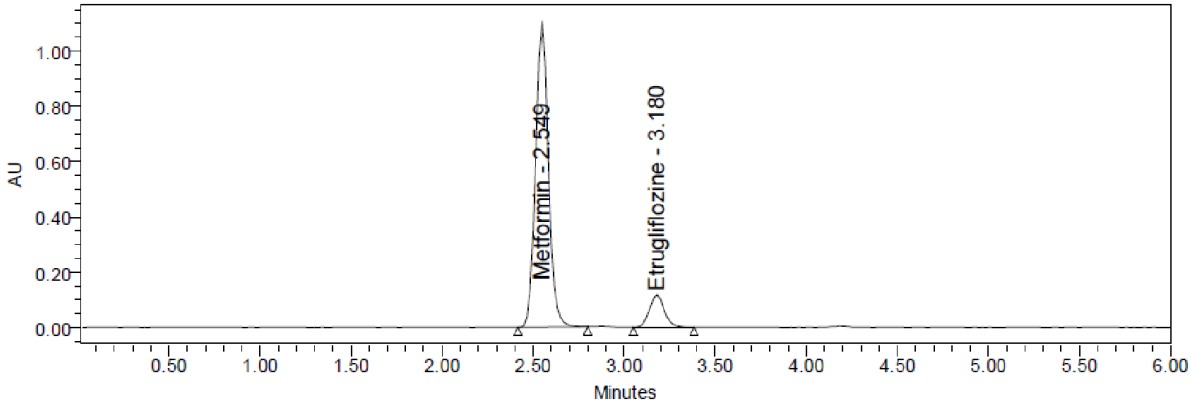
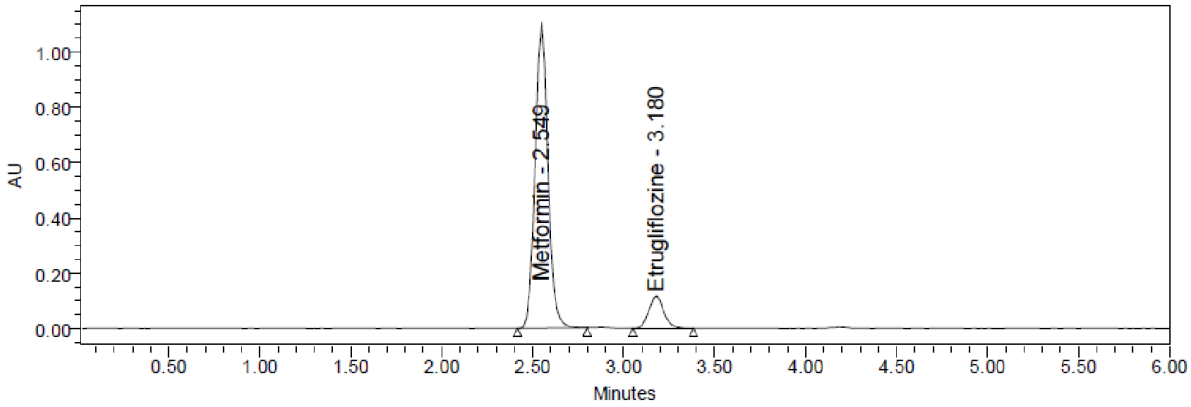


System suitability Chromatogram

Discussion: 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 system suitable parameters were passed and were within the limits.

Validation:

Specificity:



Discussion: Retention times of Metformin and Ertugliflozin were 2.549 min and 3.180 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity:

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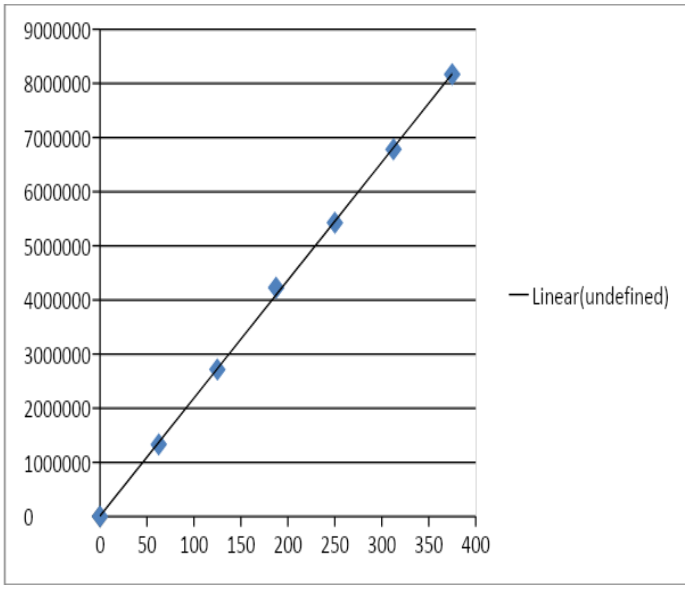
Linearity table for Metformin and Ertugliflozin

Metformin		**Ertugliflozin**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
62.5	1329694	0.9375	17154
125	2714369	1.8750	34608
187.5	4225090	2.8125	53325
250	5426596	3.7500	70607
312.5	6782048	4.6875	87622
375	8167025	5.6250	103777



Calibration curve of Metformin

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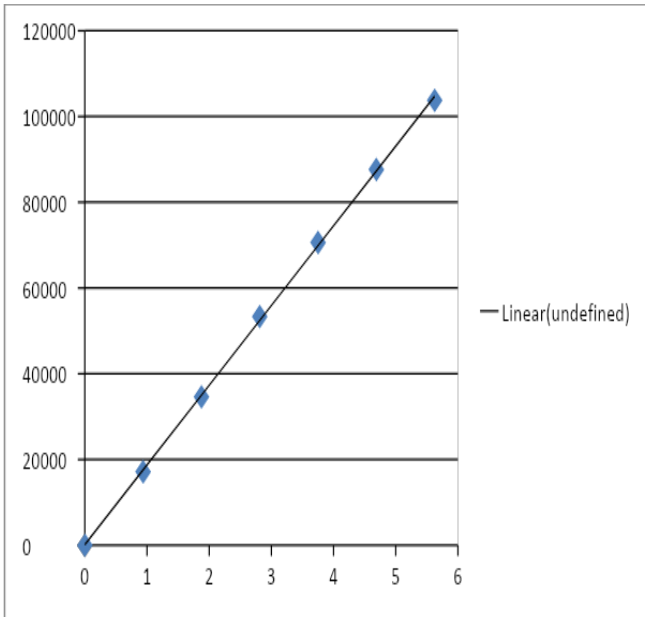
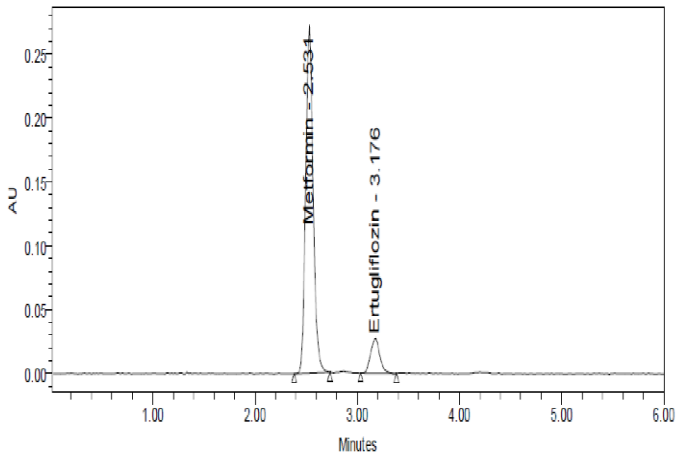
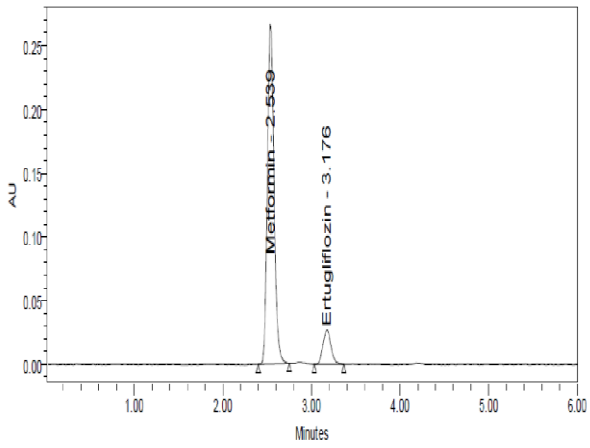


Calibration curve of Ertugliflozin

Discussion: Six linear concentrations of Metformin 62.5-375µg/ml) and Ertugliflozin (0.9375-5.6250µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Metformin was y = 21782x + 8044.9 and of Ertugliflozin was y = 18601x + 127.71. Correlation coefficient obtained was 0.999 for the two drugs.





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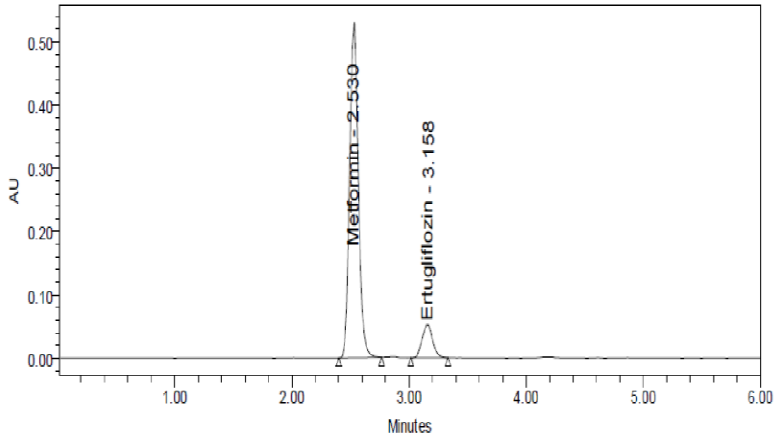
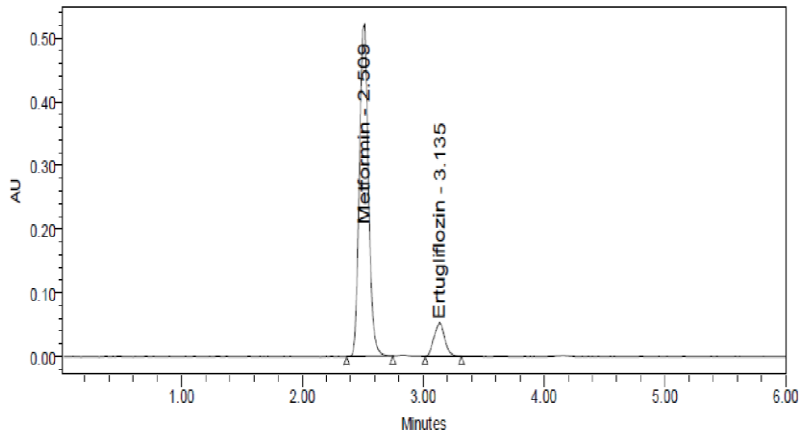
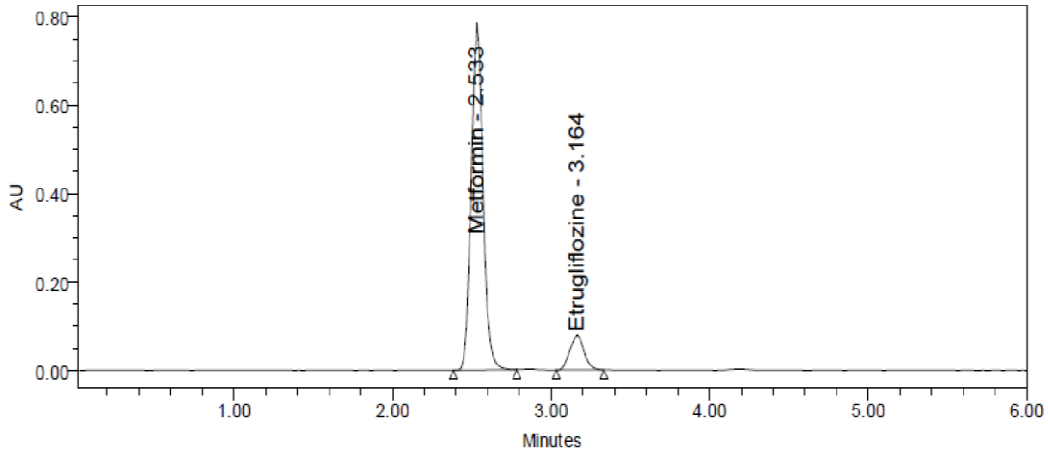
Linearity 25% Chromatogram of Metformin and Ertugliflozin





Linearity 50% Chromatogram of Metformin and Ertugliflozin



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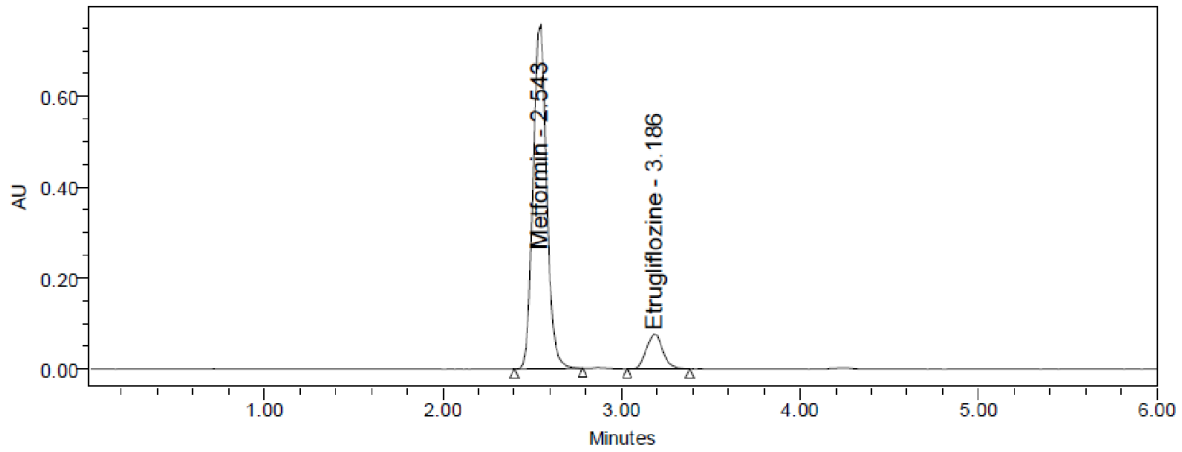
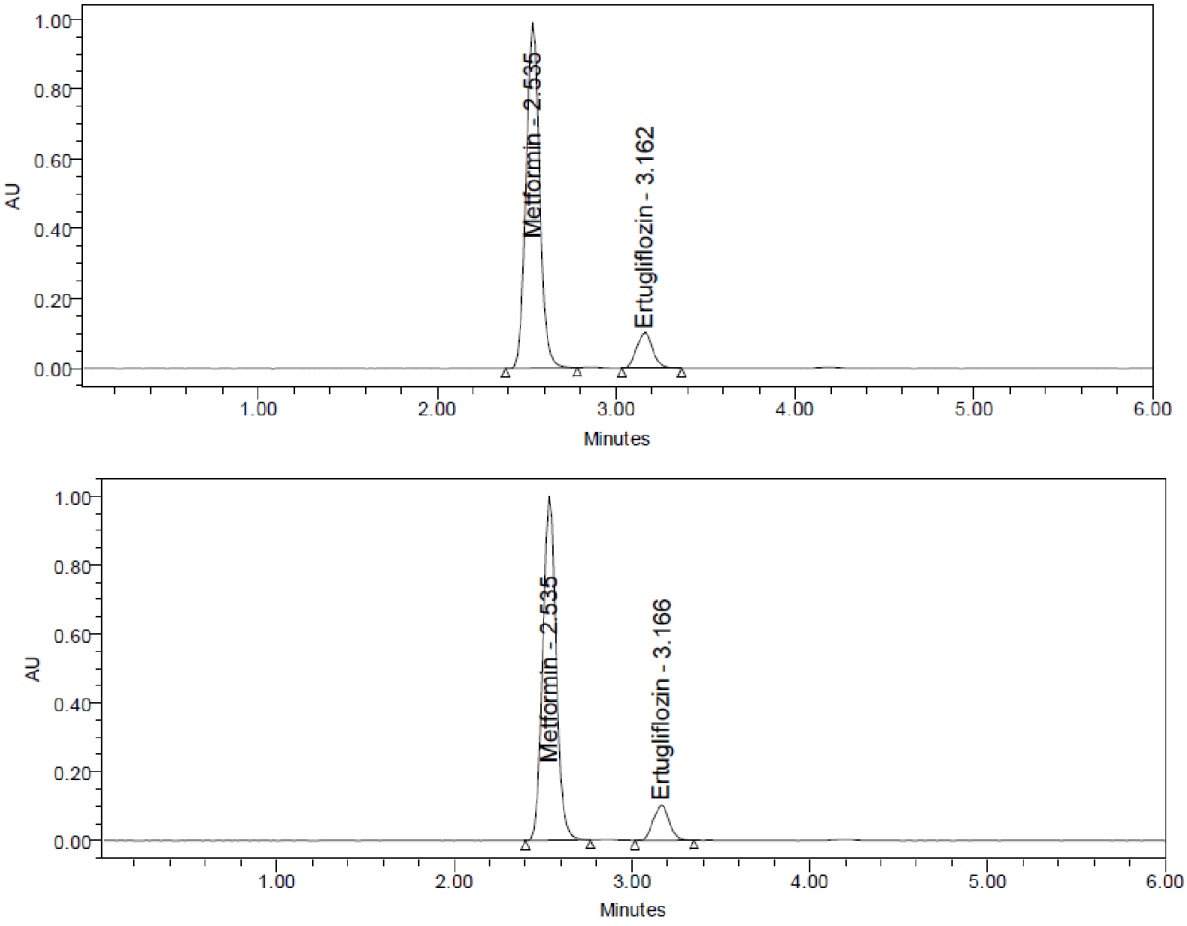


Linearity 75% Chromatogram of Metformin and Ertugliflozin



Linearity 100% Chromatogram of Metformin and Ertugliflozin

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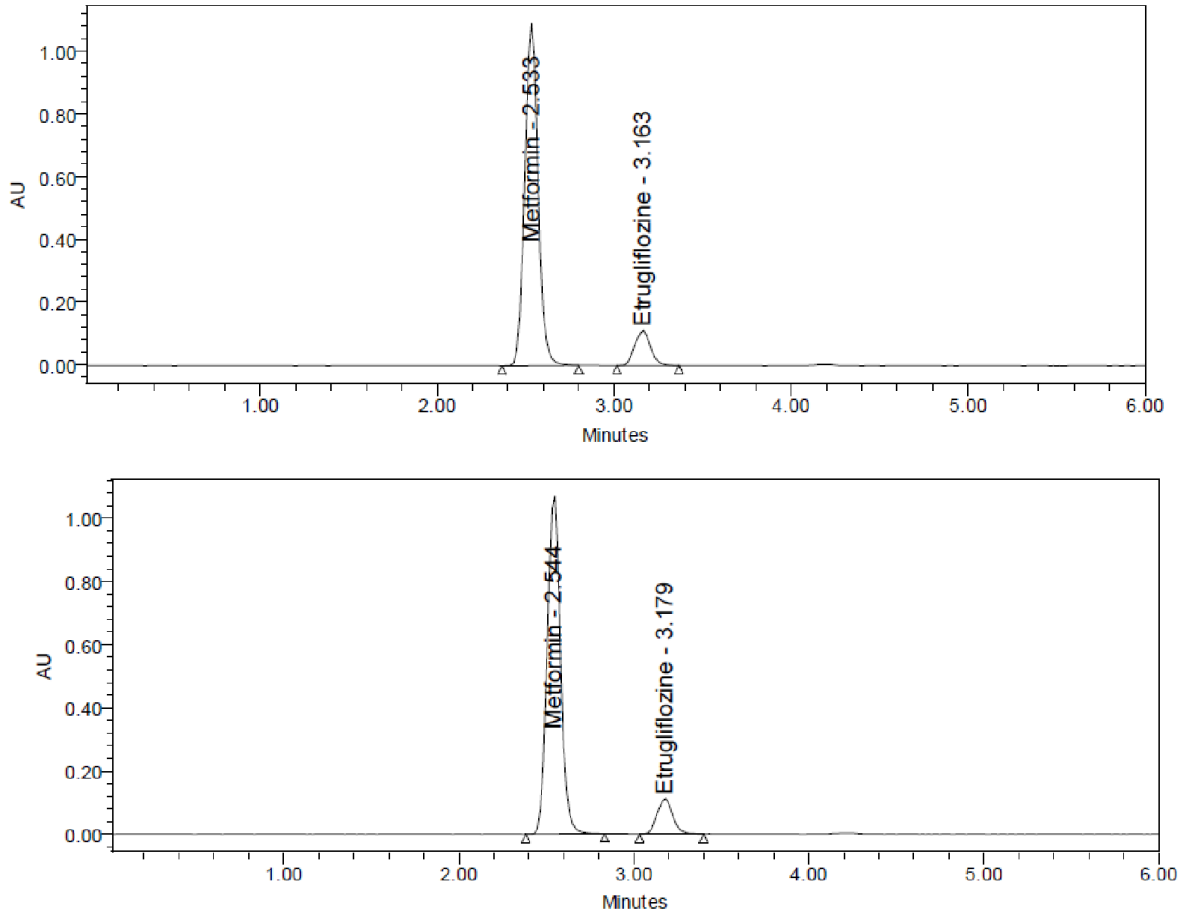
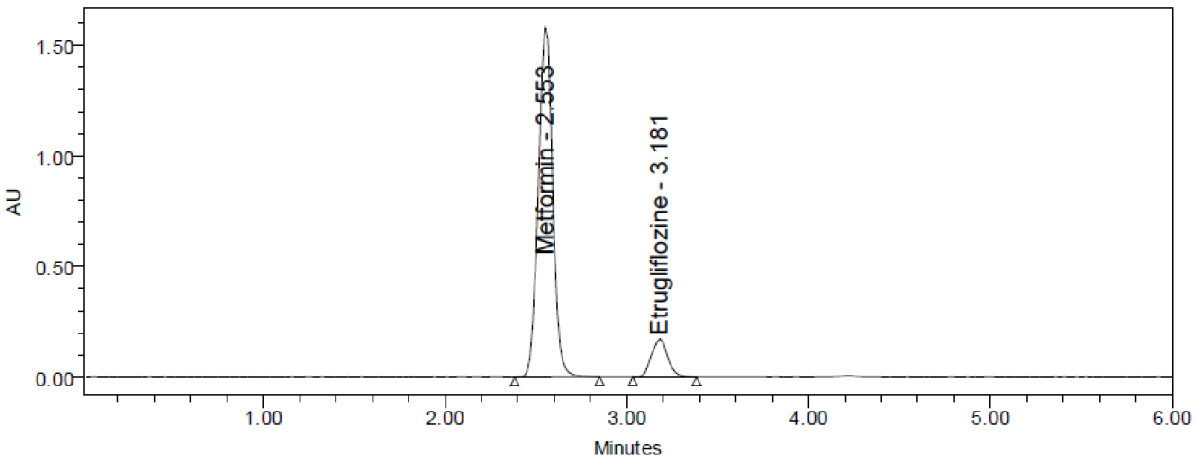


Linearity 125% Chromatogram of Metformin and Ertugliflozin



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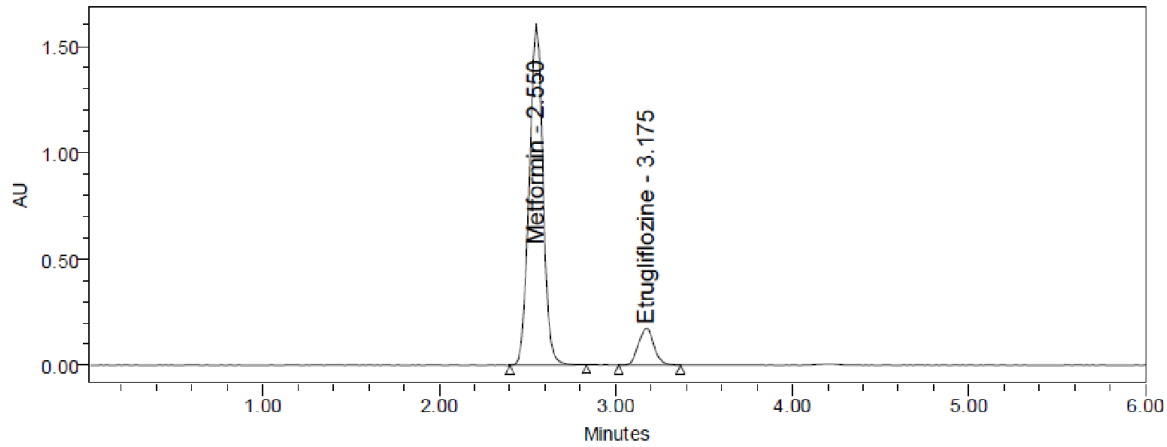


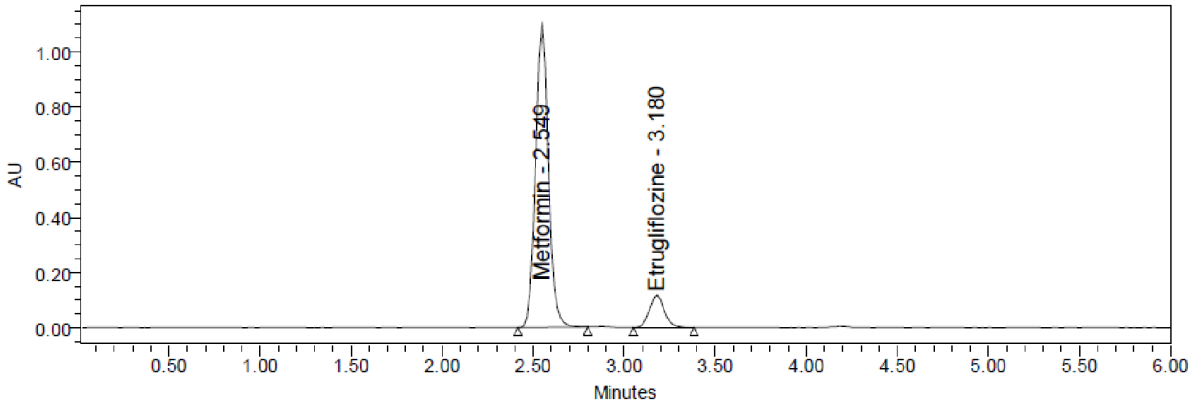
Linearity 150% Chromatogram of Metformin and Ertugliflozin

Precision:

System Precision:

System precision table of Metformin and Ertugliflozin

S. No	Area of Metformin	Area of Ertugliflozin
1.	5413850	70401
2.	5405357	70615
3.	5419250	70883
4.	5412375	70583
5.	5422266	70323
6.	5418060	70058
Mean	5415193	70477
S.D	6019.9	282.9
%RSD	0.1	0.4



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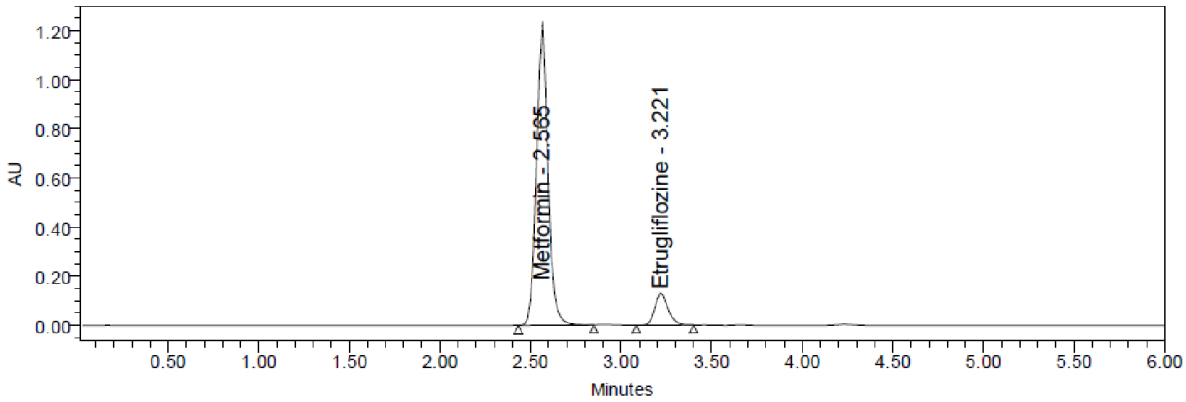

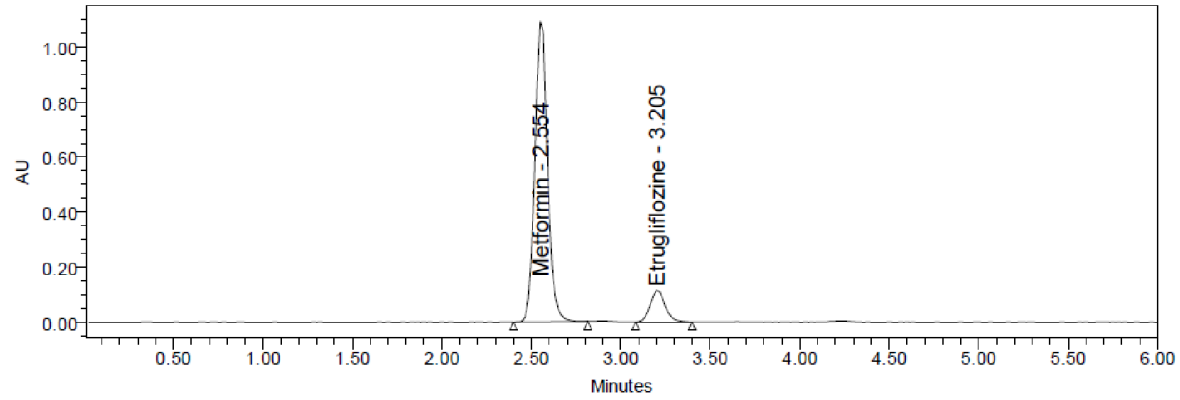

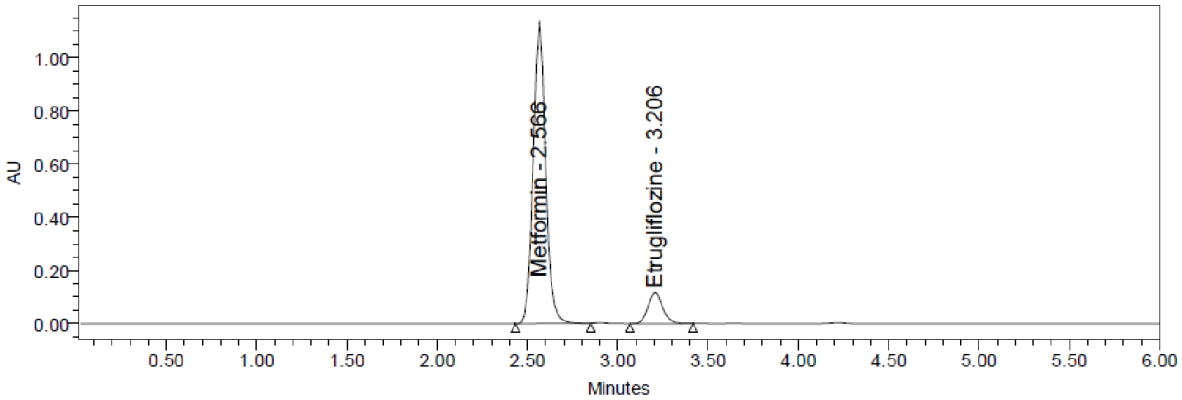

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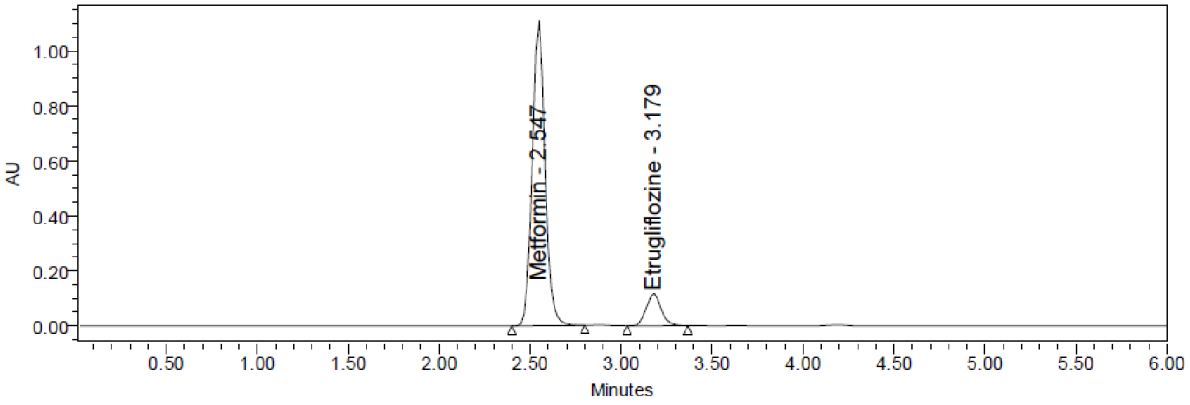

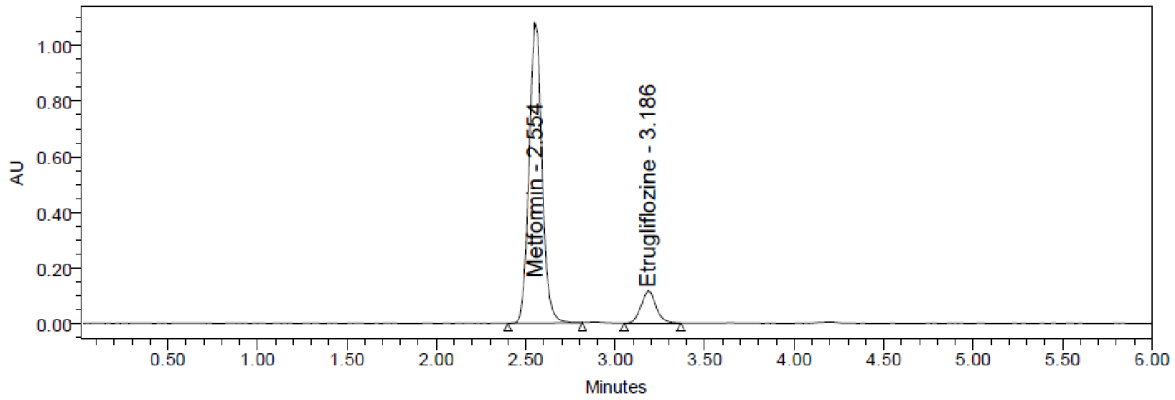

System precision chromatogram

Discussion: 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. % RSD obtained as 0.1% and 0.4% respectively for Metformin and Ertugliflozin .As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability: Repeatability table of Metformin and Ertugliflozin

S. No	**Area of Metformin**	**Area of Ertugliflozin**
1.	5438567	70768
2.	5407464	70057
3.	5403533	70951
4.	5423346	70997
5.	5475652	70785
6.	5462600	70247
Mean	5435194	70634
S.D	29363.5	388.8
%RSD	0.5	0.6

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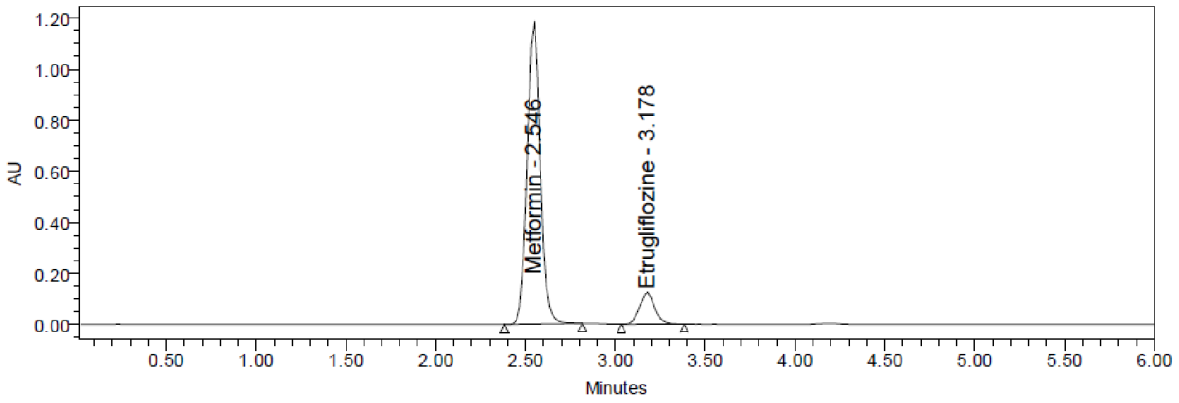

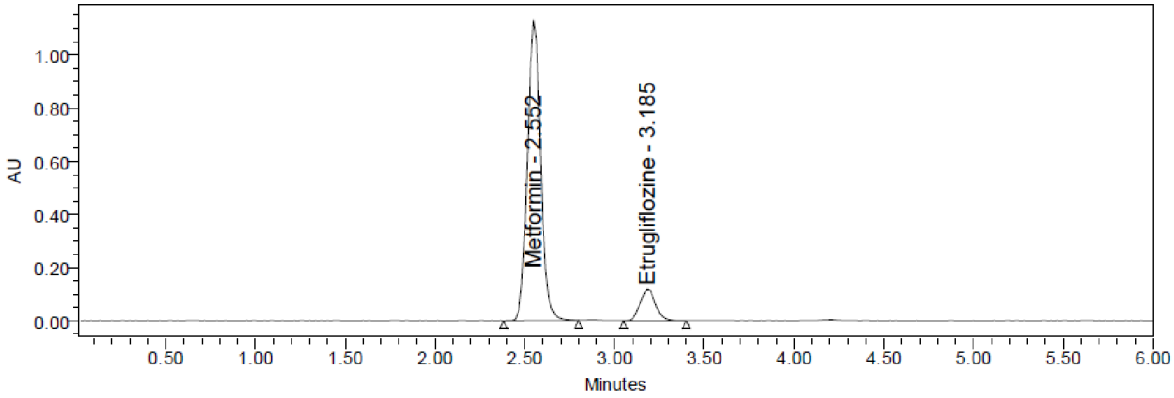

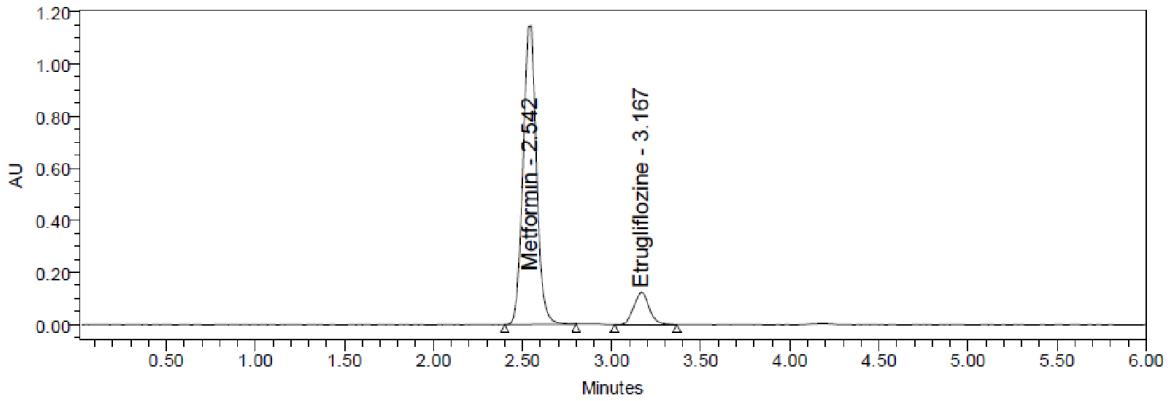

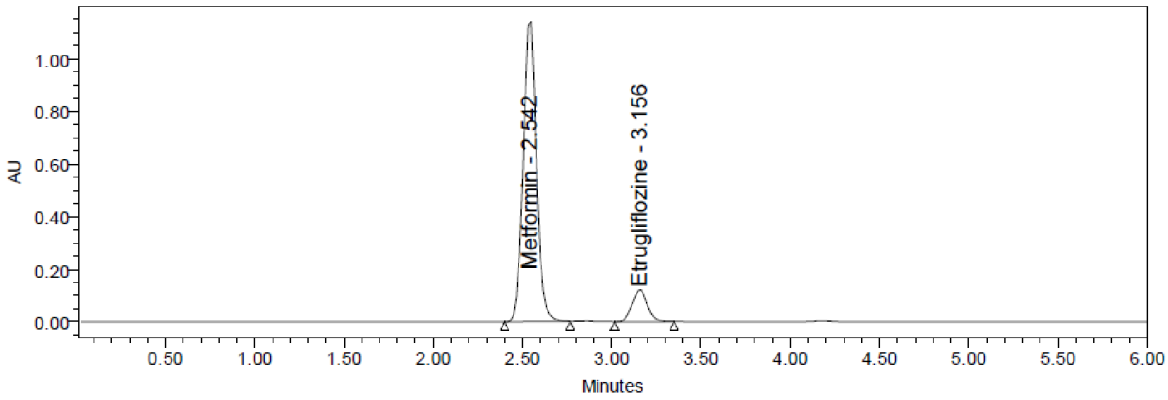

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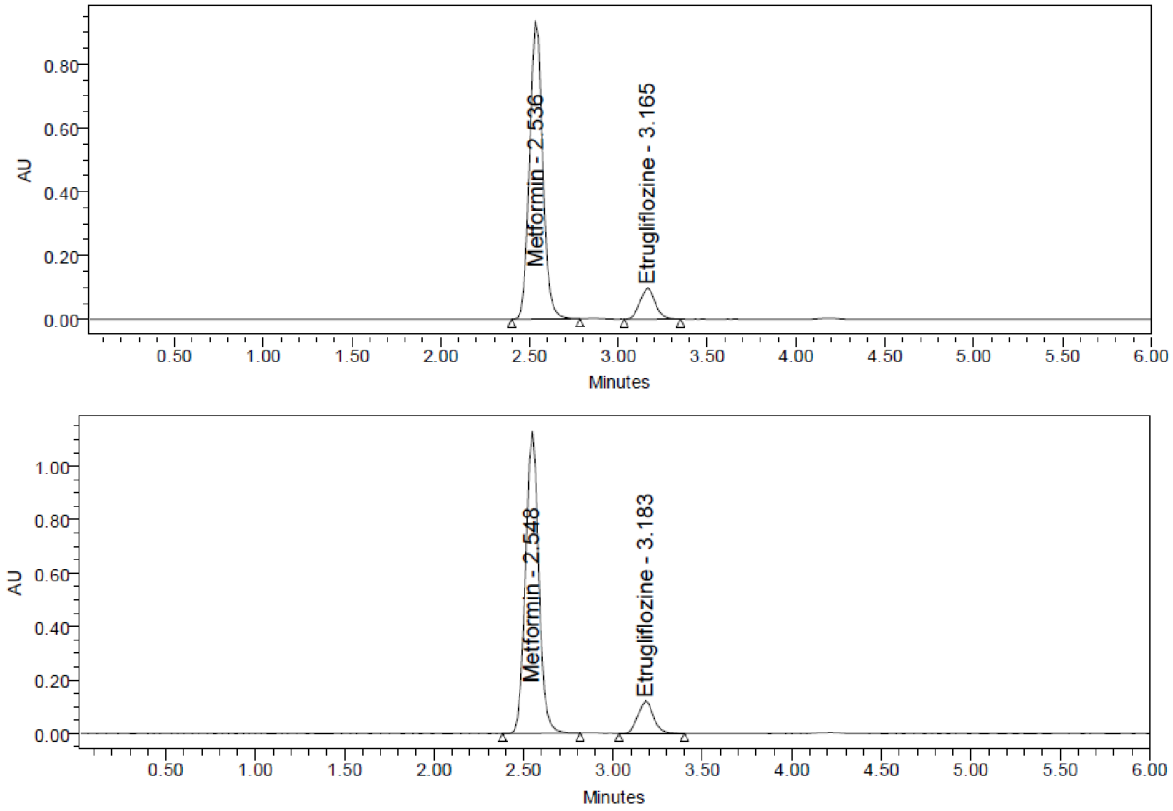

Repeatability chromatogram

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.5% respectively for Metformin and Ertugliflozin . As the limit of Precision was less than "2" the system precision was passed in this method

.Intermediate precision (Day_ Day Precision): Intermediate precision table of Metformin and Ertugliflozin

S. No	Area of Metformin	Area of Ertugliflozin
1.	5350349	68057
2.	5382262	68460
3.	5382534	67355
4.	5349702	68380
5.	5370550	67708
6.	5294015	67692
Mean	5354902	67942
S.D	33202.3	432.5
%RSD	0.6	0.6

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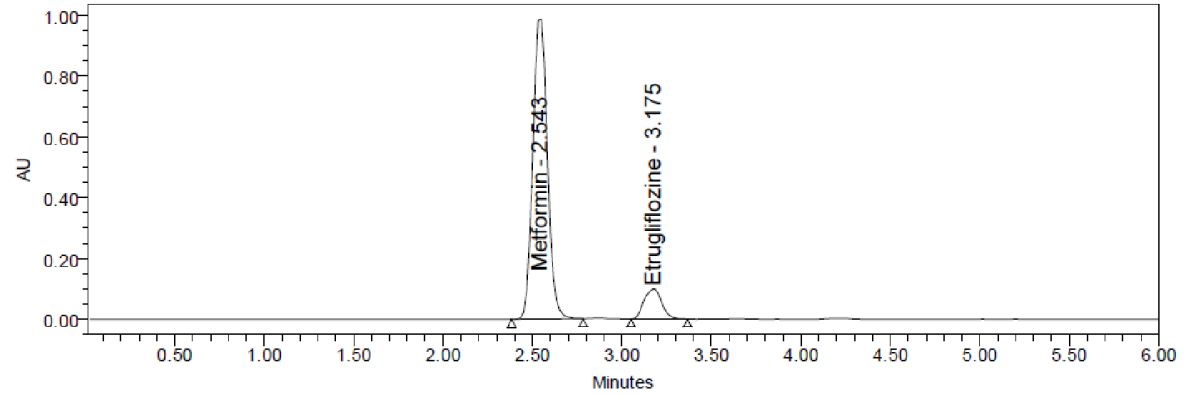

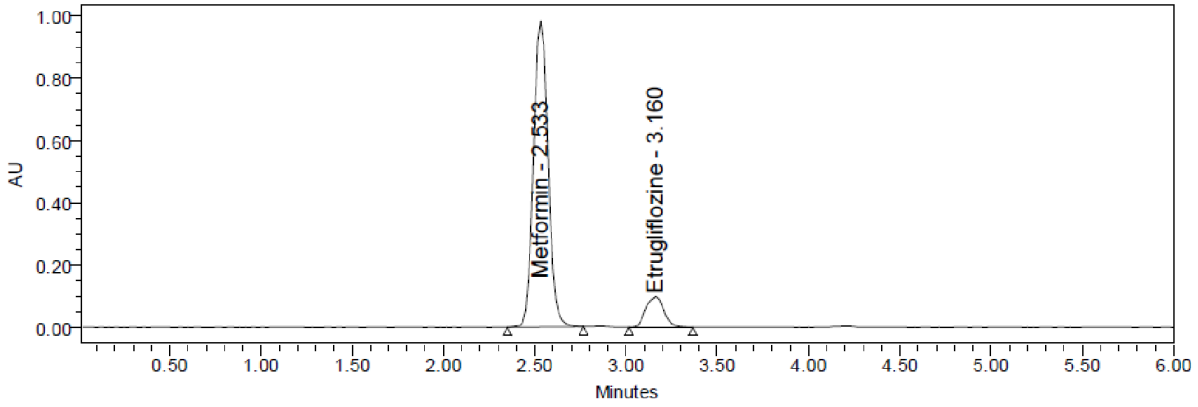

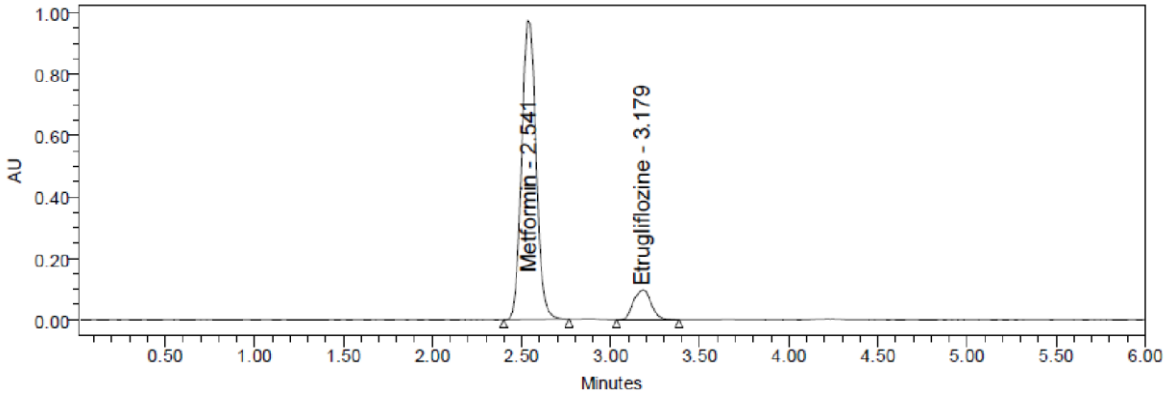

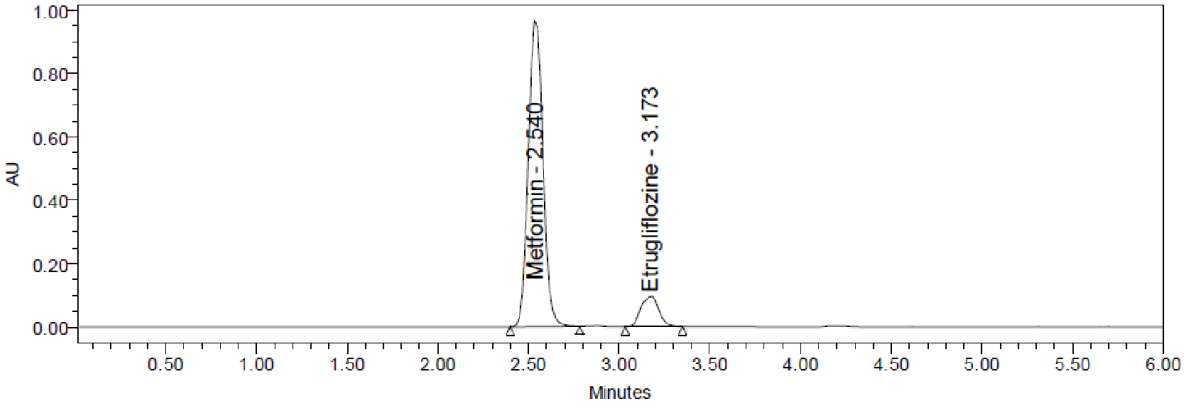

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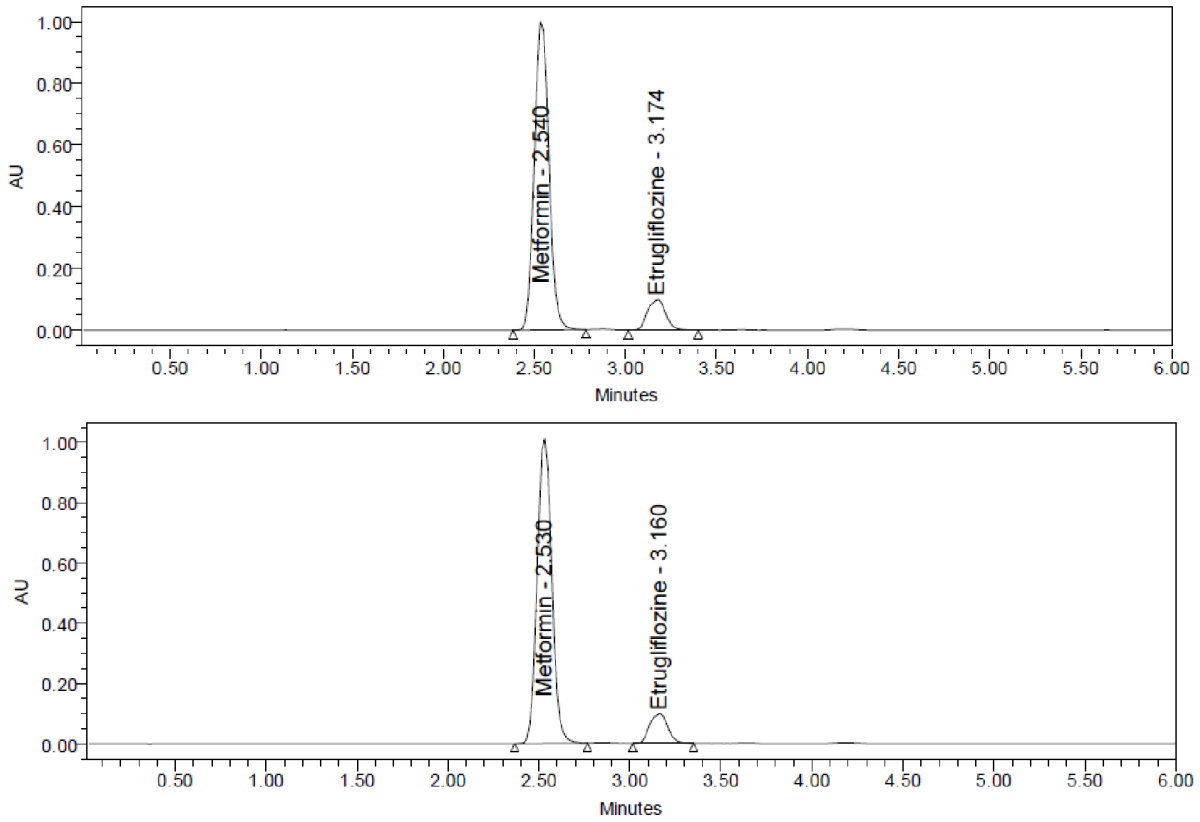

Inter Day precision Chromatogram

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.6% and 0.6% respectively for Metformin and Ertugliflozin . As the limit of Precision was less than “2” the system precision was passed in this method.

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Accuracy: Accuracy table of Metformin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	125	125.33	100.27	100.52%
	125	124.54	99.63	
	125	124.47	99.57	
100%	250	254.89	101.96	
	250	254.17	101.67	
	250	250.54	100.22	
150%	375	373.41	99.58	
	375	375.65	100.17	
	375	381.19	101.65	

Accuracy table of Ertugliflozin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	1.875	1.86	99.27	99.80%
	1.875	1.87	99.69	
	1.875	1.87	99.89	
100%	3.75	3.78	100.88	
	3.75	3.73	99.49	
	3.75	3.79	101.09	
150%	5.625	5.59	99.39	

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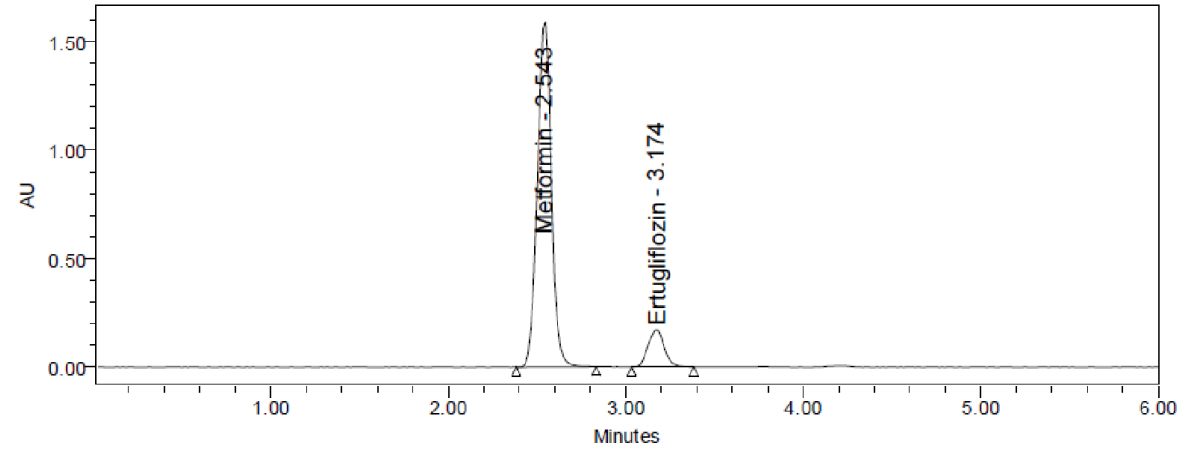
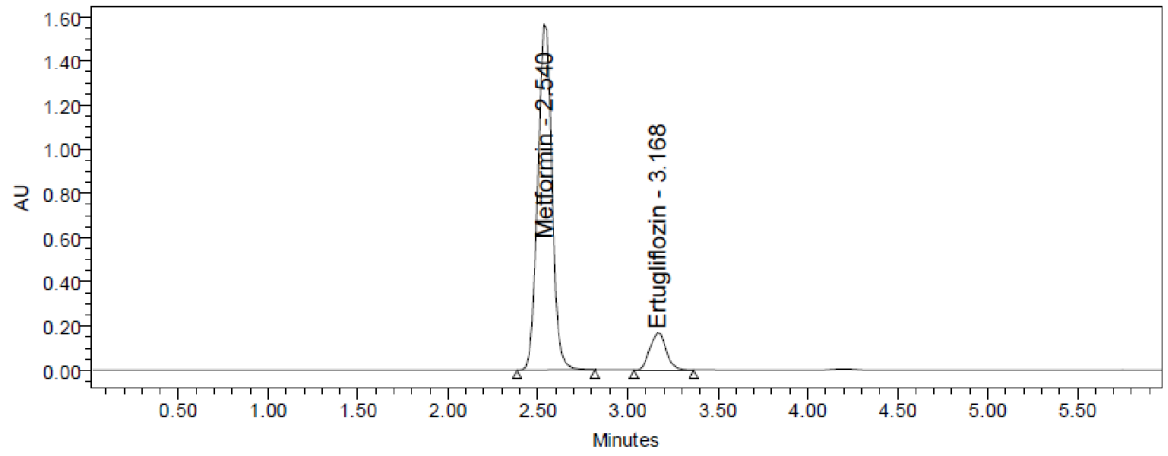
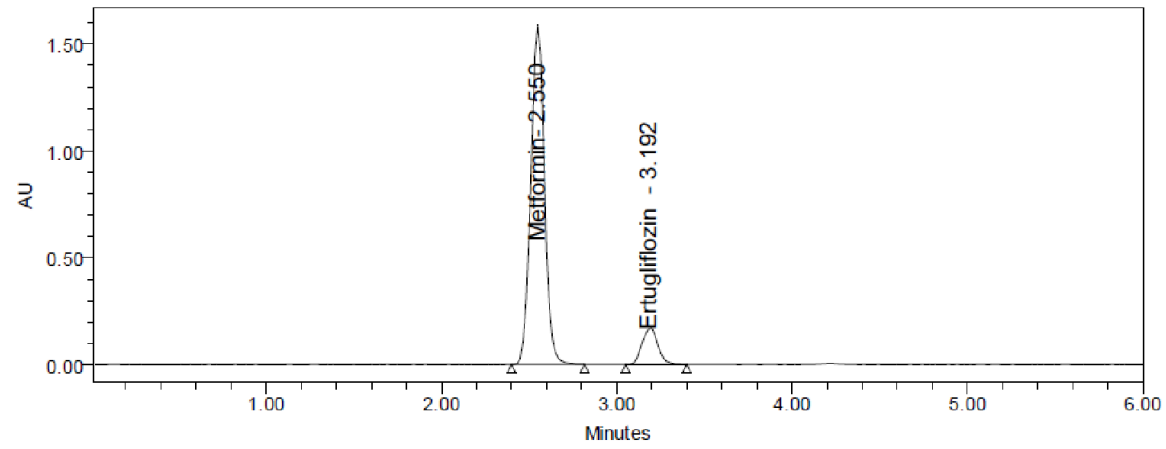
	5.625	5.58	99.14	
	5.625	5.59	99.32	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.52% and 99.80% for Metformin and Ertugliflozin respectively.







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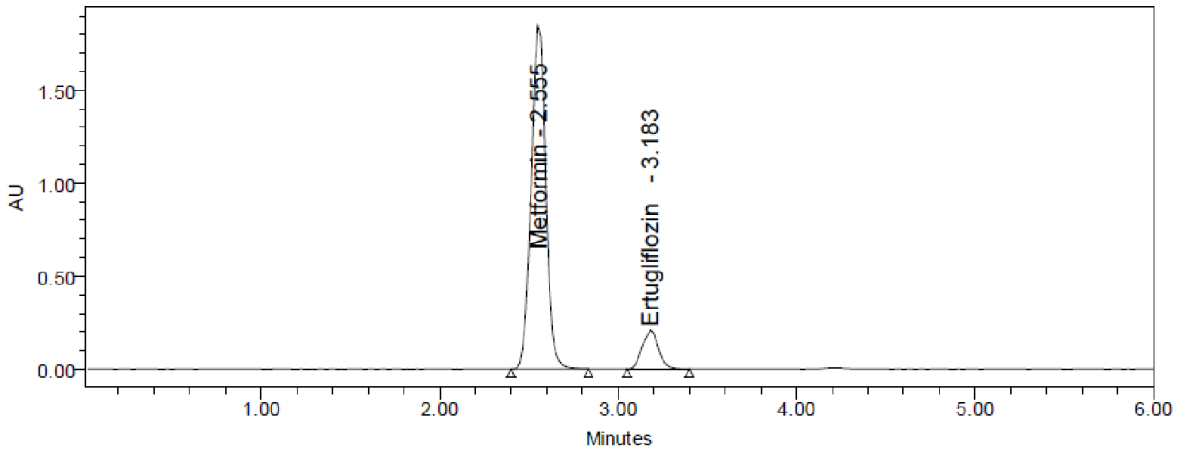
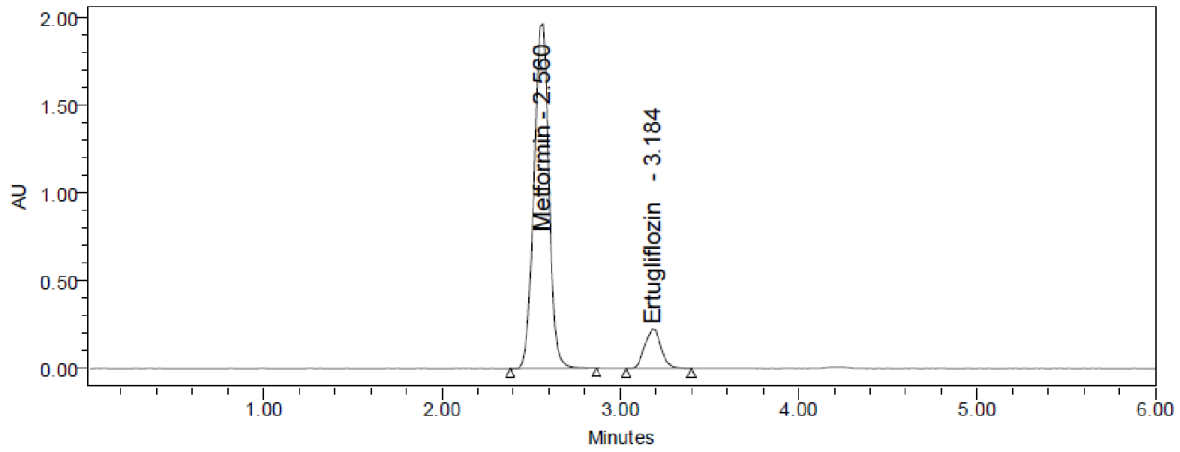
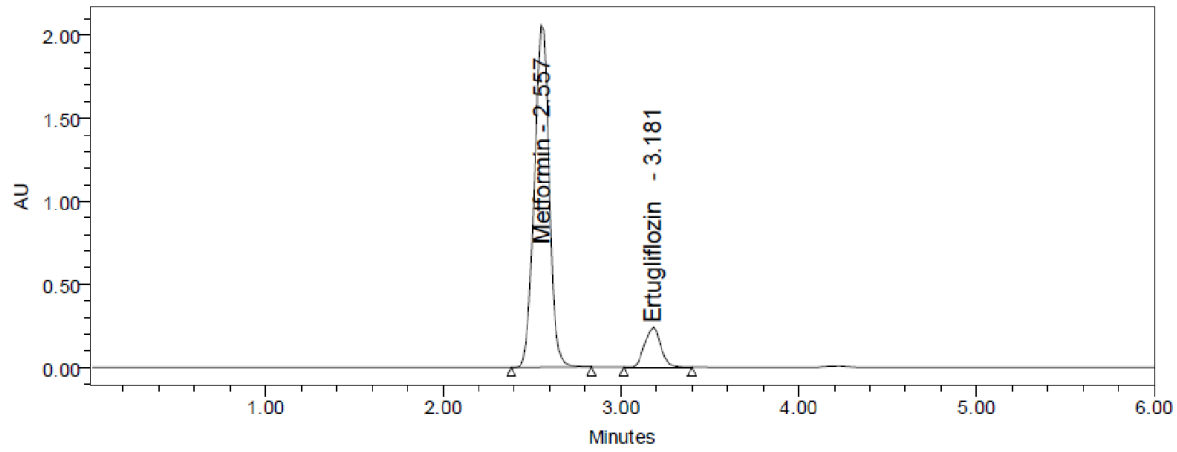
Accuracy 50% Chromatogram of Metformin and Ertugliflozin







Accuracy 100% Chromatogram of Metformin and Ertugliflozin

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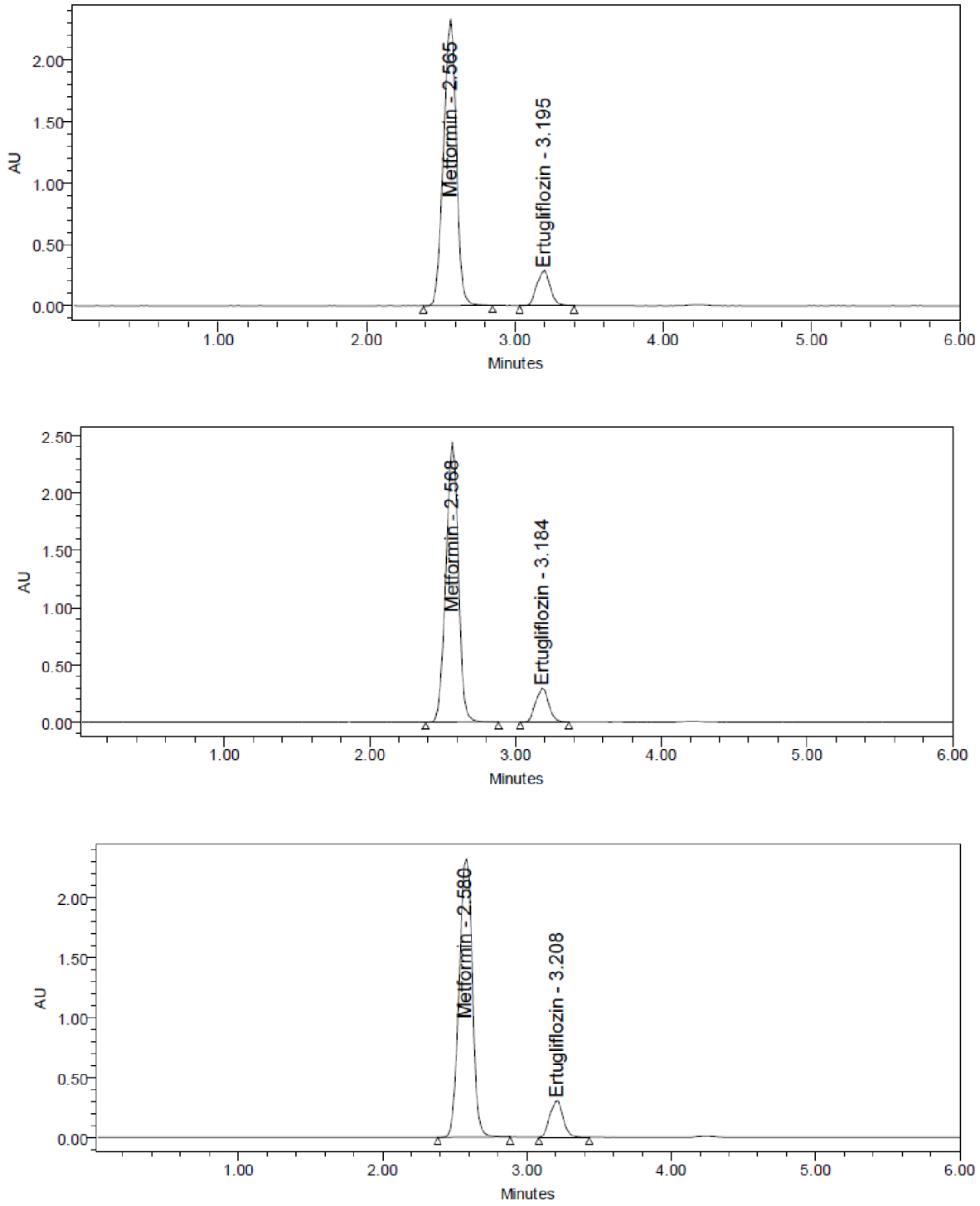

Accuracy 150% Chromatogram of Metformin and Ertugliflozin

Sensitivity: Sensitivity table of Metformin and Ertugliflozin

Molecule	**LOD**	**LOQ**
Metformin	0.36	1.08
Ertugliflozin	0.01	0.03

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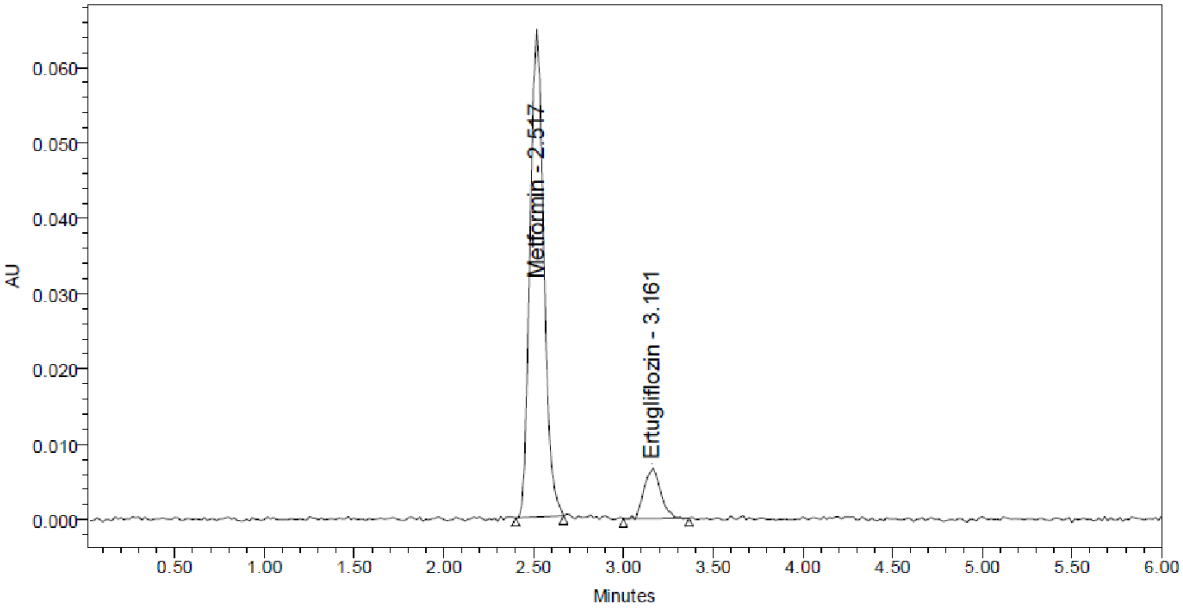
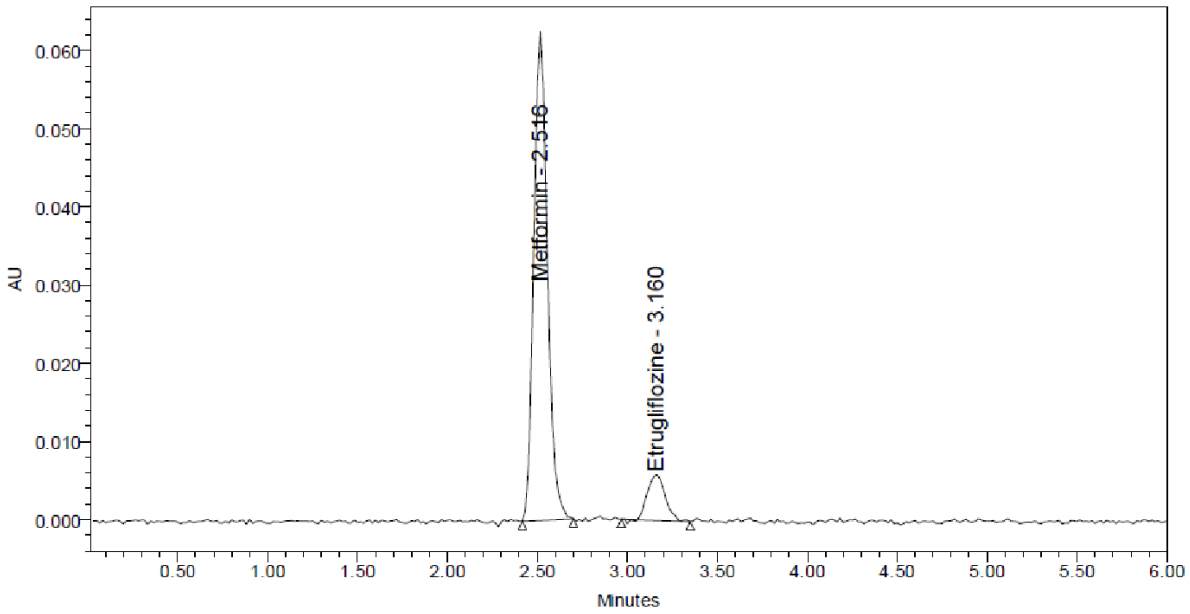


LOD Chromatogram of Standard



LOQ Chromatogram of of Standard

Robustness:

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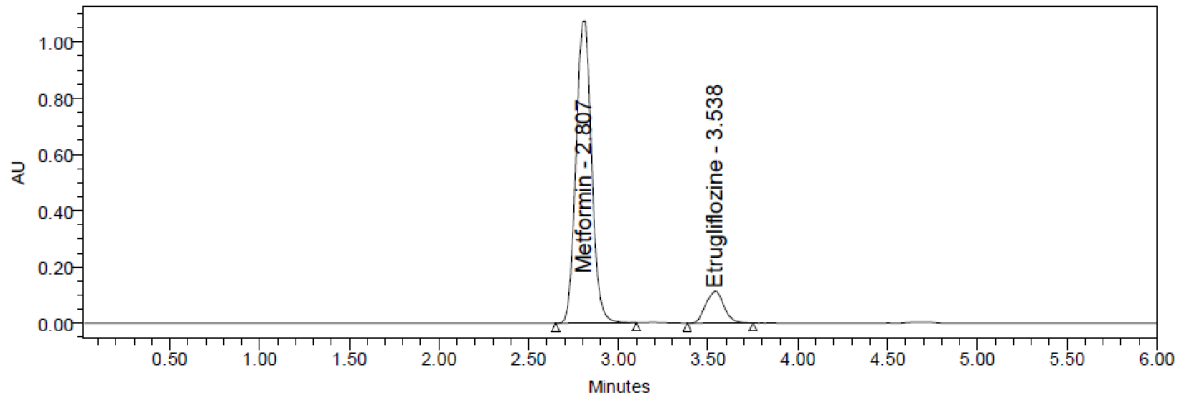
Robustness data for Metformin and Ertugliflozin .

S.no	Condition	%RSD of Metformin	%RSD of Ertugliflozin
1	Flow rate (-) 0.9ml/min	0.3	0.3
2	Flow rate (+) 1.1ml/min	0.3	0.5
3	Mobile phase (-) 40B:60A	0.8	1.5
4	Mobile phase (+) 60B:40A	0.9	0.2
5	Temperature (-) 25°C	1.0	1.3
6	Temperature (+) 35°C	0.5	0.5

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (40B:60A), mobile phase plus (60B:40A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.



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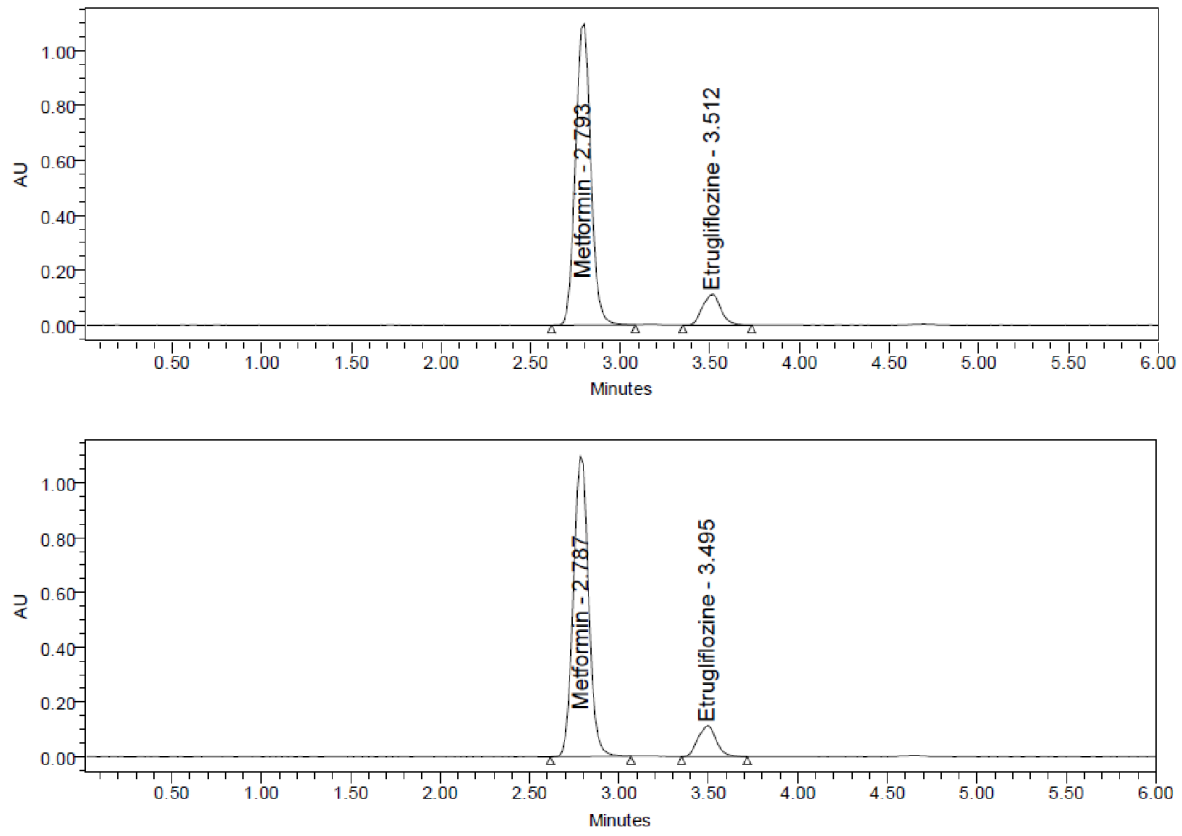
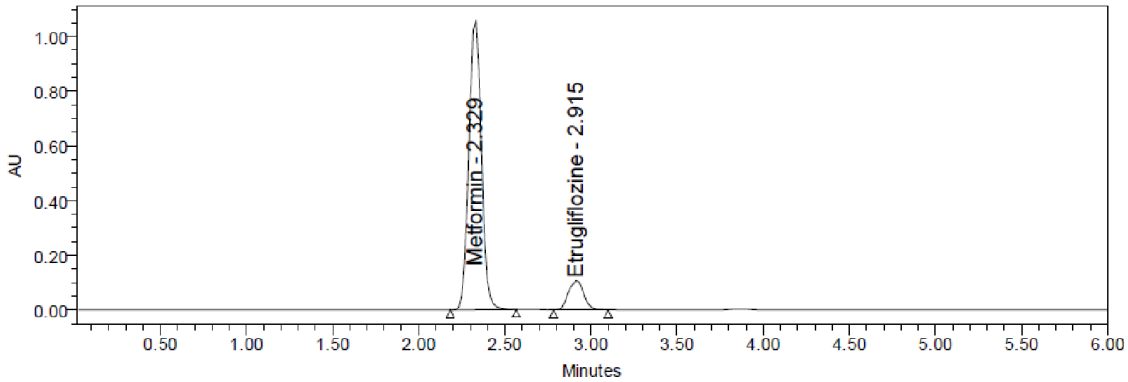


Flow minus Chromatogram of Metformin and Ertugliflozin .



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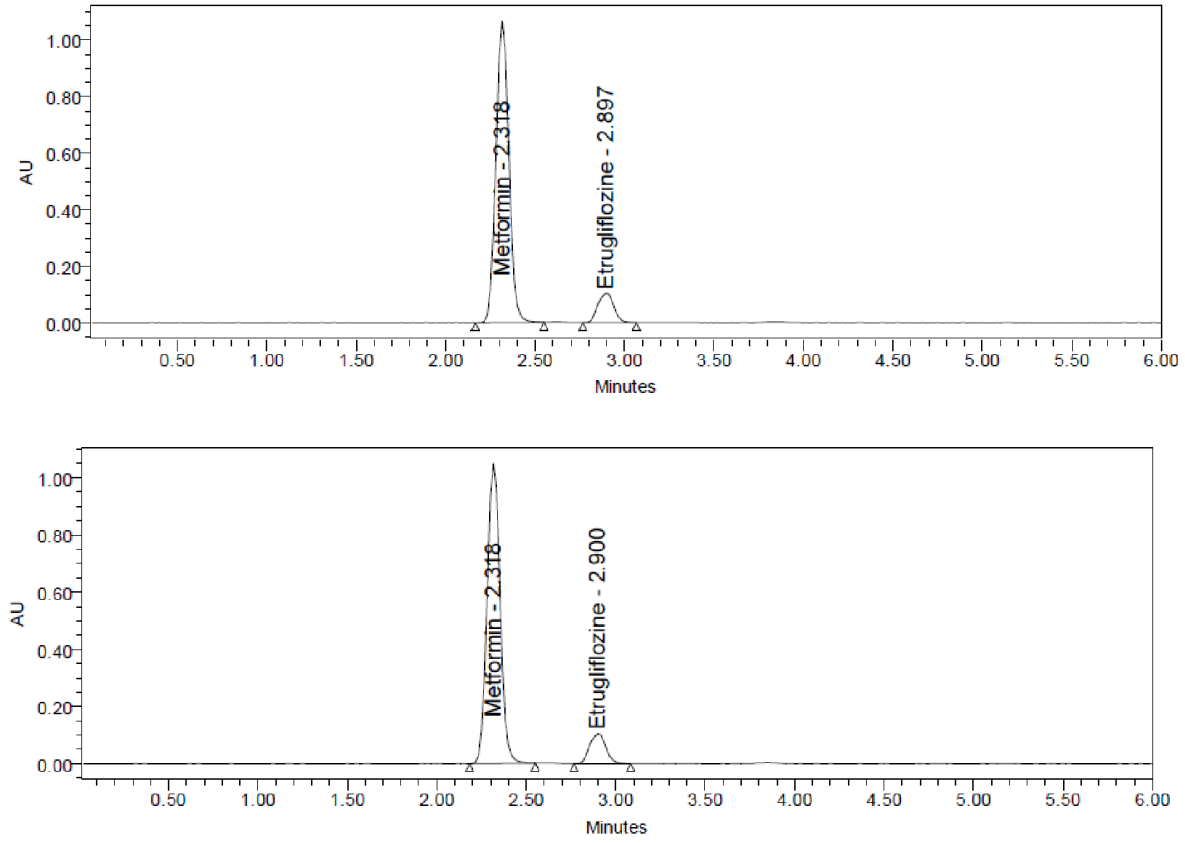
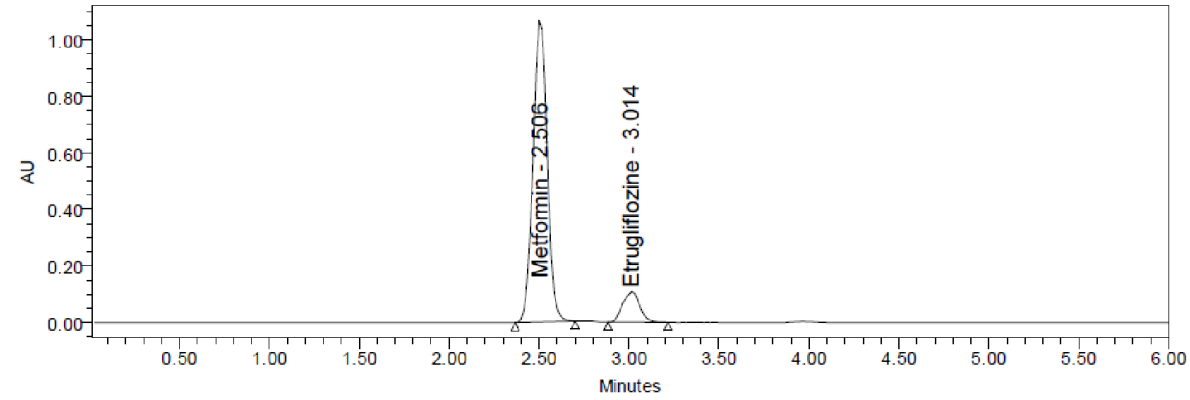


Flow plus Chromatogram of Metformin and Ertugliflozin .



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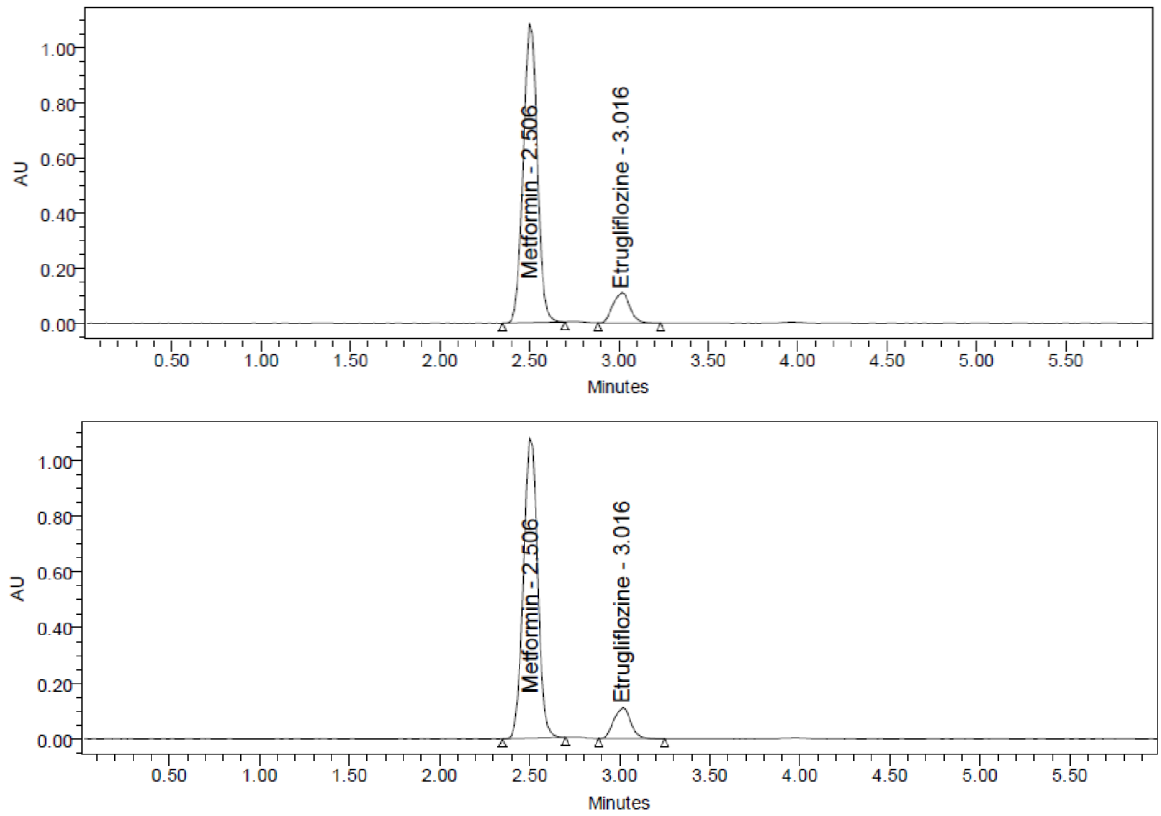
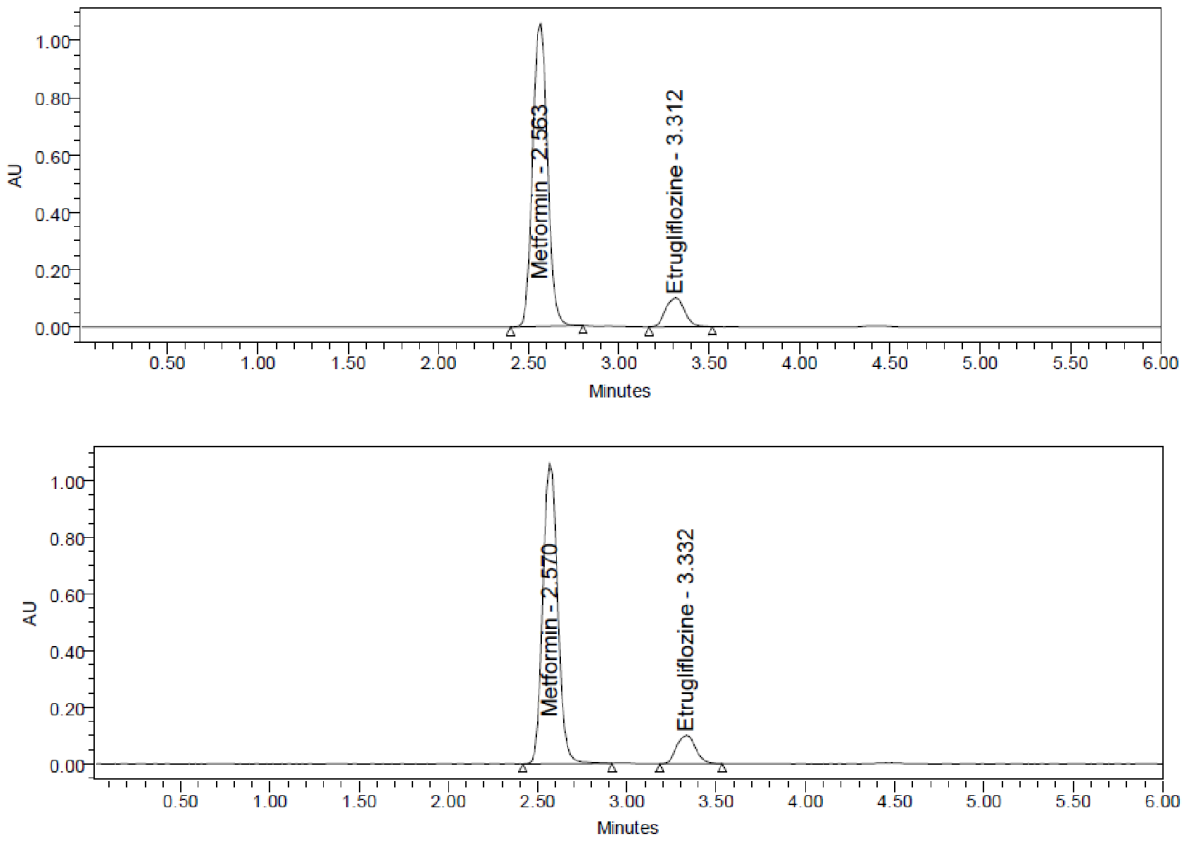


Mobile phase minus Chromatogram of Metformin and Ertugliflozin .



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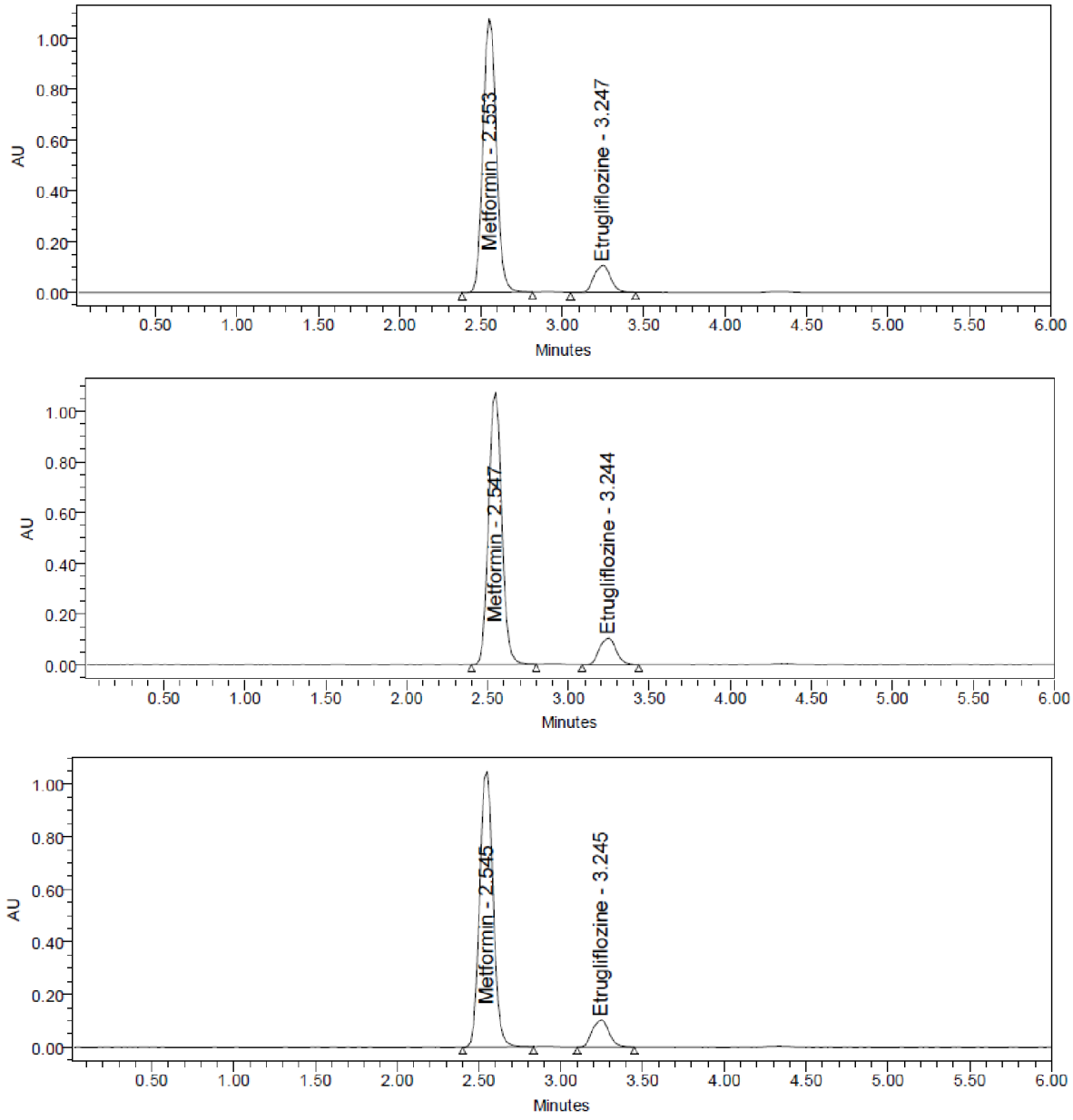
Mobile phase Plus Chromatogram of Metformin and Ertugliflozin .



Temperature minus Chromatogram of Metformin and Ertugliflozin . .

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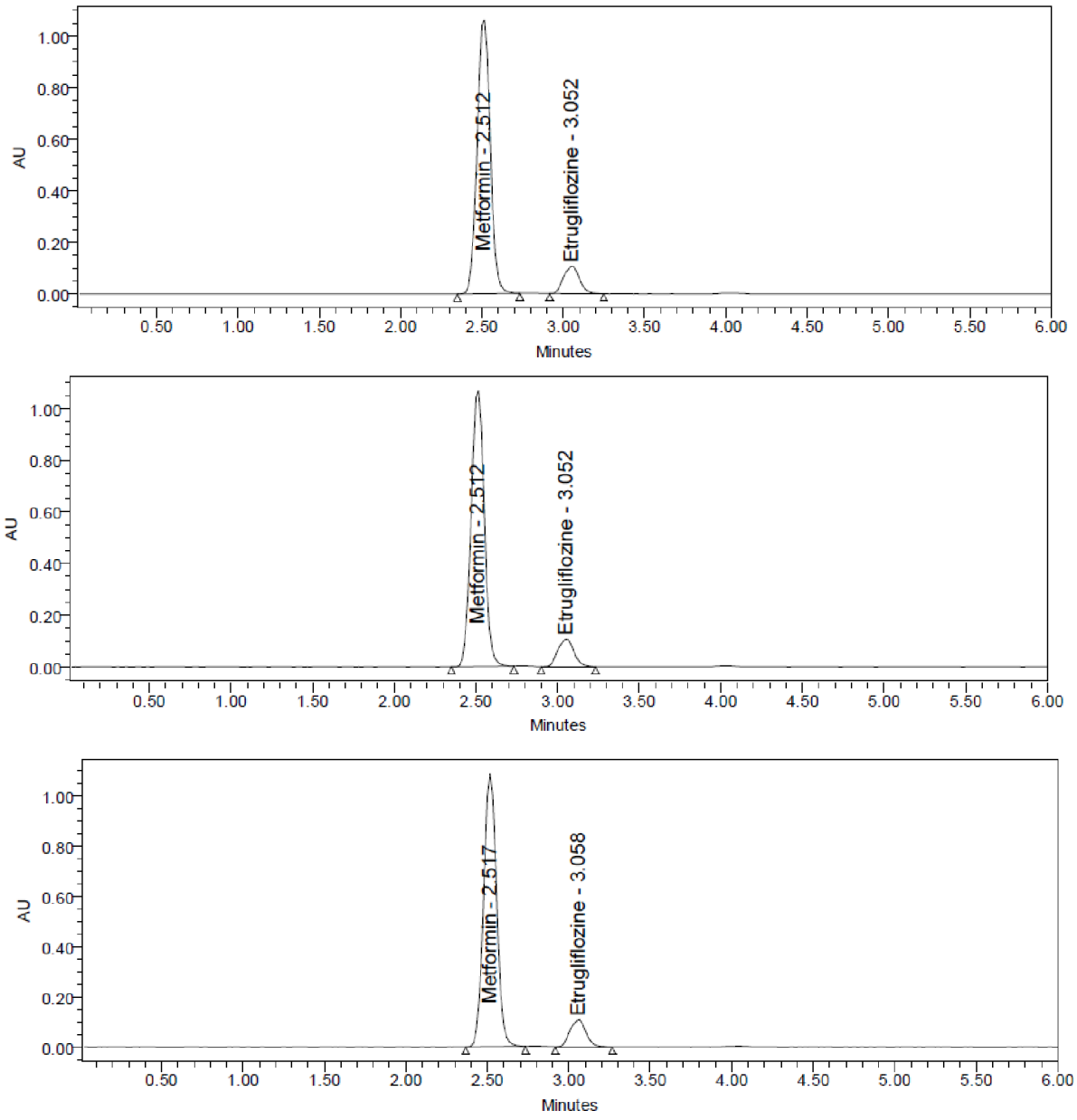

Temperature plus Chromatogram of Metformin and Ertugliflozin

Assay: Medley pharmaceuticals (U MOM), bearing the label claim Metformin 600mg, Ertugliflozin 50mg. Assay was performed with the above formulation. Average % Assay for Metformin and Ertugliflozin obtained was 100.27% and 100.12% respectively

Assay Data of Metformin

S.no	Standard Area	Sample area	% Assay
1	5413850	5438567	100.33

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2	5405357	5407464	99.76
3	5419250	5403533	99.68
4	5412375	5423346	100.05
5	5422266	5475652	101.02
6	5418060	5462600	100.77
Avg	5415193	5435194	100.27
Stdev	6019.9	29363.5	0.54
%RSD	0.1	0.5	0.5

.

Assay Data of Ertugliflozin

S. no	**Standard Area**	**Sample area**	**% Assay**
1	70401	70768	100.31
2	70615	70057	99.30
3	70883	70951	100.57
4	70583	70997	100.64
5	70323	70785	100.34
6	70058	70247	99.57
Avg	70477	70634	100.12
Stdev	282.9	388.8	0.55
%RSD	0.4	0.6	0.55

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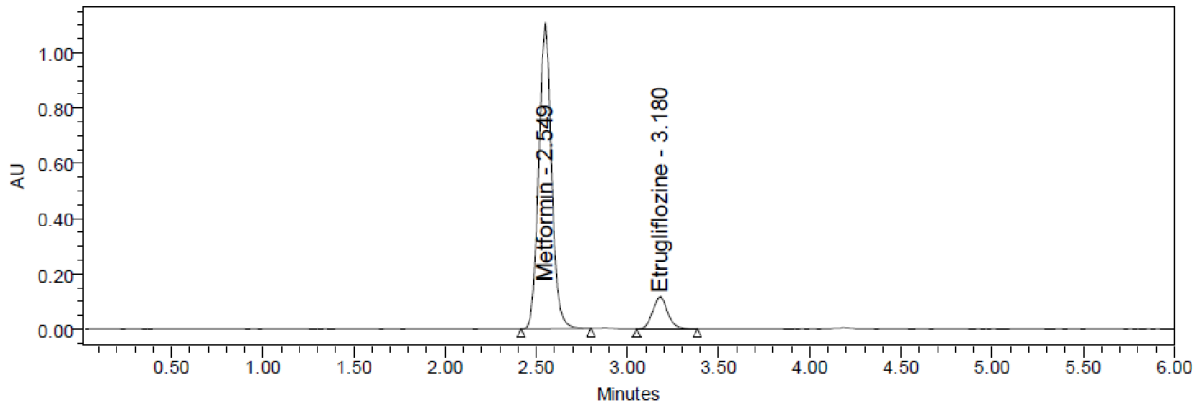
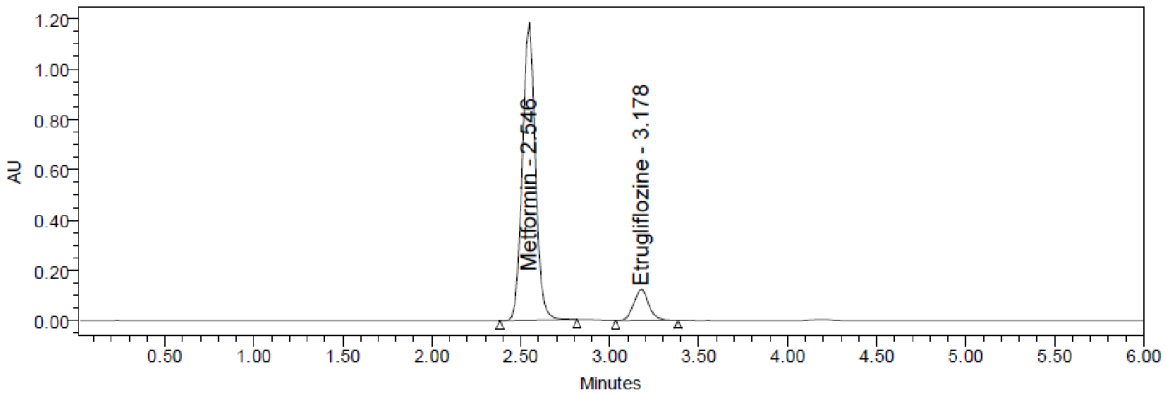


Chromatogram of working standard solution



Chromatogram of working sample solution

Degradation data

Type of degradation	**Metformin**			**Ertugliflozin**		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	5087955	93.86	6.14	70401	92.37	7.63
Base	5141099	94.84	5.16	70615	94.96	5.04
Peroxide	5231147	96.50	3.50	70883	96.20	3.80
Thermal	5296383	97.71	2.29	70583	97.65	2.35
Uv	5350761	98.71	1.29	70323	98.55	1.45
Water	5401273	98.71	1.29	70058	99.14	0.86

Degradation chromatograms

Acid degradation chromatogram

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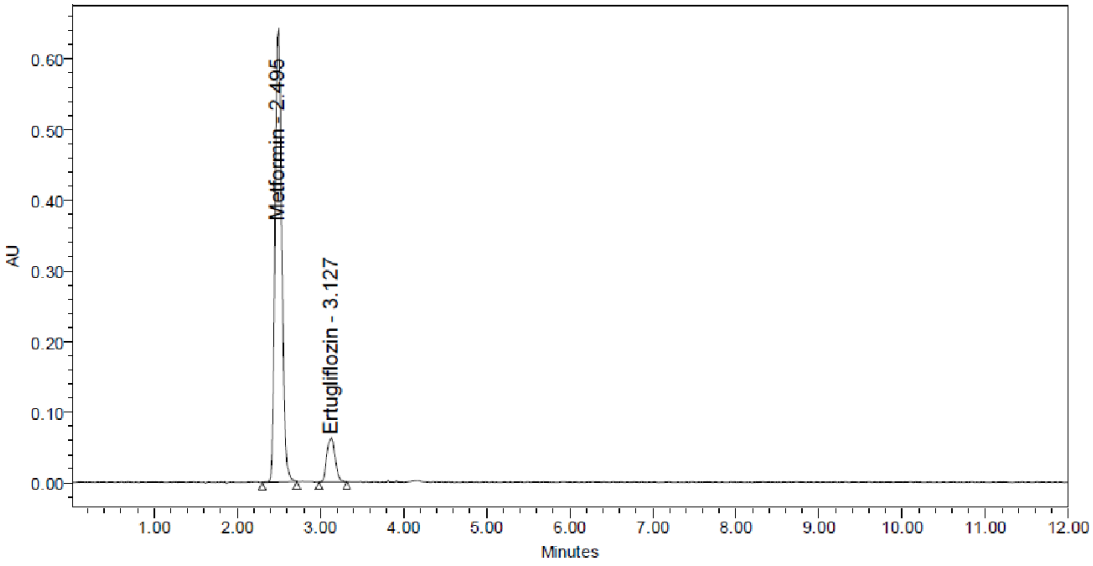
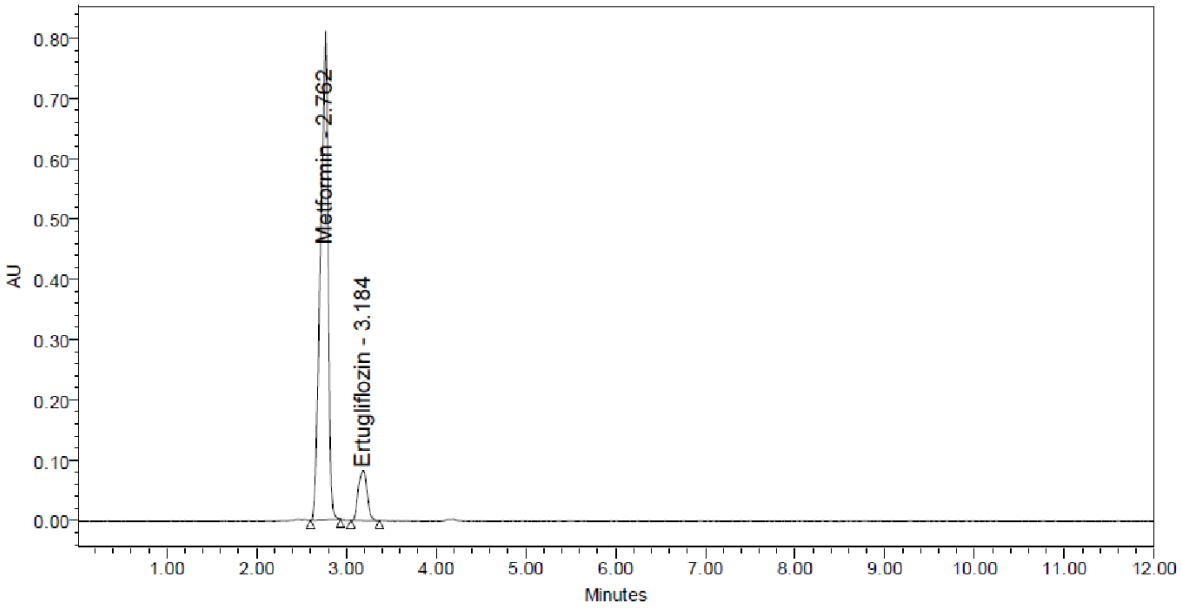


acid

Base degradation chromatogram



base

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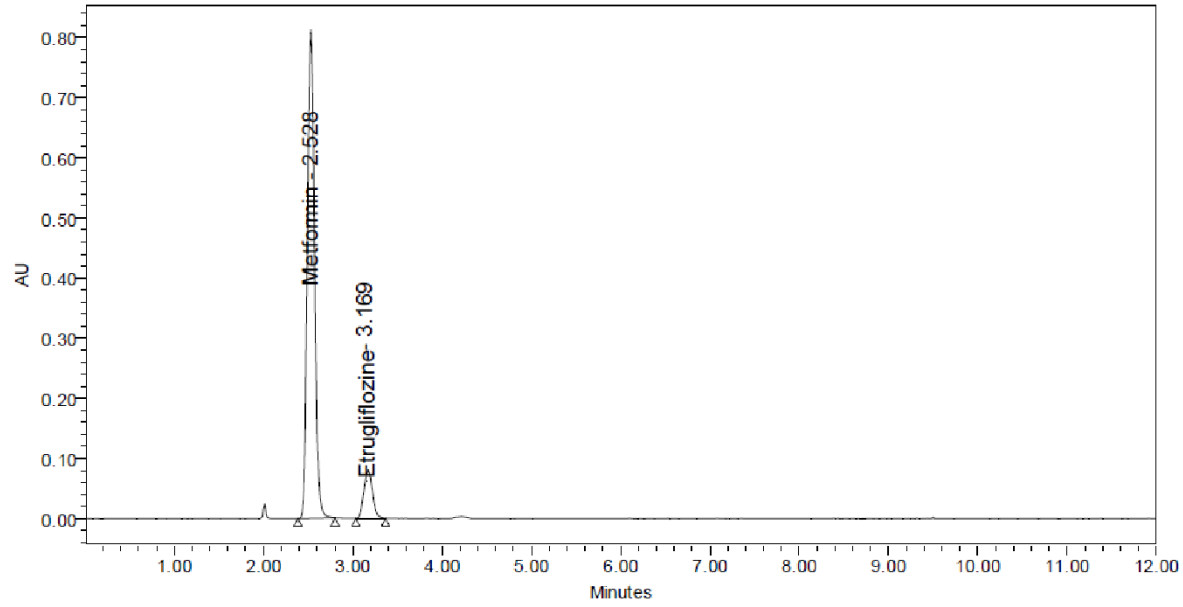
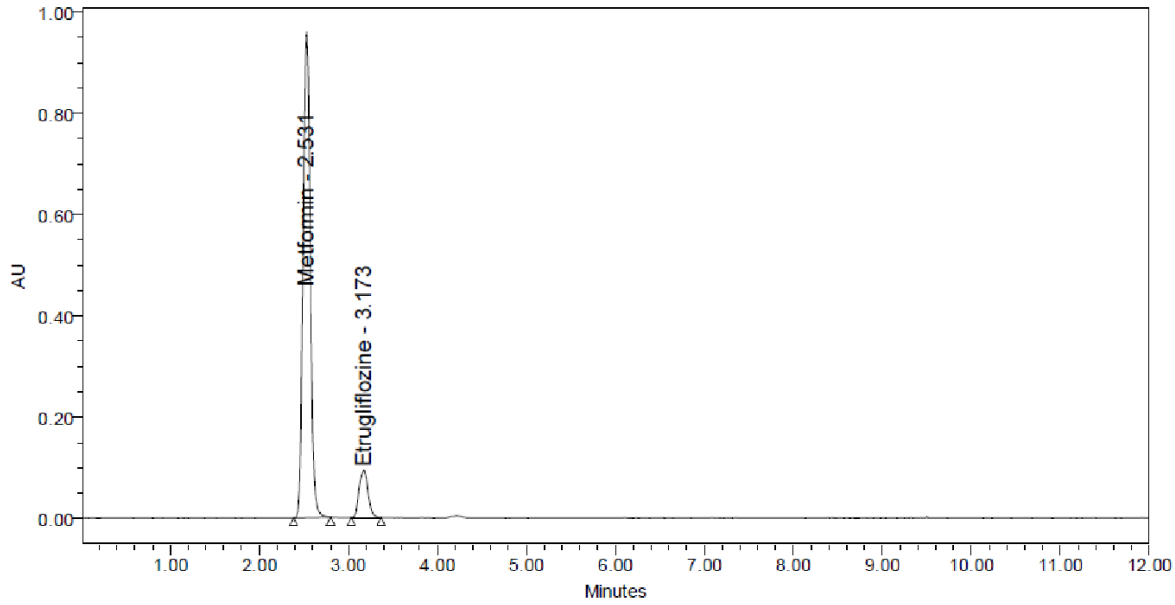
Peroxide degradation chromatogram



peroxide

Thermal degradation chromatogram



thermal

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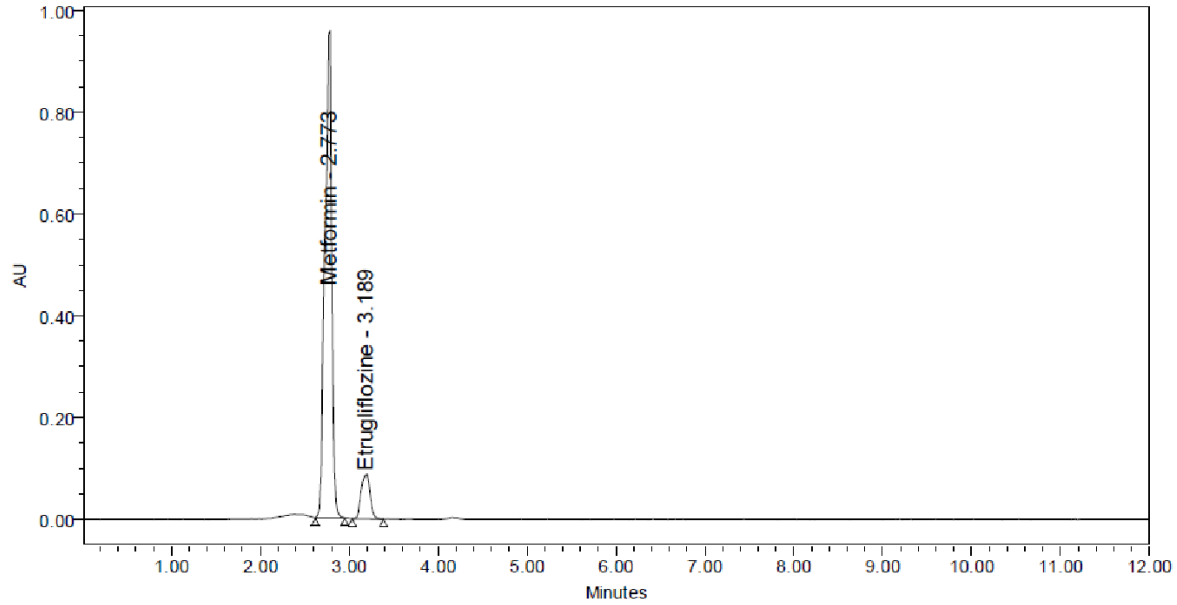
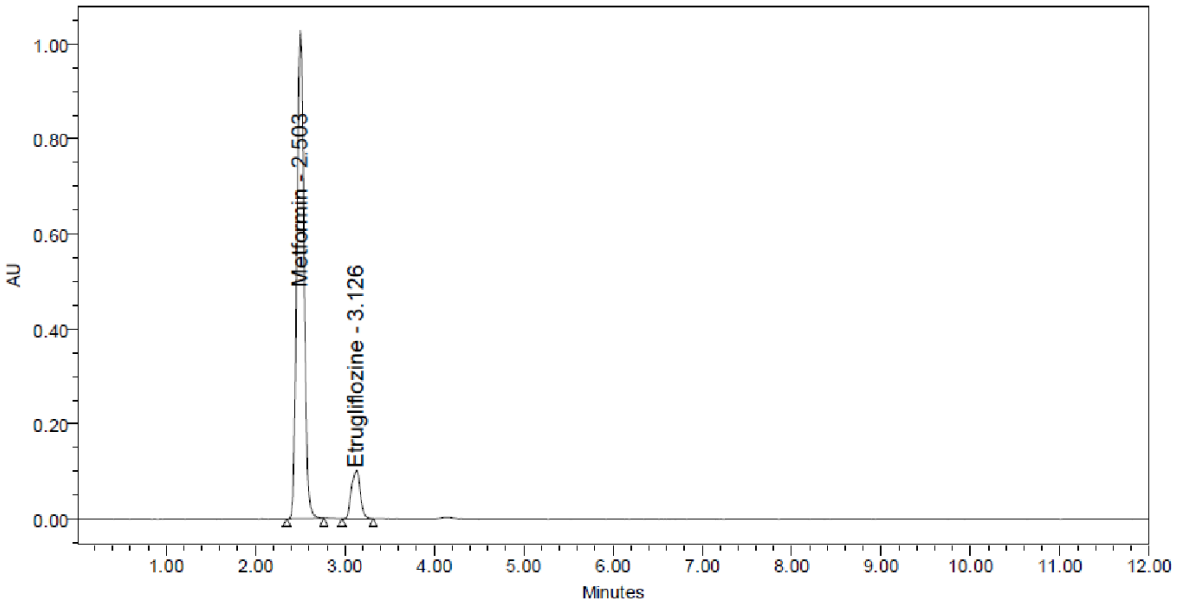
Uv degradation chromatogram



UV

Water degradation chromatogram



water

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SUMMARY AND CONCLUSION

Summary Table

Parameters		**Metformin**	**Ertugliflozin**	**LIMIT**
Linearity Range (µg/ml)		62.5-375µg/ml	0.9375-5.6250µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		21782	18601	
Intercept(c)		8044.9	127.71	
Regression equation (Y=mx+c)		y = 21782x + 8044.9.	y = 18601x + 127.71	
Assay (% mean assay)		100.27%	100.12%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.1	0.4	NMT 2.0%
Method precision %RSD		0.5	0.5	NMT 2.0%
Accuracy %recovery		100.52%	99.80%	98-102%
LOD		0.36	0.01	NMT 3
LOQ		1.08	0.03	NMT 10
Robustness	**FM**	0.3	0.3	%RSD NMT 2.0
	FP	0.3	0.5	
	MM	0.8	1.5	
	MP	0.9	0.2	
	TM	1.0	1.3	
	TP	0.5	0.5	

Conclusion

A simple, Accurate, precise 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 2.565min and 3.221min. %RSD of the Metformin and Ertugliflozin were and found to be 0.1 and 0.5 respectively. %Recovery was obtained as 100.52% and 99.80% for Metformin and Ertugliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin and Ertugliflozin were 0.36, 1.08 and 0.01, 0.03 respectively. Regression equation of Metformin is y = 21782x + 8044.9., and y = 18601x + 127.71 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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