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Research Article

Stability Indicating Method Development and Validation for Determination of Metformin and Empagliflozin in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Empagliflozin in Tablet dosage form. Chromatogram was run through Std Symmetry 150 x 4.6 mm, 5µ. Mobile phase containing 0.1% OPA Buffer: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.1% OPA buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 230 nm. Retention time of Metformin and Empagliflozin were found to be 2.276min and 2.890min. %RSD of the Metformin and Empagliflozin were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 100.87% and 100.43% for Metformin and Empagliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin and Empagliflozin were 0.23, 0.75 and 0.01, 0.03 respectively. Regression equation of Metformin is y =27483x + 7407, and y =26728x + 133.3 of Empagliflozin. 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: RP-HPLC, Metformin and Empagliflozin, ICH Guidelines

INTRODUCTION

Metformin (1, 1-dimethylbiguanide hydrochloride), a biguanide derivate, is the most widely prescribed drug to treat hyperglycemia in individuals with Type 2 diabetes especially in overweight patients and is recommended, in conjunction with lifestyle modification (diet, weight control and physical activity), as a first line oral therapy in the recent guidelines of the American Diabetes Association and European Association of the Study of Diabetes. Metformin is one of only two oral anti-diabetics in the World Health Organization Model List of Essential Medicines.

Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes. SGLT2 co-

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transporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore resulting in increased glucose excretion. Additionally, it contributes to reduced hyperglycaemia and also assists weight loss and blood pressure reduction.

MATERIALS AND METHODS

Materials: Metformin and Empagliflozin pure drugs (API), Combination Metformin and Empagliflozin tablets (SYNJARDY), 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 Empagliflozin solutions.

Methods

Diluent: Based up on 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 50mg of Metformin,0.5mg of Empagliflozin and transferred to 25ml and 100ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of MET and 10µg/ml of EMPA)

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. (100 µg/ml of MET and 1µg/ml of EMPA)

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 100) 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. (1000 µg/ml of MET and 10 µg/ml of EMPA)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of MET and 1µg/ml of EMPA)

Preparation of buffer:

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihydrogen 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.

METHOD VALIDATION

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantification (LOQ) were assessed.

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 50mg of Metformin,0.5mg of Empagliflozin and transferred to 25ml and 100ml volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of MET and 10µg/ml of EMPA)

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. (100 µg/ml of MET and 1µg/ml of EMPA)

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 100 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 MET and 1µg/ml of EMPA)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of MET and 1µg/ml of EMPA)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 50mg of Metformin,0.5mg of Empagliflozin and transferred to 25ml and 100ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of MET and 10µg/ml of EMPA)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of MET, and 0.25µg/ml of EMPA)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of MET, and 0.5µg/ml of EMPA)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of MET, and 0.75µg/ml of EMPA)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (100µg/ml of MET, and 1µg/ml of EMPA)

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

150% Standard solution: 1.5ml each from two standard stock solutions was pipette out and made up to 10ml. (150µg/ml of MET and 1.5µg/ml of EMPA)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 50mg of Metformin,0.5mg of Empagliflozin and transferred to 25ml and 100ml volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of MET and 10µg/ml of EMPA)

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 deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was 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.

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 Metformin, Empagliflozin, 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 Metformin, Empagliflozin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Assay of Metformin and Empagliflozin: Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system.

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for estimation of Metformin and Empagliflozin in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 60% Ortho phosphoric acid:40% Acetonitrile at a flow rate of 1ml/min, samples were analyzed at 230 nm detector wave length and at an injection volume of 10µL using Symmetry C18 (4.6 x 150mm, 5µm)with run time of 5min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Metformin and Empagliflozin, the optimized

chromatogram was obtained as shown in (Figure-3).

Validation: Linearity was established (25-150µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y=27483x+7407.5 for Metformin and y=26728x+133.3 for Empagliflozin. correlation coefficient (R^2) was determined as 0.999. The Linearity calibration curves were plotted as shown in (Figure-4,5). Retention time of Metformin and Empagliflozin were found to be 2.276min and 2.890min where no interfering peaks in blank and placebo were found in this method. So, this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of injections were given for each level of accuracy and mean% Recovery was obtained as 100.87% for Metformin and 100.43% was shown in (Table-3).%RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Metformin was obtained as 0.9% and Empagliflozin 0.6% .The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Metformin and Empagliflozin. The detection limit value was obtained for metformin and empagliflozin was 0.23 and 0.01. Quantitation limit was found to for metformin and empagliflozin was be 0,75 and 0.03. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65:35), mobile phase plus (55:45), temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in duplicate manner (Table - 5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6). Metformin and Empagliflozin pure drug (API) was obtained from Spectrum Pharma research solutions (SYNJARDY), bearing the label claim 250mg.Assay was performed with the above formulation. Average % Assay obtained was 101.10%, 100.61% for Metformin and Empagliflozin respectively the result was shown in (Table-7) and the chromatogram of standard drugs and pharmaceutical dosage forms were shown in (Figure-5,6) respectively.

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and the samples passed the limits of degradation (Table-8)

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Empagliflozin in Tablet dosage form. Retention time of Metformin and Empagliflozin were found to be 2.276min and 2.890min. %RSD of the Metformin and Empagliflozin were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 100.87% and 100.43% for Metformin and Empagliflozin respectively. LOD, LOQ values obtained from regression equations of Metformin and Empagliflozin were 0.23, 0.75 and 0.01, 0.03 respectively. Regression equation of Metformin is y =27483x + 7407, and y =26728x + 133.3 of Empagliflozin. 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.

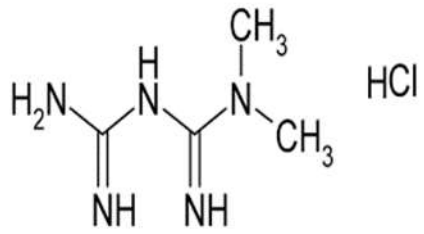

Figure-1: Structure of Metformin

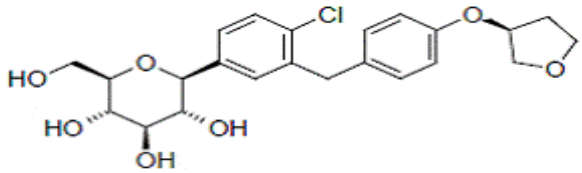

Figure-2: Structure of Empagliflozin

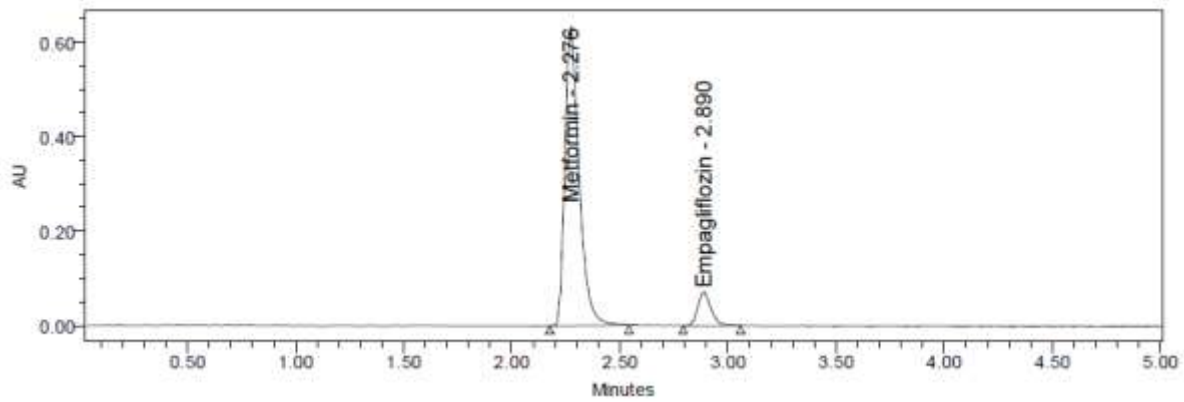

Figure-3: Optimized Chromatogram of Metformin and Empagliflozin

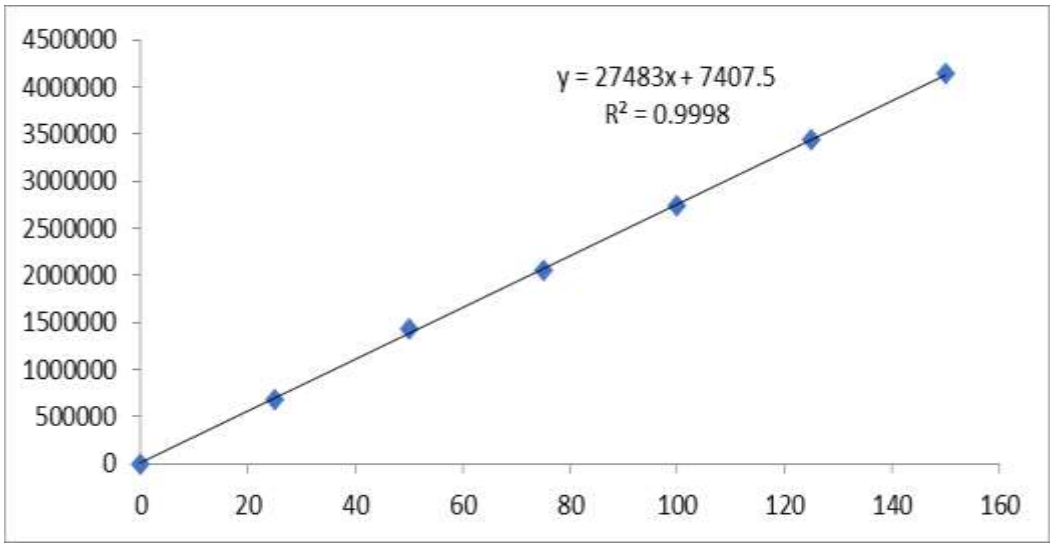

Figure-4: Linearity Curve of Metformin

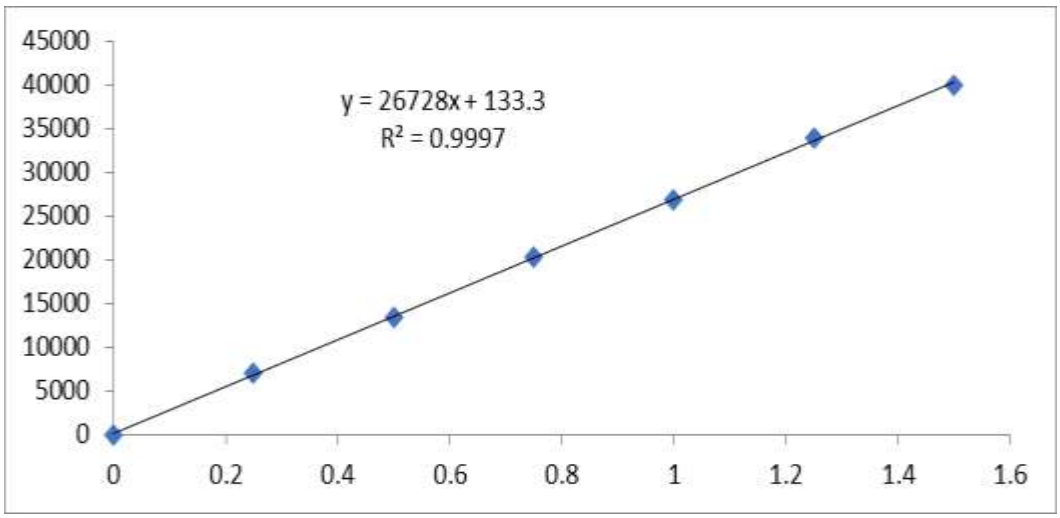

Figure-5: Linearity Curve of Empagliflozin

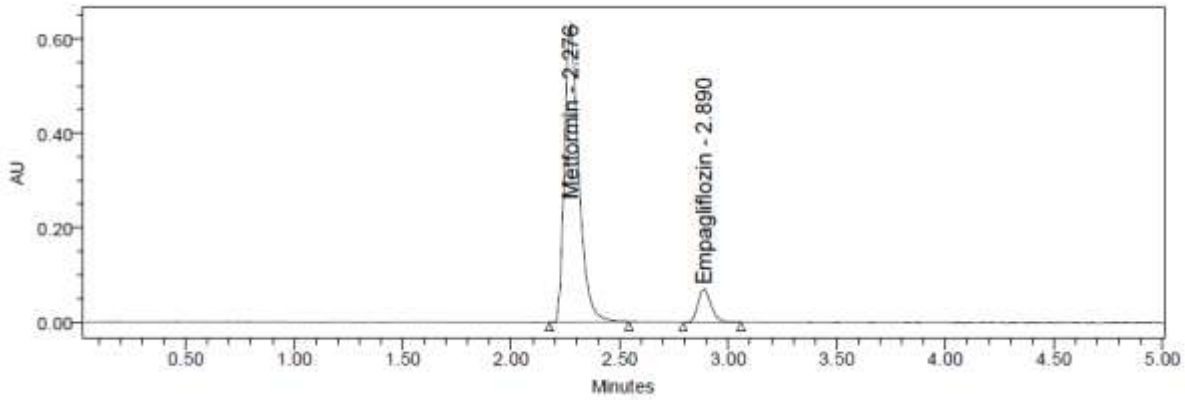

Figure-6 Standard Chromatogram of Metformin and Empagliflozin

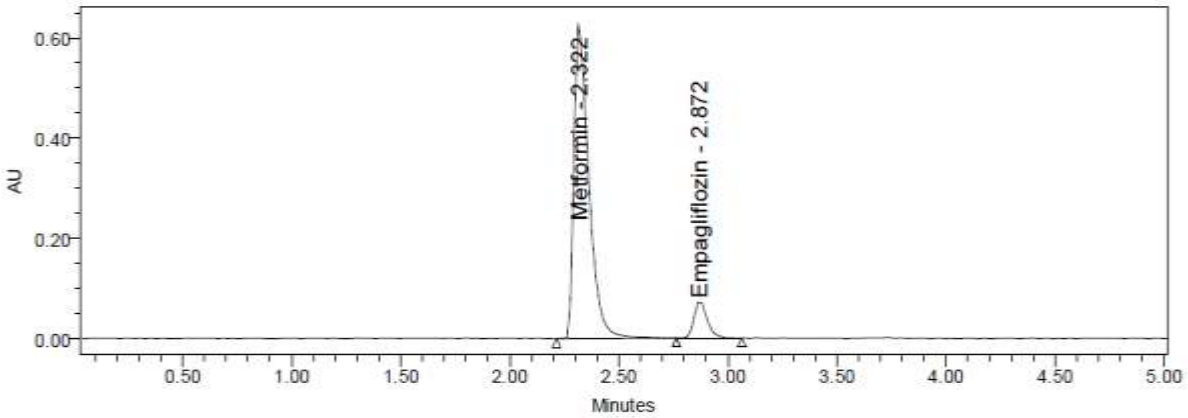

Figure-7 Sample Chromatogram of Metformin and Empagliflozin

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC 2695 SYSTEM equipped with quaternary pumps with PDA detector
Mobile Phase	60%OPA(0.1%):40%Acetonitrile
Flow Rate	1ml/min
Detector wave length	Symmetry C18(4.6×150mm,5µm)
Column Temperature	230nm
Injection Volume	25ºC
Run time	10µL
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Metformin 2.276 min, Empagliflozin 2.890 min

Table-2: LOD and LOQ values of Metformin and Empagliflozin

Molecule	LOD	LOQ
Metformin	0.23	0.75
Empagliflozin	0.01	0.03

Table-3: Accuracy results of Metformin (Drug 1) and Empagliflozin (Drug 2)

%Level	Amount Spiked(µg/ml)		Amount Recovery(µg/ml)		%Recovery		Mean% Recovery	
	Drug1	Drug2	Drug1	Drug2	Drug1	Drug2	Drug1	Drug2
50%	50	0.5	50.19	0.5071455	100.39	101.43	100.87%	100.43%
	50	0.5	50.51	0.5110363	101.02	102.21		
	50	0.5	50.84	0.5041526	101.68	100.83		
100%	100	1	101.50	1.0025065	101.50	100.25		
	100	1	100.72	0.9989525	100.72	99.90		
	100	1	100.40	0.9960344	100.40	99.60		
150%	150	1.5	151.91	0.5071455	101.27	99.50		
	150	1.5	151.81	0.5110363	101.21	100.18		
	150	1.5	149.52	0.5041526	99.68	100.01		

Table-4: Precision of Metformin and Empagliflozin

S.No.	Repeatability		Intermediate precision		System precision	
	Metformin	Empagliflozin	Metformin	Empagliflozin	Metformin	Empagliflozin
1	2757837	26495	2816160	26756	2746160	26343
2	2793377	26561	2797136	26343	2733004	26469
3	2770492	26642	2787543	26356	2757543	26585
4	2764687	26764	2766273	26438	2708370	26366
5	2804963	26558	2770492	26742	2766273	26438
6	2794516	26429	2774687	26264	2776145	26115
Mean	2780979	26575	2785382	26483	2747916	26386
S.D	19095.3	117.0	18928.1	213.2	24559.6	158.0
%RSD	0.7	0.4	0.7	0.8	0.9	0.6

Table-5 Robustness of Metformin and Empagliflozin

S.no	Condition	%RSD of Metformin	%RSD of Empagliflozin
1	Flow rate (-) 0.9ml/min	1.2	0.5
2	Flow rate (+) 1.1ml/min	0.4	1
3	Mobile phase (-) 65B:35A	1.1	1
4	Mobile phase (+) 55B:45A	0.2	0.4
5	Temperature (-) 25°C	0.6	0.6
6	Temperature (+) 35°C	0.5	1.3

Table-6:System Suitability Parameters Result of Metformin and Empagliflozin

S.No.	Metformin			Empagliflozin			
Inj	RT(min)	USP Plate count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.254	4960	1.57	2.795	11411	1.17	4.4
2	2.260	5014	1.56	2.797	11179	1.31	4.4
3	2.261	4859	1.56	2.799	10906	1.15	4.5
4	2.262	4992	1.51	2.800	11095	1.18	4.5
5	2.269	4849	1.46	2.807	10898	1.30	4.4
6	2.276	5980	1.33	2.890	10733	1.18	5.2

Table-7:Assay of Metformin and Empagliflozin

S.no	% Assay of Metformin	% Assay of Empagliflozin
1	100.26	100.31
2	101.55	100.56
3	100.72	100.87

4	100.51	101.33
5	101.97	100.55
6	101.59	100.06
Avg	101.10	100.61
Std	0.6942	0.4429
%RSD	0.7	0.4

Table-8 Degradation Data of Metformin and Empagliflozin

S.no	Degradation Condition	Metformin		Empagliflozin	
		% Drug Degraded	% drug undegraded	% Drug Degraded	% drug undegraded
1	Acid	94.93	5.07	94.38	5.62
2	Alkali	95.50	4.50	95.80	4.20
3	Oxidation	96.00	4.00	95.66	4.34
4	Thermal	98.54	1.46	98.13	1.87
5	UV	99.59	0.41	99.56	0.44
6	Water	98.21	1.79	99.03	0.97

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