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Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Dapagliflozin and Metoprolol in Synthetic Mixture

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ABSTRACT

Background: This invention of Beta-blockers and an SGLT2 inhibitor composition is used in the patients for the treatment of heart failure with reduced ejection fraction (HfrEF), with or without type-2 diabetes.

Objective: To develop simple, precise, accurate and reproducible stability study assay method for estimation of dapagliflozin and metoprolol in synthetic mixture by RP-HPLC method.

Method: The adequate seperation was carried out by using waters HPLC with Ultrasphere C18 (250×4.6 mm, 5 µm) column with the mobile phase composed of Methanol : ACN : Phosphate Buffer pH 3.0 (60:10:30) and the pH was adjusted using othrophosphoric acid. The flow was set at 0.8 ml/min with the wavelength of 223nm.

Result: Successful application of the developed method was carried out for the determination and validation of the drug according to ICH guidelines. By using the developed method, retention time of Dapagliflozin and Metoprolol was found to be 7.012 and 3.392 respectively. Linearity of Dapagliflozin and Metoprolol were in the range of 1-50 µg/mL and 5-250 µg/mL respectively. LOD and LOQ were found to be 0.15 and 0.46µg/mL for Dapagliflozin, 0.13 and 0.40µg/mL for Metoprolol. The study was evaluated at 50%,100% and 150% of the working level concentration and the % recoverieswere found to be in the range. For stability studies the drug was exposed to various conditions such as acid, base, oxidation, thermal and photolytic as per the ICH guidelines.

Conclusion: Results of these parameters demonstrated that the analytical procedures is suitable for its intended purpose and meets the criteria defined in ICH Q2R2.

KEYWORDS: Dapagliflozin, Metoprolol, RP-HPLC, Stability, Validation.

I. INTRODUCTION

The combination comprising of Dapagliflozin and Metoprolol in a fixed dosage form of SGLT2 inhibitor and Beta-blockers with one or more pharmaceutically acceptable exipients.This invention of Beta-blockers and an SGLT2 inhibitor composition is used in the patients for the prevention or treatment of heart failure with reduced ejection fraction (HfrEF), with or without type-2 diabetes, angina, myocardial infraction, arteriosclerosis, diabetic nephropathy, diabetic cardiac myopathy, renel insufficiency, peripheral vascular disease, left ventricular hypertrophy, cognitive dysfunction, and chronic heart failure. This combination is Under Clinical Trial Phase III, approved by CDSCO on 9th Feburary, 2023. Dapagliflozin((2S,3R,4R,5S,6R)-2-[4-chloro-3-[(4-ethoxyPhenyl)methyl]Phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol;(2S)-propane-1,2 diol;hydrate)(chemical formula $C_{24}H_{35}ClO_9$)belongs to the class of Oral Hypoglycemic agents, called Sodium Glucose Co-Transporter(SGLT2) inhibitor, works by reducing the amount of sugar which is present in the blood. These drugs are used to treat Diabetes Mellitus.Dapagliflozin is a sodium-glucose co-transporter 2 inhibitor indicated for managing diabetes mellitus type 2 and now a days also associated with reduction in blood pressure.

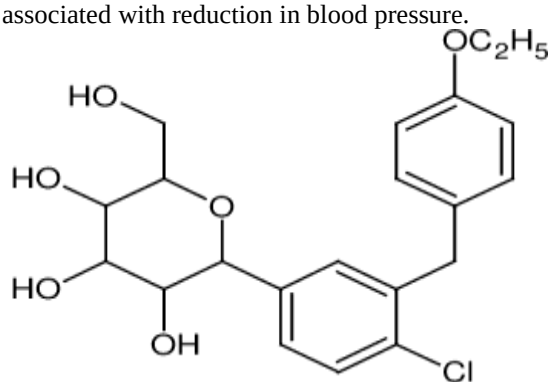

Fig.1 Chemical structure of Dapagliflozin

Metoprolol ((2R,3R)-2,3-dihydroxybutanedioic acid;1-[4-(2-methoxyethyl)Phenoxy]-3-(propan-2-ylamino)propan-2-ol)(chemical formula $(C_{15}H_{25}NO_3)_2,C_4H_6O_6$)is an Anti-Hypertensive drug seeks to prevent the high blood pressure in the

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body. Followed by heart failure, stroke, myocardial infraction and kidney failure. Hypertension is caused when consistent force is exerted by the blood against the walls of blood vessels which is higher than normal pressure.It can last for years or lifelong.

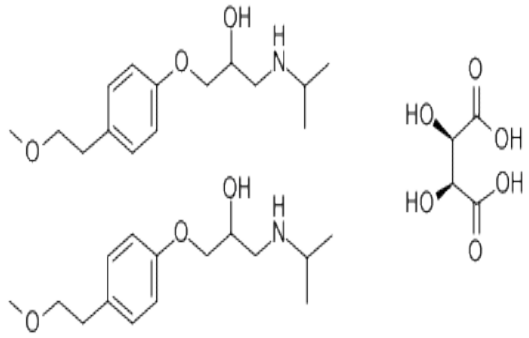

Fig.2 Chemical structure of Metoprolol

Forced degradation experiments are used to relieve the development of analytical methodology, to achieve better stability of the active pharmaceutical ingredient (API) and the drug product, and to provide information about degradation pathways and degradation products. However, no literature is available which deals with the degradation profile of Dapagliflozin and Metoprolol in accordance with ICH guidelines using any analytical techniques. High performance liquid chromatography (RP-HPLC) for analysis ofDapagliflozin and Metoprolol in pharmaceutical formulation. This paper describes an accurate, specific, repeatable, and stability-indicating method for analysis of Dapagliflozin and Metoprolol in the presence of its degradation products. The method was validated in accordance with the guidelines of International Conference on Harmonization (ICH).

II. MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals and solvents used were of analytical grade (RANKEM,INDIA). Solvents and solutions were all filtered through a membrane flter (0.45 µm pore size) and degassed by sonication before use.

Instrumentation

The analysis was performed by using Waters Alliance HPLC system equipped with PDA detector. The output signals were monitored and processed using LC Solution software. The analytical column used was Ultrasphere C18 (4.6 mm × 250 mm, 5 µ) and the samples were introduced through a injection valve with 20 µL sample loop.

Preparation of Solutions

Preparation of the Buffer Solution

Accurately weighed 2.04gm of Potassium dihydrogen phosphate was transferred into 1000ml of water.The solution was mixed well and filtered. And adjust pH 3.0 with 1% Orthophospharic acid. (Preparation of 1% Orthophospharic acid 1.66ml of Orthophospharic acid into 25ml of water)

Preparation of Mobile Phase

Methanol : ACN : Phosphate Buffer pH 3.0 (60:10:30%v/v/v)

Preparation of Standard Solutions

Preparation of Standard Stock Solution of Dapagliflozin and Metoprolol:

Accurately weighed Dapagliflozin (10mg) was transferred into 100ml of volumetric flask and sonicated to dissolve and make upto the mark with diluent. (Dapagliflozin 100µg/ml). Accurately weighed Metoprolol (50mg) was transferred into 100ml of volumetric flask and make upto the mark with diluent. (Metoprolol 500µg/ml)

Preparation of Working Standard of Dapagliflozin and Metoprolol:

From the above prepared solutions take 1ml of Dapagliflozin stock solution and 1ml of Metoprolol stock solution in 10ml of volumetric flask and make upto the mark with diluent to give a solution of Dapagliflozin 10µg/ml and Metoprolol 50µg/ml.

PreparationofSampleSolutionof Dapagliflozinand Metoprolol:

Preparation of Sample Stock Solution of Dapagliflozin and Metoprolol:

(Label claim: Dapagliflozin-10mg; Metoprolol-50mg)

Here, synthetic mixture equivalent to 10mg of Dapagliflozin and 50mg of Metoprolol were added into 100 ml of volumetric flask. Volume was made up to the mark with diluent.

Preparation of Working Standard of Dapagliflozin and Metoprolol:

1 ml of this solution was transferred to 10 ml volumetric flask and volume was made up to the mark by diluent, which gives Dapagliflozin (10 µg/ml) and Metoprolol (50 µg/ml). The quantification was carried out by keeping these

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values to be straight line equation of calibration curve.

The placebo for synthetic mixture preparation is described under in table 1.

Table 1 Synthetic Mixture

Ingredient	mg/tablet
Dapagliflozin	10
Calcium hydrogen Phosphate anhydrrous	76.3
Lactose monohydrate	97.84
Low substituted hydroxy-propyl cellulose	4.06
Hydroxy propyl cellulose	1.21
Low substituted hydroxy-propyl cellulose	1.626
Sodium stearyl fumarate	2.439
Iron oxide red	0.813

Ingredient	mg/tablet
Metoprolol	50
Microcrystalline cellulose	38.421
Methyl cellulose	1.8421
Polyvinyl pyrolidine	0.789

Base Granule

MCC	82.64
Croscarmellose sodium	5.43
Polyvinylpyrrolidone	7.242

Lubrication

MCC	27.03
Purified talc	2.84
Silicone dioxide	0.921
Magnesium stearate	0.710

ANALYTICAL METHOD VALIDATION

Analytical validation ensures that a selected analytical procedure will give accurate results that are adequate for the intended use.

1. Specificity:

Specificity is required to show that the procedure is unaffected by the presence of impurities or excipients. It indicates that the analytical method is able to measure accurately and specifically the analyte of interest without any interference from blank. So here, the specificity was determined by the comparison of the chromatograms of, Blank (mobile phase), Standard solutions Dapagliflozin and Metoprolol, and Sample solution of Dapagliflozin and Metoprolol.

2. Linearity:

A linear relationship should be evaluated across the range of the analytical procedure.

Here, the linearity was evaluated by analysis of standard solution in range of 1-50µg/mL for Dapagliflozin and 5 – 250µg/ml for Metoprolol. 1, 2, 3, 4, 5ml solutions were pipetted out from the standard stock solution of Dapagliflozin and Metoprolol were transfer to 10 ml volumetric flask and volume was make up with mobile phase to obtain 10, 20, 30, 40, 50 µg/ml for Dapagliflozin and 50, 100, 150, 200, 250 µg/ml for Metoprolol. And by pipetting out 1 and 5ml from 10:50 µg/ml solution, the obtained µg/ml were 1 and 5 µg/ml for Dapagliflozin and 5 and 25 µg/ml for Metoprolol .In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted. Acceptance criteria: value of r^2 should be nearer to 1 or 0.999.

3. Precision:

Results should be expressed as Relative Standard Deviation (RSD) or co-efficient of variance.

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Repeatability:

Standard solution containing Dapagliflozin and Metoprolol (10 and 50µg/ml respectively) was injected six times and areas of peaks were measured and RSD was calculated.

Interday Precision:

Standard solution containing Dapagliflozin and Metoprolol (10, 20, 30µg /ml) and 50, 100, 150µg/ml respectively) were injected three times in same day and areas of peaks were measured and RSD was calculated.

Intraday Precision:

Standard solution containing Dapagliflozin and Metoprolol (10, 20, 30µg /ml) and 50, 100, 150µg/ml respectively) were injected three times in different days and areas of peaks were measured and RSD was calculated.

Acceptance criteria: RSD of area should not be more than 2.0%.

4. Accuracy:

Preparation of Standard Stock Solution of Dapagliflozin and Metoprolol:

Accurately weighed Dapagliflozin (10mg) was transferred into 100ml of volumetric flask and make upto the mark with diluent. (Dapagliflozin 100µg/ml) Accurately weighed Metoprolol (50mg) was transferred into 100ml of volumetric flask and make upto the mark with diluent. (Metoprolol 500µg/ml)

Preparation of Working Standard of Dapagliflozin and Metoprolol:

From the above prepared solutions take 1ml of Dapagliflozin stock solution and 1ml of Metoprolol stock solution in 10ml of volumetric flask and make upto the mark with diluent. (Dapagliflozin 10µg/ml and Metoprolol 50µg/ml)

Preparation of Sample for Recovery:

Dapagliflozin and Metoprolol (10µg/ml and 50µg/ml respectively) drug solution was taken in three different flask labeled as A, B and C. Spiked 50%, 100%, 150% of working standard solution in it and diluted up to 10ml. The area of each solution peak was measured.The amount of Dapagliflozin and Metoprolol was calculated at each level and % recoveries were calculated.

5. LOD and LOQ:

The LOD was estimated from the set of 3 calibration curves used to determination linearity. The LOD may be calculated as, LOD = 3.3 × (SD/Slope)

Where, SD= Standard deviation of Y-intercepts of 3calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine linearity.The LOQ may be calculated as, LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

6. Robustness:

Dapagliflozin and Metoprolol (10 and 50µg/ml respectively) drug solution was taken and injected by applying little deliberate changes of the following method conditions and evaluated by RSD.

(i) Mobile phase pH change: 3 ± 0.1

(ii) Column Temperature: 25±1°C

(iii) Flow rate:0.8 ± 0.1ml/min.

Acceptance criteria:

Number of theoretical plates for the analyte peak should not be less than 2000.

Asymmetry value for the analyte peak should not be more than 2.0.

RSD for the analyte peak should not be more than 2.0%.

7. Application of Method on Synthetic Mixture:

(Label claim: Dapagliflozin-10mg; Metoprolol-50mg)

Here, synthetic mixture equivalent to 10mg of Dapagliflozin and 50mg of Metoprolol were added into 100 ml of volumetric flask. Volume was made up to the mark with diluent. 1 ml of this solution was transferred to 10 ml volumetric flask and volume was made up to the mark by diluent, which gives Dapagliflozin (10 µg/ml) and Metoprolol (50 µg/ml). The quantification was carried out by keeping these values to be straight line equation of calibration curve.

Forced Degradation study of Dapagliflozin and Metoprolol:

Degradation conditions

1) Hydrolysis (a) Acid Hydrolysis

(b) Base Hydrolysis

2) Oxidative

3) Photolytic

4) Thermal

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Preparation of Reagent:

0.1 N HCl Solution: 0.84 ml conc. hydrochloric acid was taken in 100ml volumetric flask and volume was made upto the mark with water and mixed well**.**

0.1 N NaOH Solution: 0.4gm of NaOH pellets were taken in 100ml volumetric flask and volume was made upto the mark with water and mixed well.

3%H_2O_2 Solution: 30ml of the 30% H_2O_2 solution was taken in 100ml volumetric flask and volume was made upto the mark with water and mixed well.

Acid Degradation

For Standard Solution of Metoprolol and Dapagliflozin

Transferring 1ml of stock solution of Metoprolol and Dapagliflozin into 10ml of volumetric flask. Add 2ml of 0.1N HCl solution, mixed well and kept for 2 hour at RT (25°C).The solution was neutralized with 2ml of 0.1N NaOH solution. Then the volume was adjusted with the diluent to get sample solution concentration.(Metoprolol 50µg/ml Dapagliflozin 10µg/ml)

Base Degradation:

For Standard Solution of Metoprolol and Dapagliflozin

Transferring 1ml of stock solution of Metorpolol and Dapagliflozin into 10ml of volumetric flask. Add 2ml of 0.1N NaOH solution. Mixed well and kept for 2 hour at RT (25°C).The solution was neutralized with 2ml of 0.1N HCl solution. Then the volume was adjusted with the diluent to get sample solution concentration.(Metoprolol 50µg/ml and Dapagliflozin 10µg/ml)

Oxidative Degradation:

For Standard solution of Metoprolol and Dapagliflozin:

Transferring 1ml of stock solution of Metoprolol and Dapagliflozin into 10ml of volumetric flask. Add 2ml of 3% H_2O_2 solution and mixed well and kept for 2 hour at RT (25°C). The solution was neutralized with 2ml water.Then the volume was adjusted with the diluent to get sample solution concentration. (Metoprolol 50µg/ml and Dapagliflozin 10µg/ml)

Photo Degradation:

For Standard solution of Metoprolol and Dapagliflozin

Transferring 1ml of Metoprolol and Dapagliflozin from standard stock solution to 10ml volumetric flask in UV chamber for 24 hours under 1.2 million lux h for visible light. After which, makeup with diluent upto the markto get standard working solution concentration. (Metoprolol 50µg/ml and Dapagliflozin 10µg/ml)

Thermal Degradation:

For Standard solution of Metoprolol and Dapagliflozin

Metoprolol (50mg) and Dapagliflozin (10mg) were taken in perti plateand was kept in oven for 1 hour at 60°C temperature.After which, it was kept to cool down at room temperature.100ml volumetric flask was used to dilute the solution by adding diluent upto the mark.1ml of Metoprolol and Dapagliflozin was transferred in 10ml volumetric flask and volume was made up with methanol to get working solution concentration. (Metoprolol 50µg/ml and Dapagliflozin 10µg/ml)

III. RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of Dapagliflozin and Metoprolol were found to be accurate, precise, effective and specific method for the analysis of drug. It was found that Methanol : ACN : Phosphate buffer pH 3.0 gives satisfactory results, in the ratio of (60:10:30%v/v/v) at the flow rate of 0.8 ml/min to get a better reproducibility and repeatability. Quantification was achieved with UV detection wavelength at 223nm and the retention time for Dapagliflozin and Metoprolol were found to be 7.012 and 3.392min respectively.The optimized method was validated as per ICH guidelines. Chromatogram of Dapagliflozin and Metoprolol is shown in Fig. No. 1

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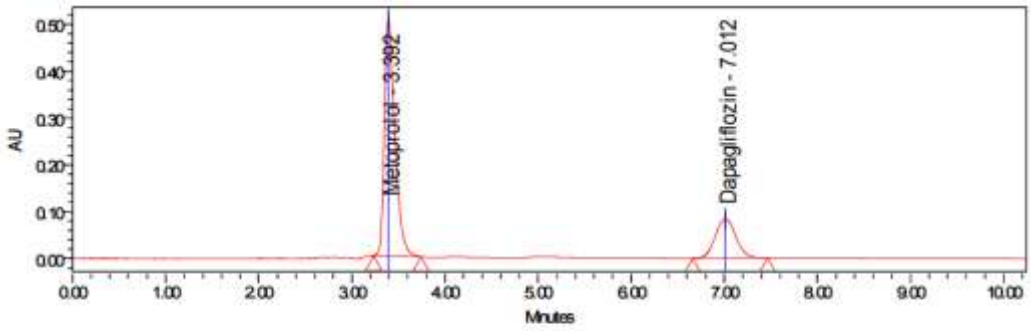

Peak Results

	Name	RT	Area	Height	USP Tailing	USP Plate Count	Symmetry Factor	Purity1 Threshold	Purity1 Angle	Resolution
1	Metoprolol	3.392	3912446	506999	1.55	4535	1.55	0.260	0.196	
2	Dapagliflozin	7.012	1277526	83666	1.07	4808	1.07	0.312	0.256	12.03

Fig. 1Chromatogram of Standard Metoprolol(50µg/ml) and Dapagliflozin(10µg/ml)

Linearity

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 1-50 µg/mL for Dapagliflozin and 50-250 µg/mL for Metoprolol shown in table 1, 2. The Correlation coefficient value should not be less than 0.995 for given range and we obtained 0.999 and 0.999 for Dapagliflozin and Metoprolol respectively. So, the method is found to be in the specified range.

Precision

The precision of the method was studied by determining the concentrations of each ingredient for six times. The RSD value for Dapagliflozin and Metoprolol was 0.46 and 0.81% respectively which should not be more than 2% shown in table 3, 4. The inter-day and intra-day precision was assessed by analyzing six samples. The RSD values obtained for Dapagliflozin and Metoprolol for inter-day is in range of 0.22-0.79 and 0.10-0.15% respectively and for intra-day is in range of 0.27-0.88 and 0.10-0.21% respectively shown in table 5, 6, demonstrating that the method is accurate within the desired range.

Accuracy

Accuracy was evaluated by determining the analyte in solutions prepared according to the standard addition method and expressed in terms of percentage recoveries (i.e. the recovery is 98% - 102% and the RSD is NMT 2.0%) of Dapagliflozin and Metoprolol which were found to be as 98.99-101.95and 100.55-101.97 respectively shown in table 7, 8.The results of accuracy study indicate that the method is reliable.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were obtained by using the signal to noise ratio calculations The experimental LOD and LOQ data were 0.15 and 0.46µg/ml for Dapagliflozin and 0.13 and 0.40µg/ml for Metoprolol shown in table 9, 10.

Robustness

To evaluate the robustness of the proposed method, small but delibrate variations in the optimized method parameters were done. The effect of changes in mobile phase, flow rate, and pH was studied. The results indicate that less variability were observed shown in table11, 12.

Assay

Assay % determination is done by injecting solutions for three times into the chromatographic system, from which the mean of % assay was found 101.90% and 101.12% for Dapagliflozin and Metoprolol respectively shown in table 13. (limits for %assay is 98% - 102%)

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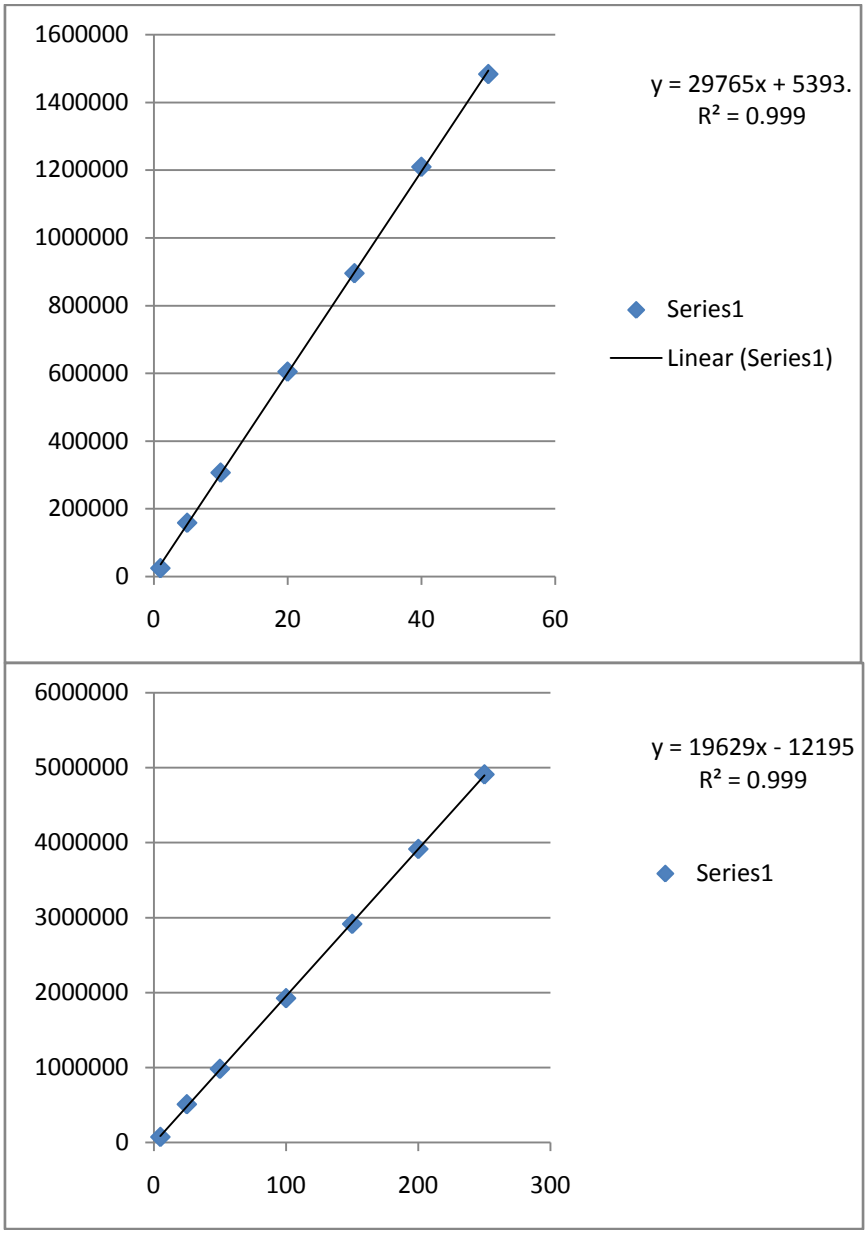

Fig. 2 and Fig. 3Calibration Curve of Dapagliflozin(1-50µg/ml)&Metoprolol (5-250µg/ml)

Table 2 Linearity of Dapagliflozin and Metoprolol

Dapagliflozin (1-50 µg/ml)			**Metoprolol (5-250µg/ml)**	
Sr. No	**Concentration (µg/ml)**	**Area**	**Concentration (µg/ml)**	**Area**
1	1	24639	5	72323
2	5	158512	25	508529
3	10	306628	50	980673

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4	20	604573	100	1923825
5	30	895008	150	2915143
6	40	1208994	200	3914915
7	50	1482797	250	4910220

Table 3Repeatability data of Dapagliflozin(10µg/ml)

Sr.No.	Conc.(µg/ml)	Area	Mean ± SD(n=6)	RSD (%)
1	10	305219	306678 ± 1436.023	0.46
2		308156		
3		305630		
4		308761		
5		306201		
6		306101		

Table 4Repeatability data of Metoprolol(50µg/ml)

Sr.No.	Conc.(µg/ml)	Area	Mean ± SD(n=6)	RSD (%)
1	50	976807	987022.2 ± 8064.846	0.81
2		987346		
3		983337		
4		997342		
5		995556		
6		981745		

Table 5Intraday Data for Dapagliflozin and Metoprolol

Dapagliflozin				**Metoprolol**		
Sr. No.	**Conc. (µg/ml)**	**Area Mean ± SD (n=3)**	**RSD (%)**	**Conc. (µg/ml)**	**Area Mean ± SD (n=3)**	**RSD (%)**
1	5	148561.3±1307.86	0.88	25	480195± 1041.18	0.21
2	10	306092.7 ±1573.43	0.51	50	984834± 1251.73	0.12
3	15	465547± 1266.02	0.27	75	1460652± 1598.51	0.10

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Table 6 Interday Data for Dapagliflozin and Metoprolol

Dapagliflozin				Metoprolol		
Sr. No.	**Conc. (µg/ml)**	**Area Mean ± SD (n=3)**	**RSD (%)**	**Conc. (µg/ml)**	**Area Mean ± SD (n=3)**	**RSD (%)**
1	5	148509± 1185.42	0.79	25	480750± 767.26	0.15
2	10	306002.3 ±1325.94	0.43	50	984242± 1044.76	0.10
3	15	466266.3 ±1028.93	0.22	75	1461177± 2180.50	0.14

Table7RecoverydataforDapagliflozin (10 µg/ml)

Sr.No.	**Conc. Level(%)**	**Sample amount(µg/ml)**	**Amount Added(µg/ml)**	**Amountrecovered (µg/ml)**	**% Recovery**
1	50	10	5	15.12	100.82
2				15.29	101.95
3				15.02	100.18
1	100	10	10	19.80	99.00
2				20.03	100.17
3				20.20	101.04
1	150	10	15	25.28	101.12
2				24.90	99.62
3				24.74	98.99

Table8RecoverydataofMetoprolol (50 µg/ml)

Sr.No.	**Conc. Level(%)**	**Sample amount(µg/ml)**	**Amount Added(µg/ml)**	**Amountrecovered (µg/ml)**	**% Recovery**
1				75.96	101.29

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2	50	10	25	76.47	101.97
3				75.90	101.21
1	100	10	50	101.00	101.00
2				100.75	100.75
3				100.55	100.55
1	150	10	75	126.68	101.34
2				126.11	100.88
3				125.77	100.62

Table 9 Limit of Detection data for Dapagliflozin and Metoprolol

Dapagliflozin	**Metoprolol**
LOD = 3.3 x (SD / Slope) = 3.3 x (1379.15 / 29765) = 3.3 x (0.046) = 0.15	LOD = 3.3 x (SD / Slope) = 3.3 x (785.46 / 19629) = 3.3 x (0.040) = 0.13

Table 10 Limit of Quantitation data for Dapagliflozin and Metoprolol

Dapagliflozin	**Metoprolol**
LOQ = 10 x (SD / Slope) =10 x (1379.15 / 29765) = 10 x (0.046) = 0.46	LOQ = 10 x (SD / Slope) =10 x (785.46 / 19629) = 10 x (0.040) = 0.40

Table 11 Robustness data for Dapagliflozin

SrNo.	**Area atFlow rate(-0.1 ml/min)**	**Area at Flowrate (+0.1 ml/min)**	**Area atTemp of column (-1°C)**	**AreaatTemp of column (+1°C)**	**Areaat pH(-0.1)**	**Area at pH (+0.1)**
1	305700	284930	294507	283174	303575	283622
2	297729	284995	301728	285445	299245	285577
3	298121	284052	305689	285962	302456	285494
Avg. area	297257	289328	297319.1667	289428.7	297877.8	289447.3
SD	4714.18	5259.91	5243.884874	5226.821	4635.734	5169.571
RSD	1.58	1.81	1.763722444	1.80591	1.556253	1.786014

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Condition		MeanArea	Mean	SD	RSD(%)
Flowrate (ml/min)	0.7	297257	293527	3985.23	1.35
	0.8	293997			
	0.9	289328			
Temp of Column (^oC)	24	297319	293582	3961.62	1.34
	25	293997			
	26	289429			
pH ofMobilephase	2.9	297878	293774	4219.67	1.43
	3.0	293997			
	3.1	289447			

Table 12 Robustness data for Metoprolol

SrNo.	Area atFlow rate(-0.1 ml/min)	Area at Flowrate (+0.1 ml/min)	Area atTemp (-1^{o}C)	AreaatTemp (+1^{o}C)	Areaat pH(-0.1)	Area atpH (+0.1)
1	981149	954611	997670	965837	996345	954255
2	989624	964551	997304	941223	984541	952464
3	994819	945614	984224	965652	995875	955998
Avg. area	982216.2	965413.5	984483.8333	966736.2	984077.7	965070.3
SD	8591.467	13221.68	10898.27066	13707.85	10247.16	12204.53
RSD	0.874702	1.369536	1.107003517	1.417952	1.041296	1.264626

Condition		MeanArea	Mean	SD	RSD(%)
Flowrate (ml/min)	0.7	982216	974510	8487.29	0.87
	0.8	975902			
	0.9	965414			
Temp of Column (^oC)	24	984483	975707	8875.43	0.90
	25	975902			
	26	966736			
pH ofMobilephase	2.9	984078	975017	9534.53	0.97
	3.0	975902			
	3.1	965070			

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Table 13 Assay of Dapagliflozin and Metoprolol

	Dapagliflozin		Metoprolol	
Sr. No.	**Area of samples**	**%Assay**	**Area of samples**	**%Assay**
1	300588	100.6272	979876	101.082
2	299532	100.2601	98921	100.984
3	301696	101.0125	980912	101.187
	Avg. Assay	100.633	**Avg. Assay**	101.084
	SD	0.376	**SD**	0.101
	RSD of Assay	0.373	**RSD of Assay**	0.100

Forced degradation studies:

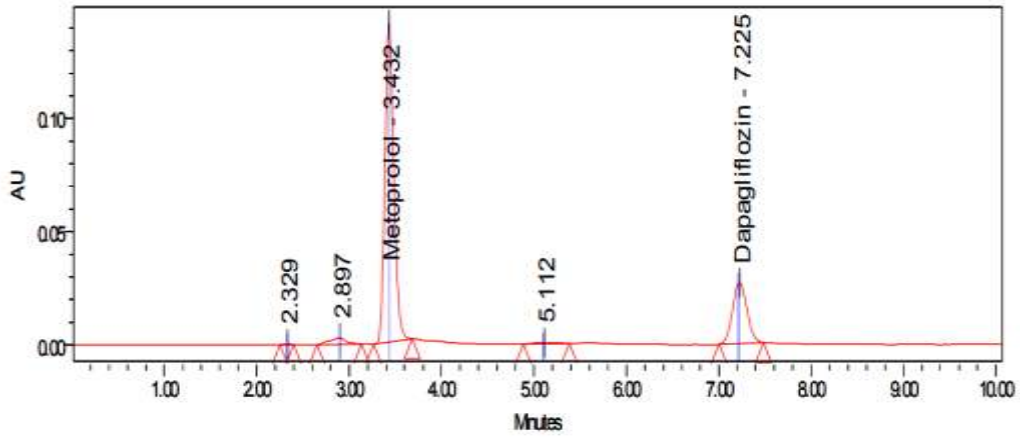

Fig. 4 Chromatogram of Metoprolol(50µg/ml) and Dapagliflozin(10µg/ml) for Acid Degradation

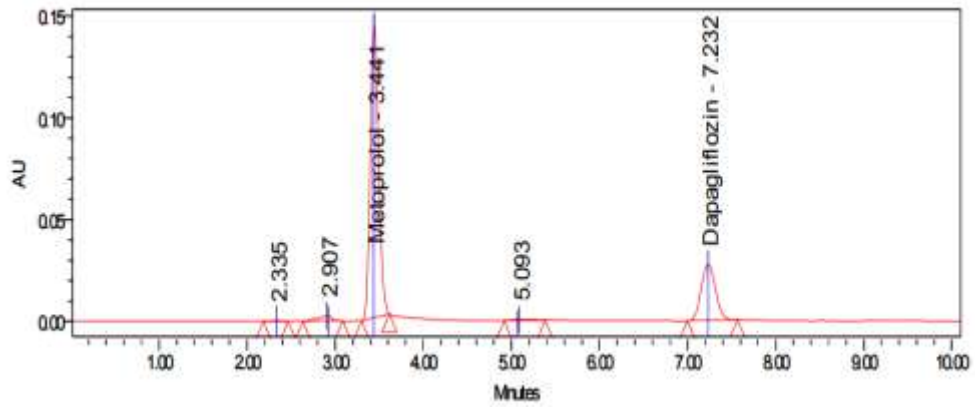

Fig. 5 Chromatogram of Metoprolol(50µg/ml) and Dapagliflozin(10µg/ml) for Base Degradation

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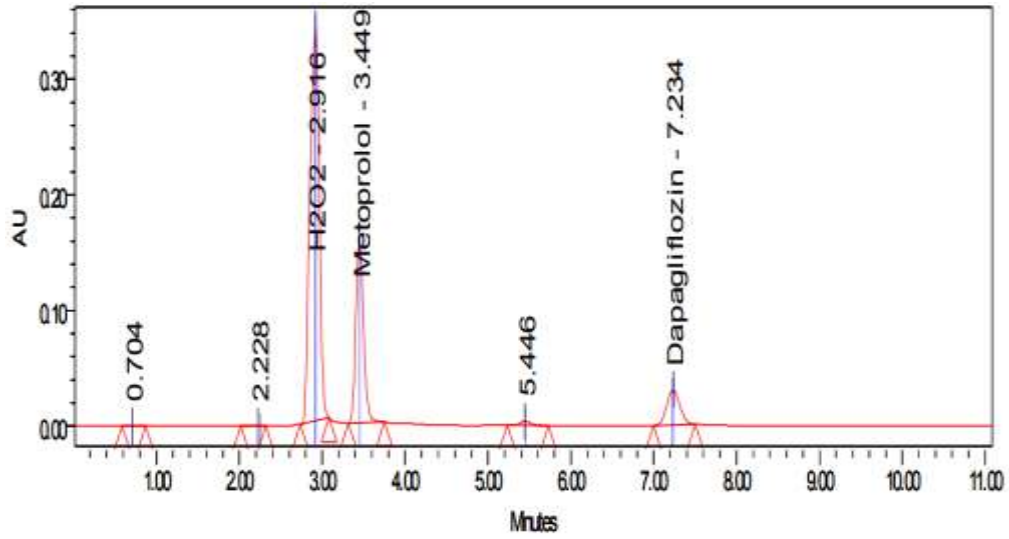

Fig. 6 Chromatogram of Metoprolol(50µg/ml) and Dapagliflozin(10µg/ml) for Oxidative Degradation

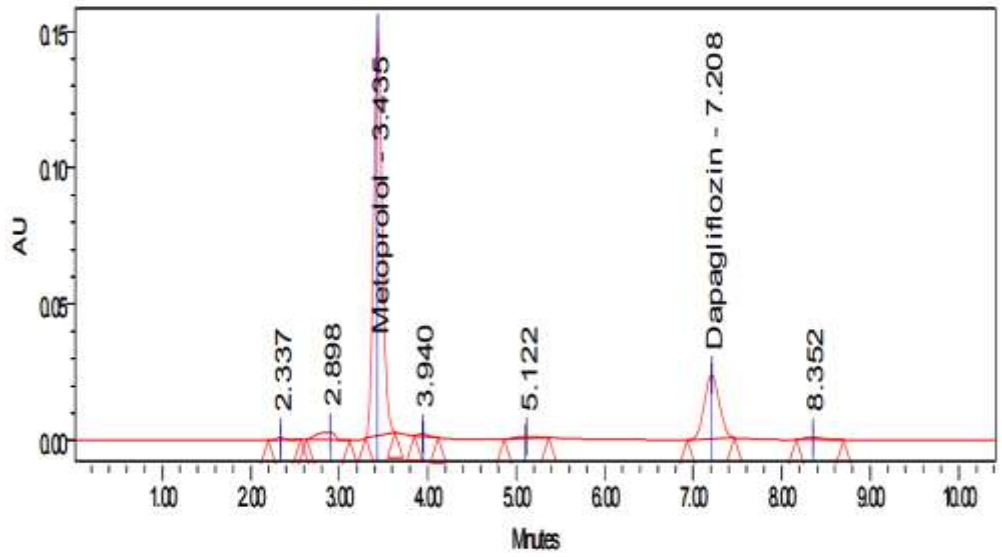

Fig. 7 Chromatogram of Metoprolol(50µg/ml) and Dapagliflozin(10µg/ml) for Photo Degradation

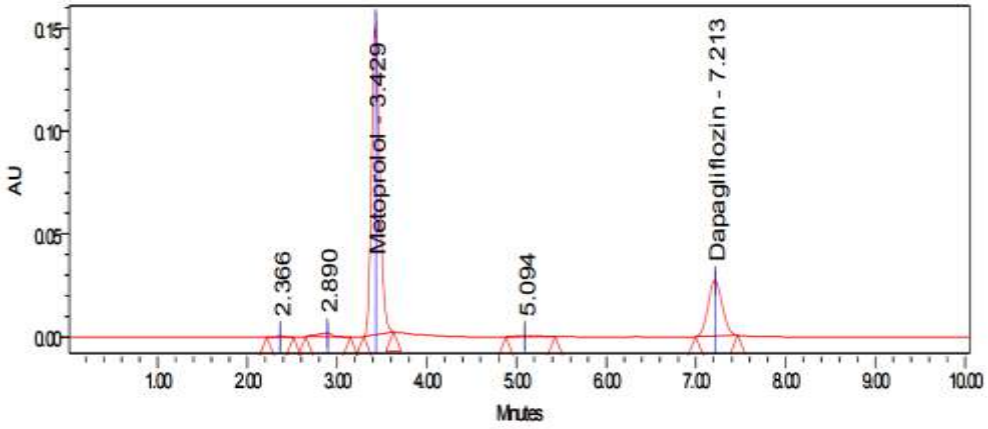

Fig. 8 Chromatogram of Metoprolol(50µg/ml) and Dapagliflozin(10µg/ml) for Thermal Degradation

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Table 13 Summary of Forced Degradation

Sr. No.	Types of Degradation	Condition	Duration	Solution	Area	%Degradation
1	Acid Degradation	0.1 N HCl	1 Hour	Metoprolol	947657	16.61
				Dapagliflozin	293749	25.42
2	Base Degradation	0.1 N NaOH	1 Hour	Metoprolol	957093	15.78
				Dapagliflozin	301472	23.46
3	Oxidative Degradation	1% H_2O_2	1 Hour	Metoprolol	1006029	11.48
				Dapagliflozin	331442	15.85
4	Photo Degradation	UV chamber	24 Hours	Metoprolol	994476	12.49
				Dapagliflozin	303973	22.86
5	Thermal Degradation	60°C	2 Hours	Metoprolol	963652	15.20
				Dapagliflozin	296774	24.66

IV. CONCLUSION

From above observations, it can be concluded that developed Stability indicating method and validation of Dapagliflozin and Metoprolol in synthetic mixture by RP-HPLC is, specific, linear, accurate, precise and robust. Thus above developed RP-HPLC method can be applied for routine analysis.

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