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REVERSE PHASE-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE AND METOPROLOL SUCCINATE

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Abstract: A simple, rapid, precise, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Dapagliflozin Propanediol Monohydrate (DAP) and Metoprolol Succinate (MET) which was used in combined ratio of (1: 5) for treatment of patient suffering with Heart Failure.[1] The separation was achieved on a Shim-pack solar C18 (250 mm × 4.6 mm, particle size of 5 µ) using a mobile phase [Acetonitrile: Methanol: Water (pH 3.0 adjusted with 1% (OPA) Orthophosphoric Acid)](72:3:25 %v/v/v). HPLC separation of DAP and MET was carried out detection of at 223 nm. The mobile phase was pumped at a flow rate of 1ml/min. The retention times of DAP and MET were found to be 4.245 min and 2.186 min respectively. Excellent linearity range was found between 2-6 µg/ml for DAP and 10-30 µg/ml for MET. The method was validated with respect to linearity, precision, accuracy, specificity and ruggedness. Method was successfully applied for the simultaneous determination of Dapagliflozin Propanediol Monohydrate (DAP) and Metoprolol Succinate (MET) in combined pharmaceutical dosage form synthetic mixture.

Keywords: Dapagliflozin Propanediol Monohydrate, Metoprolol Succinate, RP-HPLC, Method Validation

INTRODUCTION:

Congestive Cardiac failure is a syndrome caused by cardiac problems that impairs the heart's ability to provide enough blood to satisfies the body's needs. Cardiac failure can be caused by either the right or left or both ventricles failing. Heart failure causes blood to travel more slowly through the heart and body, resulting in higher pressure in the cardiac tissues. As a result, the heart is unable to supply adequate oxygen and nutrients to the body. Thus, the heart chambers either extend to hold more blood to pump through the body or stiffen and thicken. Such process helps to keep the blood flowing for a short period, but the heart muscle walls weaken with time and become unable to pump with sufficient force.

Congestive heart failure (CHF) is the chronic form of heart failure in which the patient exhibits signs of peripheral circulation and lung congestion; CHF is the end result of several types of significant cardiac illnesses. [2-4]

TYPES OF HEART FAILURE: [5]

1. Systolic Heart Failure
2. Diastolic Heart Failure
3. Left-sided Heart Failure
4. Right-sided Heart Failure
5. Biventricular Heart Failure

Dapagliflozin Propanediol Monohydrate (DAP):

Dapagliflozin Propanediol Monohydrate (DAP) is chemically known as (2S)-propane-1,2-diol(2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl} 6(hydroxymethyl) oxane-3,4,5-triol hydrate with Molecular Formula and Molecular Weight, $C_{24}H_{35}ClO_9$ and 502.98 gm/mole respectively belonging to the Antidiabetic category, shown in Fig. 1. This API is freely soluble in Water and soluble in Ethanol. MOA of this drug is, it inhibits the Sodium-Glucose Co-Transporter 2(SGLT2) which is primarily located in the proximal tubule of the nephron. SGLT2 facilitates 90% of glucose reabsorption in the kidneys and so its inhibition allows for glucose to be excreted in the urine. [6,7]

Metoprolol Succinate (MET):

Metoprolol Succinate (MET) is chemically known as 1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate with Molecular Formula and Molecular Weight, $C_{34}H_{56}N_2O_{10}$ and 652.8 gm/mole respectively belonging to the Beta Blockers category, shown in Fig. 2. This API is freely soluble in Water and soluble in Methanol. MOA of this drug is, Metoprolol is a beta-1-adrenergic receptor inhibitor specific to cardiac cells with negligible effect on beta-2 receptors. This inhibition decreases cardiac output by producing negative chronotropic and inotropic effects without presenting activity towards membrane stabilization nor intrinsic sympathomimetics. [8,9]

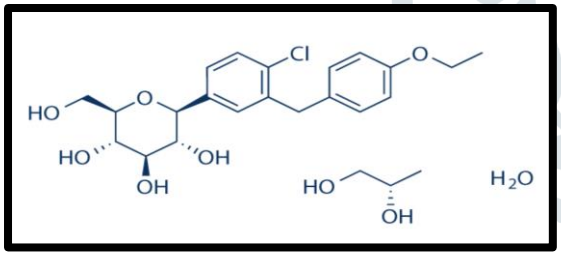

Fig. 1: Chemical Structure of DAP

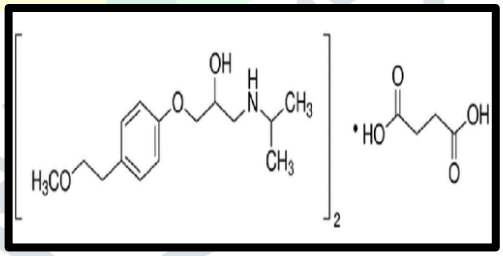

Fig. 2: Chemical Structure of MET

A study of the Literature Review on Analytical Method Development and Validation for Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate, it was found that though several analytical techniques have been developed for each medication alone or in combination with other drugs, but no analytical method on the combination of these two specific drugs has been reported to date.

Thus, there is a scope to Develop and Validate Spectrophotometric and Chromatographic techniques for the combination of Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate in compliance with ICH Q2(R2) specifications. [10]

The present work was undertaken with an aim to develop and validate of analytical methods as per ICH guidelines for simultaneous estimation of Dapagliflozin Propanediol and Metoprolol Succinate in synthetic mixture dosage form.

INTRODUCTION TO HPLC METHOD: [11-17]

High pressure liquid chromatography, also known as high-performance liquid chromatography is a method used to separate ions or molecules in a diluent. If the sample solution comes into contact with a solid or liquid phase, the individual solutes will interact to varying degrees with the other phase due to differences in adsorption, ion exchange, partitioning, or size.

Principle: Adsorption is the basic principle at work in both normal and reverse phase modes. When a sample is injected into an HPLC column, it moves to the stationary phase based on the drug's affinity. When a component with a greater affinity for adsorbent (stationary phase) moves slowly on it, and a component with a lower affinity for adsorbent (stationary phase) moves quickly on it, and the components do not have the same affinity for the stationary phase, the components are separated.

HPLC is the preferred technology for analysing of:

• Non-volatile substances

• Substances with high polarity or ionic samples

• High molecular weight chemicals

• Thermally unstable and decomposable substances

MATERIALS AND METHODS:

Apparatus and Instrumentation:

- Model: SHIMADZU LC-2010 CHT
- Column: Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm)
- Injector: Auto injector
- Detector: UV PDA Detector
- Software: LC solution
- Electronic analytical balance (Shimadzu 0.1 mg)
- Digital pH meter (Systronic pH system)
- Ultrasonic cleaner (Athena Technology)
- Filter paper: **a.** Vacuum filter: Membrane filter 0.45 micron

  b. Syringe filter: Membrane filter 0.27 micron

Chemicals:

Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate, is obtained as gift sample from Merril Pharma Pvt. Ltd., Modasar, Bavla, Gujarat.

Methanol (HPLC grade), Acetonitrile (HPLC grade), Water (HPLC grade) – Rankem

Ortho Phosphoric Acid (HPLC grade) – Thomas baker

Instrumentation and Chromatographic Conditions:

SHIMADZU LC-2010 CHT attached to PDA detector, which is having an Auto injector and autosampler opted for chromatography. A degasser to remove the dissolved air and column oven to maintain the desired temperature is also available in the system. Operation data acquisition and analysis were performed by using LC Solution software. The mobile phase [Acetonitrile: Methanol: Water (pH 3.0 adjusted with 1% (OPA) Orthophosphoric Acid)](72:3:25 %v/v/v). HPLC separation of DAP and MET was carried out at detection wavelength of 223 nm, the mobile phase is in low pressure gradient mode, at a flow rate of 1.0 ml/min. Stationary phase is Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm). A Flow rate of the mobile phase was 1.0 ml/min and all chromatographic experiments were performed at room temperature (25 ºC ± 2 ºC). The detector wavelength was fixed at 223 nm.

Spectrophotometric Conditions:

Mode: Scan

Scan speed: Medium

Wavelength range: 200 - 400 nm

Scale of Absorbance: 0.00 - 2.00 A

Baseline Correction: Distilled water

SELECTION OF WAVELENGTHS:

Aliquots of 0.2 ml from working stock solution of DAP (10 µg/ml) and 0.1 ml from working stock solution of MET (100 µg/ml) were pipette out and taken into two separate volumetric flasks of 10 ml and volume was made up to mark with distilled water to give a solution containing 2 µg/ml and 10 µg/ml of DAP and MET. Each solution was scanned between 200-400 nm against distilled water as blank using UV-Visible Spectrophotometer. 223 nm Wavelength was selected from the overlay spectra of above solution.

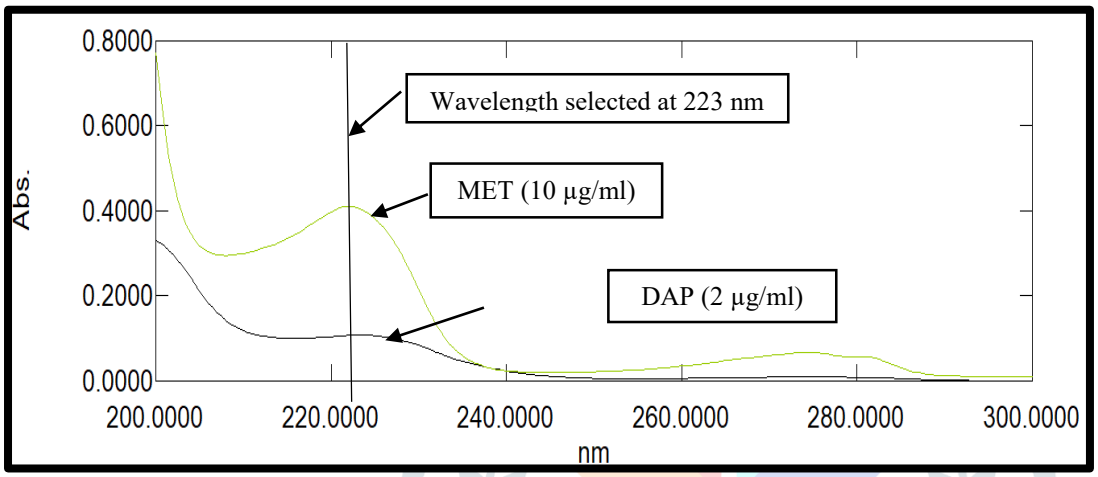

Fig. 3: Overlay UV spectrum of DAP (2 µg/ml) and MET (10 µg/ml) showing selection of wavelength detection at 223 nm

PREPARATION OF STANDARD SOLUTION:

1. Preparation of DAP standard stock solution (1000 µg/ml):

10 mg of DAP was weighed and transferred to 10 ml volumetric flask. It was dissolved in distilled water and volume was made up to the mark with distilled water to give a solution containing 1000 µg/ml.

2. Preparation of DAP standard stock solution (100 µg/ml):

Aliquot of 1 ml from above standard stock solution was pipetted out into 10 ml of volumetric flask and volume was made up to the mark with distilled water to give a solution containing 100 µg/ml.

3. Preparation of DAP standard stock solution (10 µg/ml):

Aliquot of 2.5 ml from above standard stock solution was pipetted out into 25 ml of volumetric flask and volume was made up to the mark with distilled water to give a solution containing 10 µg/ml.

4. Preparation of MET standard stock solution (1000 µg/ml):

10 mg of MET was weighed and transferred to 10 ml volumetric flask. It was dissolved in distilled water and volume was made up to the mark with distilled water to give a solution containing 1000 µg/ml.

5. Preparation of MET standard stock solution (500 µg/ml):

Aliquot of 5 ml from above standard stock solution was pipetted out into 10 ml of volumetric flask and volume was made up to the mark with distilled water to give a solution containing 500 µg/ml.

6. Preparation of MET standard stock solution (50 µg/ml):

Aliquot of 2.5 ml from above standard stock solution was pipetted out into 25 ml of volumetric flask and volume was made up to the mark with distilled water to give a solution containing 50 µg/ml.

7. Preparation of Binary Mixture of DAP and MET:

Aliquots of 2 ml from working solution of DAP (10 µg/ml) and 2 ml from workingsolution of MET (50 µg/ml) were taken into common volumetric flask and diluted upto10 ml with distilled water to make final concentration DAP (2 µg/ml) and MET (10 µg/ml).

Preparation of Calibration Curves:

I. Calibration curve of DAP:

Calibration curve for DAP consisted of five different concentrations solution ranging from 2-6 µg/ml. Calibration curve of Peak area vs Conc. was plotted and regression equation was determined.

II. Calibration curve of MET:

Calibration curve for MET consisted of five different concentrations solution ranging from 10-30 µg/ml. Calibration curve of Peak area vs Conc. was plotted and regression equation was determined.

PREPARATION OF ASSAY SAMPLE:

A synthetic mixture (tablet) equivalent to 10 mg of DAP and 50 mg of MET was takeninto 100 ml of volumetric flask and added 10 ml of distilled water, the solution was warmedfor 5-10 mins, ultrasonicated for 20 mins to completely disperse the tablet, followed by addition of 50 ml distilled water and ultrasonicated for 15 min and was make up to themark with distilled water. The solution was filtered through Whatman filter paper no. 41. Thus, resulting solution gave 100 µg/ml of DAP and 500 µg/ml of MET respectively. From the above solution, 1 ml was pipette out and transferred to 10 ml volumetric flask and volume was made up to mark with distilled water in order to give a solution containing DAP (10 µg/ml) + MET (50 µg/ml). From the above solution, 4 ml was pipette out andtransferred to 10 ml volumetric flask and volume was made up to mark with distilled water in order to give a solution containing DAP (4 µg/ml) + MET (20 µg/ml).

PREPARATION OF SOLUTIONS FOR ANALYTICAL METHOD VALIDATION:

1. System Suitability Studies: Evaluation of system suitability was done by analyzing six replicates of DAP and MET in a mixture at concentration of 6 µg/ml of DAP and 30 µg/ml of MET. The column efficiency, peak asymmetry and resolution were calculated for each replicate.

2. Specificity: Specificity involves quantitative detection of analyte in the presence of those components that may be expected to be part of sample matrix. Specificity of developedmethod was established by spiking of DAP and MET in hypothetical placebo (i.e., might be expected to be present) and expressing that analytes peak were not interfered from excipients.

3. Linearity: The linearity response was determined by analyzing 5 independent levels of concentration in the range of 2-6 µg/ml and 10-30 µg/ml for DAP and MET respectively.

4. Precision

A. Repeatability: Repeatability of the developed method was assessed by analysing samples from the same batch 6 times with standard solutions containing concentrations 4 µg/ml for DAP and 20 µg/ml for MET and % R.S.D. was calculated.

B. Intraday Precision: It was assessed by analyzing samples from the same batch with three standard solutions containing concentrations 3, 4, 5 µg/ml for DAP and 15, 20, 25 µg/ml for MET. Solutionswere analyzed thrice (n=3) on the same day within short interval of time and % R.S.D.was calculated.

C. Interday Precision: It was assessed by analyzing samples from the same batch with three standard solutions containing

concentrations 3, 4, 5 µg/ml for DAP and 15, 20, 25 µg/ml for MET. Solutionswere analyzed thrice (n=3) on the three different day and % R.S.D. was calculated.

5. Accuracy:

A. Preparation of sample solution for DAP:

Mixture Solution X: DAP (100 µg/ml) + MET (500 µg/ml), Solution Y: DAP (100 µg/ml)

Table 1: Steps for Accuracy Measurement for DAP

Sr. No.	Step 1	Step 2	Step 3	Total DAP Conc. (µg/ml)
1.	Take 0.2 ml of solution X	-	Make up the volume to10 ml with distilled water	2 µg/ml
2.	Take 0.2 ml of solution X	Add 0.16 ml of solution Y	Make up the volume to10 ml with distilled water	3.6 µg/ml
3.	Take 0.2 ml of solution X	Add 0.2 ml of solution Y	Make up the volume to10 ml with distilled water	4 µg/ml
4.	Take 0.2 ml of solution X	Add 0.24 ml of solution Y	Make up the volume to10 ml with distilled water	4.4 µg/ml

B. Preparation of Sample Solution for MET:

Mixture Solution X: DAP (100 µg/ml) + MET (500 µg/ml), Solution Z: MET (100 µg/ml)

Table 2: Steps for Accuracy Measurement for MET

Sr. No.	Step 1	Step 2	Step 3	Total MET Conc. (µg/ml)
1.	Take 0.2 ml of solution X	-	Make up the volume to10 ml with distilled water	10 µg/ml
2.	Take 0.2 ml of solution X	Add 0.8 ml of solution Z	Make up the volume to10 ml with distilled water	18 µg/ml
3.	Take 0.2 ml of solution X	Add 1 ml of solution Z	Make up the volume to10 ml with distilled water	20 µg/ml
4.	Take 0.2 ml of solution X	Add 1.2 ml of solution Z	Make up the volume to10 ml with distilled water	22 µg/ml

Each solution was scanned from 200-400 nm against distilled water as a blank. Absorbance of solution was measured at selected wavelengths for DAP and MET. The amount of DAP and MET was calculated at each level (80%, 100% and 120%) and % recoveries were computed.

6. LOD and LOQ: The LOD (Limit of Detection) was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula: **LOD: 3.3 x S.D. / Slope**

The LOQ (Limit of Quantitation) was estimated from the set of 5 calibration curves that were used to determine linearity of the method.

The LOQ was calculated by using the formula:

LOQ: 10 x S.D. / Slope

Where, S.D. = Standard deviation of the Y – intercepts of 5 calibration curvesSlope = Mean slope of 5 calibration curves

7. Robustness: Robustness of the method was determined by subjecting the method to slight change in the method conditions like, Mobile Phase Ratio and Flow rate.

Three replicates were prepared for the same of concentration 6 µg/ml for DAP and 30 µg/ml for MET and % R.S.D. was calculated.

8. Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate in Synthetic Mixture (Tablet):

A synthetic mixture (tablet) equivalent to 10 mg of DAP and 50 mg of MET was takeninto 100 ml of volumetric flask and added 10 ml of distilled water, the solution was warmedfor 5-10 mins, ultrasonicated for 20 mins, followed by addition of 50 ml distilled water and ultrasonicated for 15 min and was makeup up to themark with distilled water. The solution was filtered through Whatman filter paper no. 41. Thus, resulting solution gave 100 µg/ml of DAP and 500 µg/ml of MET respectively. From the above solution, 1 ml was pipette out and transferred to 10 ml volumetric flaskand volume was made up to mark with distilled water in order to give a solution containing DAP (10 µg/ml) + MET (50 µg/ml). From the above solution, 4 ml was pipette out andtransferred to 10 ml volumetric flask and volume was made up to mark with distilled water in order to give a solution containing DAP (4 µg/ml) + MET (20 µg/ml). Chromatogramwas recorded and the concentration of DAP and MET was obtained by solving the regression equation.

RESULT AND DISCUSSION:

OPTIMIZED CHROMATOGRAPHIC CONDITION:

After Optimization of mobile phase, a well resolved peak of DAP and MET was observed at 4.245 and 2.186 minutes respectively., final chromatographic condition is given in the table.

Table 3: Optimized Chromatographic Condition

Sr. No.	Parameters	Condition
1.	Mobile Phase	ACN: Methanol: Water (pH-3 adjusted with 1% OPA) (72:3:25 v/v/v)
2.	Flow Rate	1 mL/min
3.	Run time	10 min
4.	Volume of Injection	10 µL
5.	Detection Wavelength	223 nm
6.	Diluent	Distilled Water

METHOD VALIDATION:

1. SYSTEM SUITABILITY DATA:

Table 4: System Suitability Data for DAP and MET

Drugs	Parameters	Mean ± S.D. (n=6)	%R.S.D.
DAP	Retention Time	4.245 ± 0.02662	0.6271
	Theoretical Plate	23943.2 ± 180.556	0.7541

	Tailing Factor	1.004 ± 0.00552	0.5494
MET	**Retention Time**	2.186 ± 0.0128	0.5855
	Theoretical Plate	9153.1 ± 44.1088	0.4819
	Tailing Factor	1.187 ± 0.00422	0.3556
	Resolution	7.932 ± 0.05171	0.6519

2. SPECIFICITY:

The specificity of the method was determined by analyzing standard drugs and sampleof DAP and MET. The results suggested that proposed method is specific, the excipientspresent in the synthetic mixture does not affect the result. The chromatogram taken byrunning only with the mobile phase, placebo and after injection of the sample are given in figure given below.

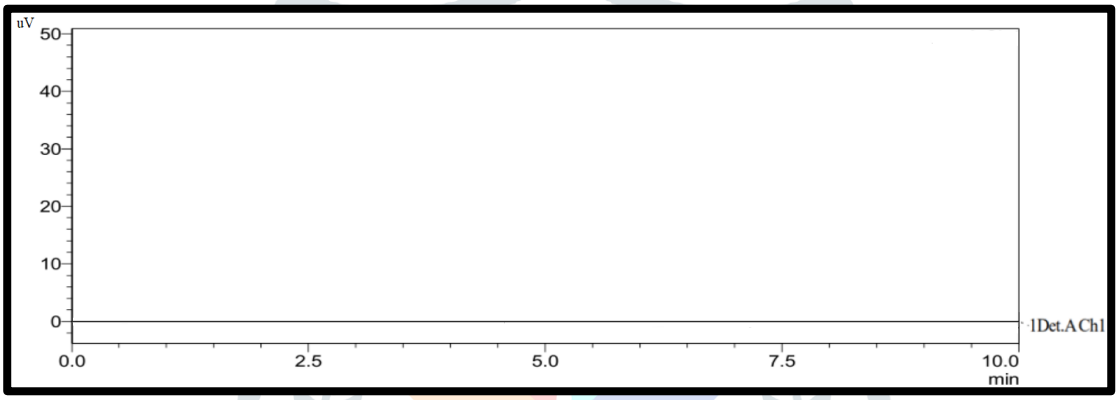

Fig. 4: Chromatogram of Placebo

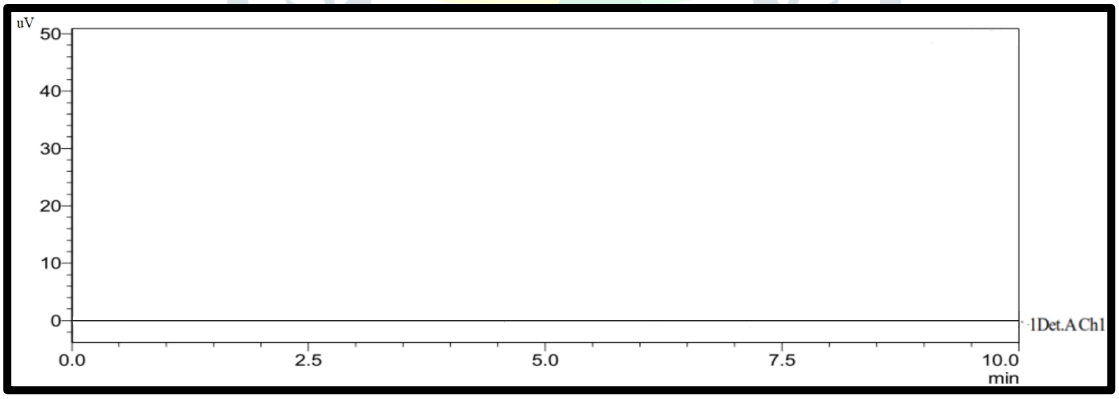

Fig. 5: Chromatogram of Mobile Phase

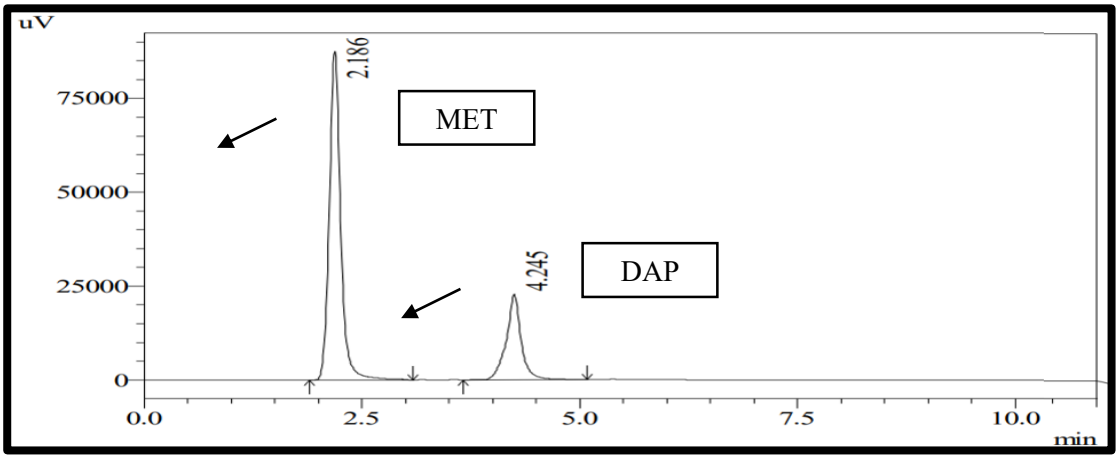

Fig. 6: Chromatogram of DAP (6 µg/ml) and MET (30 µg/ml)

3. LINEARITY:

The linearity study was carried out for both drugs at five different concentration levels.The linearity of DAP and MET was in the range of 2-6 µg/ml and 10-30 µg/ml are depicted in table.

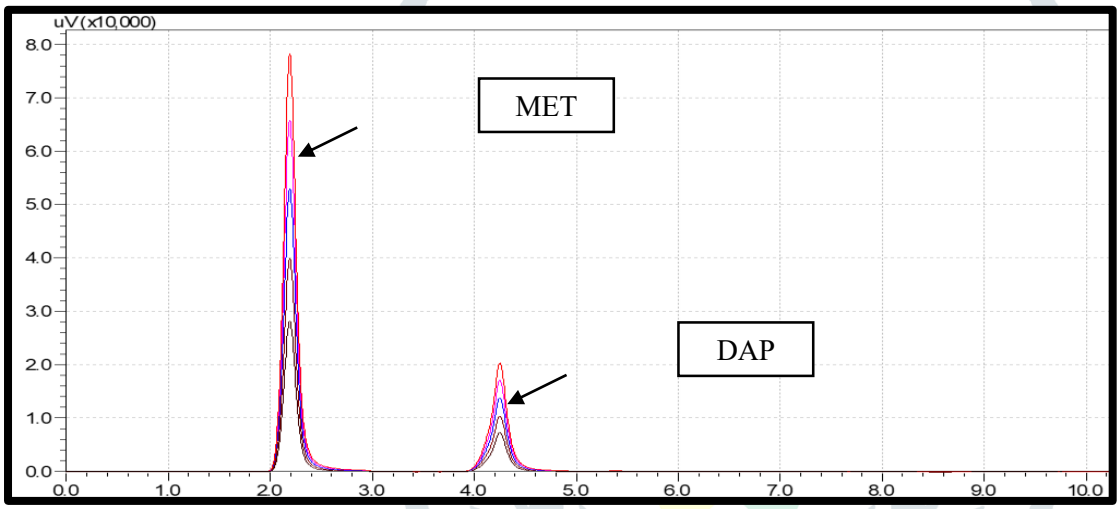

Fig. 7: Overlain Chromatogram of DAP (2-6 µg/ml) and MET (10-30 µg/ml)

Table 5: Linearity Data of DAP

Sr.No.	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=6)	%R.S.D.
1.	2	93920 ± 677.633	0.7215
2.	3	127206 ± 795.419	0.6253
3.	4	176162 ± 940.102	0.5692
4.	5	222681 ± 1008.3	0.4528
5.	6	273498 ± 974.2	0.3562

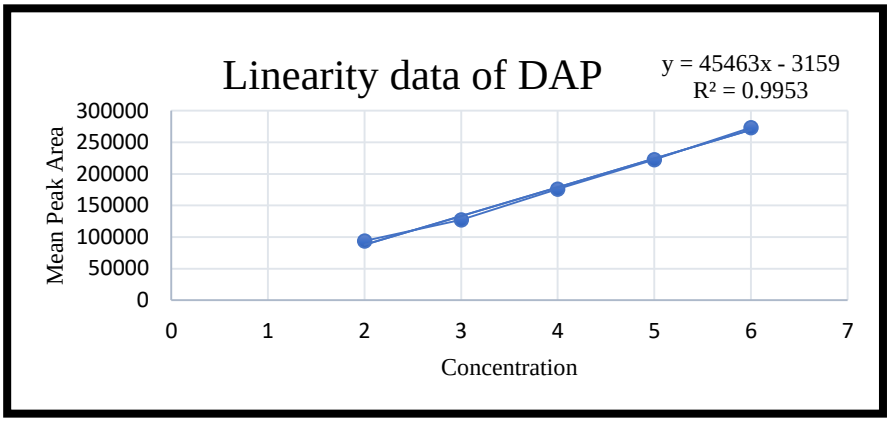

Fig. 8: Calibration Curve of DAP

Table 6: Linearity Data of MET

Sr.No.	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=6)	%R.S.D.
1.	10	271010 ± 2173.23	0.8019
2.	15	398573 ± 2877	0.7215
3.	20	524001 ± 3325.32	0.6346
4.	25	664278 ± 3347.96	0.5040
5.	30	799310 ± 3670.43	0.4592

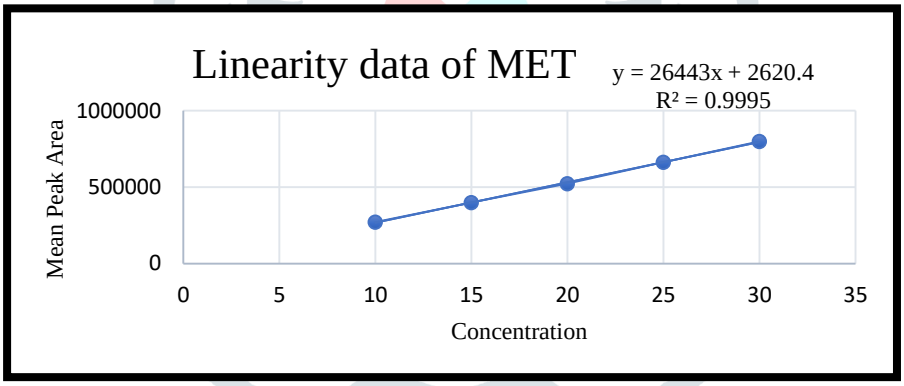

Fig. 9: Calibration Curve of MET

4. PRECISION

a) Repeatability (n= 6)

The data of Repeatability for DAP and MET is shown in table.

Table 7: Repeatability Data for DAP and MET

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=6)	%R.S.D.
DAP	4	176175 ± 1001.146	0.5683
MET	20	524003 ± 3317.987	0.6332

b) Intraday Precision (n=3)

The data of Intraday Precision for DAP and MET is shown in table.

Table 8: Intraday Precision for DAP and MET

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=3)	%R.S.D.
DAP	3	127209 ± 793.657	0.6239
	4	176168 ± 999.578	0.5674
	5	222684 ± 1005.641	0.4516
MET	15	398759 ± 2872.262	0.7203
	20	524006 ± 3314.862	0.6326
	25	664283 ± 3338.687	0.5026

c) Interday Precision (n=3)

The data of Interday Precision for DAP ansd MET is shown in table.

Table 9: Interday Precision for DAP and MET

Drugs	Concentration (µg/ml)	Mean Peak Area ± S.D. (n=3)	%R.S.D.
DAP	3	127212± 791.768	0.6224
	4	176174 ± 998.379	0.5667
	5	222689 ± 1002.769	0.4503
MET	15	398764 ± 2865.917	0.7187
	20	524011 ± 3310.178	0.6317
	25	664289 ± 3330.081	0.5013

5. ACCURACY:

The % Recovery study was performed by the Standard Addition Method. Known amounts of standard solutions of DAP and MET were added at 80%, 100% and 120% level to pre-quantified sample solutions of DAP (2 µg/ml) and MET (10 µg/ml). The amounts of DAP and MET were estimated by using regression equation of the calibration curve. The low value of standard deviation indicates that the proposedmethod is accurate. Results of recovery studies are shown in table.

Table 10: Accuracy Data for DAP and MET

Drugs	Level	Amountof sample (µg/ml)	Amountof std. spiked (µg/ml)	Total amount (µg/ml)	Mean Peak Area ± S.D. (n=3)	Amount of sample found (µg/ml)	Mean % Recovery ± S.D. (n=3)
DAP	0%	2	0.0	2.0	95643 ± 688.2471	1.990	99.50
	80%	2	1.6	3.6	165865 ± 942.6108	3.586	99.61
	100%	2	2.0	4.0	184295 ± 1021.178	3.989	99.73
	120%	2	2.4	4.4	202724 ± 1113.360	4.406	100.14

MET	0%	10	0.0	10	269573 ± 2143.915	9.940	99.40
	80%	10	8.0	18	483673 ± 3132.267	17.932	99.62
	100%	10	10	20	537538 ± 3341.874	19.960	99.80
	120%	10	12	22	581382 ± 3570.849	22.154	100.70

6. LOD and LOQ:

The LOD for DAP and MET was found to be 0.1264 µg/ml and 0.1840 µg/ml respectively.

The LOQ for DAP and MET was found to be 0.3875 µg/ml and 0.5960 µg/ml respectively.

7. ROBUSTNESS:

The robustness of the method was established by making deliberate minor variations inthe following method parameters.

Flow rate: ± 0.2 units, Mobile Phase Ratio: ± 2.0 ml

Table 11: Robustness Data for DAP

Parameters	Level	Mean Peak Area ± S.D. (n=3)	%R.S.D.	Rt ± S.D. (n=3)	%R.S.D.
Mobile Phase (A: M: W) (72: 3: 25 v/v/v)	70: 3: 27 v/v/v	273729 ± 2450.423	0.8952	4.482 ± 0.03646	0.8134
	74: 3: 23 v/v/v	273568 ± 2085.136	0.7622	4.312 ± 0.02594	0.6016
Flow Rate (1.0 ml/min)	0.8 ml	273421 ± 1908.753	0.6981	4.327 ± 0.03304	0.7635
	1.2 ml	273539 ± 1921.886	0.7026	4.159 ± 0.02477	0.5955

Table 12: Robustness Data for MET

Parameters	Level	Mean Peak Area ± S.D. (n=3)	%R.S.D.	Rt ± S.D. (n=3)	%R.S.D.
Mobile Phase (A: M: W) (72: 3: 25 v/v/v)	70: 3: 27 v/v/v	799697 ± 6116.882	0.7649	2.369 ± 0.01807	0.7626
	74: 3: 23 v/v/v	799878 ± 5699.93	0.7126	2.273 ± 0.01498	0.6590
Flow Rate (1.0 ml/min)	0.8 ml	799047 ± 5579.746	0.6983	2.261 ± 0.01638	0.7244
	1.2 ml	799661 ± 5966.27	0.7461	2.087 ± 0.01403	0.6723

8. APPLICABILITY OF PROPOSED METHOD:

Table 13: Determination of Assay of DAP and MET

Synthetic Mixture (Tablet)	Actual conc. (%w/w)		Amount obtained Mean ± S.D. (n=5) (%w/w)		DAP %Purity ± S.D. (n=5)	MET %Purity ± S.D. (n=5)
	DAP	MET	DAP	MET	DAP	MET
	4	20	3.993 ± 0.00158	19.972 ± 0.00381	99.825 ± 0.03953	99.86 ± 0.01904

SUMMARY OF VALIDATION PARAMETERS FOR PROPOSED METHOD:

Table 14: Summary of RP-HPLC Method

Parameters	DAP	MET
Linearity (µg/ml) (n=5)	2-6 (µg/ml)	10-30 (µg/ml)
Regression Equation (y= mx+c)	y = 45463x - 3159	y = 26443x + 2620.4
Regression Coefficient (R^2)	0.9953	0.9995
Correlation coefficient(r)	0.9976	0.9997
Repeatability (n=6) (%RSD)	0.5683	0.6332
Intraday precision (n=3) (%RSD)	0.4516 – 0.6239	0.5026 – 0.7203
Interday precision (n=3) (%RSD)	0.4503 – 0.6224	0.5013 – 0.7187
LOD (µg/ml)	0.1264	0.1840
LOQ (µg/ml)	0.3875	0.5960
% Recovery (n=3)	99.50 – 100.14	99.40 – 100.70
% Assay ± S.D. (n=5)	99.825 ± 0.03953	99.86 ± 0.01904

SUMMARY AND CONCLUSION:

The proposed RP-HPLC Method were developed for simultaneous estimation of Dapagliflozin Propanediol Monohydrate (DAP) and Metoprolol Succinate (MET) in their synthetic mixture are simple, precise, accurate, and sensitive. These methods have wider range with good accuracy and precision. They can be used for the routine analysis of both drugs in pharmaceutical formulations.

The method was developed and validated as per **ICH guidelines Q2(R2).**

The precision of the developed methods was confirmed by intra-day and inter-day analysis and the accuracy of the developed method was confirmed by the recovery study. The **%RSD** was found to be **<2.0%.** It indicates that the method has good precision and accuracy.

Hence the developed RP-HPLC method is simple, rapid, precise and accurate.

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