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UV SPECTROSCOPIC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AREA UNDER CURVE METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE AND METOPROLOL SUCCINATE

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ABSTRACT
In the present study Area Under Curve Method was developed for the estimation of Dapagliflozin Propanediol Monohydrate (DAP) and Metoprolol Succinate (MET) in Synthetic Method. The Area Under Curve Method for DAP and MET were found to be linear over the range of 2-6 µg/ml and 10-30 µg/ml respectively. The wavelength range 210-218 nm and 219-227 nm was selected for DAP and MET respectively in Area Under Curve Method. The method validated for different validation parameter such as Linearity, Accuracy, Precision, LOD, LOQ and the results were found to be within the acceptance limit as per the guideline of International Conference on Harmonization (ICH) Q2(R2) specifications.
KEYWORDS: *Dapagliflozin Propanediol Monohydrate, Metoprolol Succinate, Area Under Curve Method, 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) 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]

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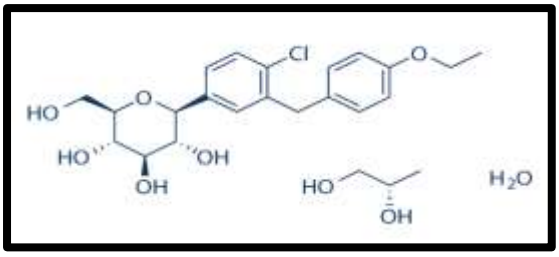


Fig. 1: Chemical Structure of DAP

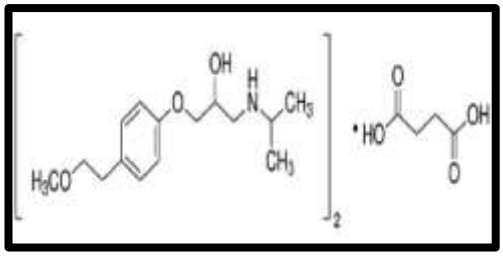


Fig.2: Chemical Structure of MET

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 UV-VIS SPECTROSCOPY METHOD: [11-16]

The wavelength and absorption intensity of near ultraviolet and visible light are measured in ultraviolet (UV)-visible spectroscopy. It is primarily based on the component in ultraviolet (190-380 nm) or visible light (380-800 nm) radiation absorbed by an element present in solution. Light absorbs in both the ultraviolet and visible parts of the electromagnetic spectrum when the energy of the light equals the energy necessary to induce an electronic transition and its accompanying vibrational and rotational transition in the molecule.

Principle

When a molecule is exposed to electromagnetic radiation (EMR), it absorbs a specific amount of radiation energy.
This is referred to as absorption. Absorption spectrophotometry is the study of this phenomena. UV wavelengths vary from 200 to 800 nm. Radiation may be absorbed by a molecule by three processes: electronic, vibrational, and rotational transformation.
Therefore, the total energy of a molecule can be: E Total= E (Electronic) + E (Vibration) + E (Rotational)

MATERIALS AND METHODS

Apparatus and Instrumentation

Model: SHIMADZU LC-2010 CHT
Column: Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm)
Detector: UV Detector
Software: LC Solution
Electronic Analytical Balance (SHIMADZU- 0.1 mg)
Digital pH Meter (Systronic pH System)
Ultrasonic Cleaner (Athena Technology)
Filter paper:

- Vacuum filter: Membrane filter 0.45 micron
- Syringe filter: Membrane filter 0.27 micron

Volumetric flask and Pipettes (Borosil)
The absorbance and spectral measurements were done on a double-beam Shimadzu UV-Visible Spectrophotometer with software Lab Solution, 1cm quartz cells were used for sample handling and a digital analytical balance was used for weighing.

Chemicals

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

Preparation of Standard Stock Solutions

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

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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.

Assay of Marketed Formulation

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 warmed for 5-10 mins, ultrasonicated for 20 mins, followed by addition of 50 ml distilled water and ultrasonicated for 15 min and was make up to the mark 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).

Area of the resulting solution was measured at 210-218 nm and 219-227 nm against distilled water. The concentration of Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate can be obtained as,

Estimation of Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate

A set of equation were established using mean absorptivity of coefficient of Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate at selected wavelength range interval.

A1= ax_1C_1 + ay_1C_1…Eq (1) at 210-218 nm, **A2**= ax_2C_2 + ay_2C_2…Eq (2) at 219-227 nm

By applying Cramer's rule to Eq 1 and 2; Where, A1 is area of sample solution at AUC 210-218 nm A2 is area of sample solution at AUC 219-227 nm ax_1 and ax_2 are mean absorptivity of coefficient of Dapagliflozin Propanediol Monohydrate and ay_1 and ay_2 are mean absorptivity of coefficient of Metoprolol Succinate.

C_1 and C_2 are concentration (gm/l) of Dapagliflozin Propanediol Monohydrate and Metoprolol Succinate which is obtained using Cramer's Rule.

METHOD DEVELOPMENT

Calibration Curve and Selection of Wavelengths

Appropriate dilutions were prepared for drugs from the working stock solution were scanned in the wavelength range of 200-400nm. The absorption spectra of Area UnderCurve (AUC) in absorption spectra of DAP were measured between the wavelength range 210-218 nm and for MET were measured between the wavelength range 219-227 nm.

Calibration curve for DAP and MET consisted of five different concentrations of standard solution of DAP ranging from 2-6 µg/ml and MET ranging from 10-30 µg/ml respectively. The AUC of the solution was measured under wavelength range 210-218 nm for DAP and 219-227 nm for MET against distilled water as blank. Calibration Curve was plotted at both wavelength and equation were formed using specific absorbance.

METHOD VALIDATION

The Proposed method was validated according to ICH guidelines. The parameters assessed were Linearity, Precision, Accuracy, LOD and LOQ.

1. Linearity (n=5)

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2-6 µg/ml for DAP and 10-30 µg/ml for MET. The Calibration curve of $dA/d\lambda$ absorbance vs. concentration was plotted and correlation coefficient and regression line equation for DAP and MET were calculated.

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2. Precision

A. Repeatability (n=6)

Aliquot of 4 ml of working stock solution of DAP (10 µg/ml) were taken into series of 10 ml volumetric flask. Aliquot of 4 ml of working stock solution of MET (50 µg/ml) were taken into series of 10 ml volumetric flask. Using distilled water, volume was made up to mark to give a solution containing 4 µg/ml of DAP and 20 µg/ml of MET. Solution was analyzed six times (n=6) and % R.S.D. was calculated.

B. Intraday (n=3)

Aliquots of 3, 4 and 5 ml of working stock solution of DAP (10 µg/ml) were taken into series of 10 ml volumetric flask. Aliquots of 3, 4 and 5 ml of working stock solution of MET (50 µg/ml). Using distilled water, volume was made up to mark, to give a solution containing 3, 4 and 5 µg/ml of DAP and 15, 20 and 25 µg/ml of MET. Solution was analyzed for three times (n=3) on the same day within short interval of time and % R.S.D. was calculated.

C. Interday (n=3)

Aliquots of 3, 4 and 5 ml of working stock solution of DAP (10 µg/ml) were takeninto series of 10 ml volumetric flask. Aliquots of 3, 4 and 5 ml of working stock solution of MET (50 µg/ml). Using distilled water, volume was made up to mark, to give a solution containing 3, 4 and 5 µg/ml of DAP and 15, 20 and 25 µg/ml of MET. Solution was analysed for three times (n=3) on three different days and % R.S.D. was calculated.

Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Limit of Detection (L.O.D.): From the linearity curve equation, the standard deviation (S.D.) of the intercepts (response) was calculated. The limit of detection (L.O.D.) of the drug was calculated by using the following equation designated by ICH guideline: L.O.D. = 3.3 σ / S

Limit of Quantitation (L.O.Q.): The limit of quantitation (L.O.Q.) of the drug was calculated by using the following equation designated by ICH guideline: L.O.Q. = 10 σ / S

Where, σ = the standard deviation of the response S = slope of the calibration curve.

RESULTS AND DISCUSSION

Area Under Curve Method Selection of Wavelengths:

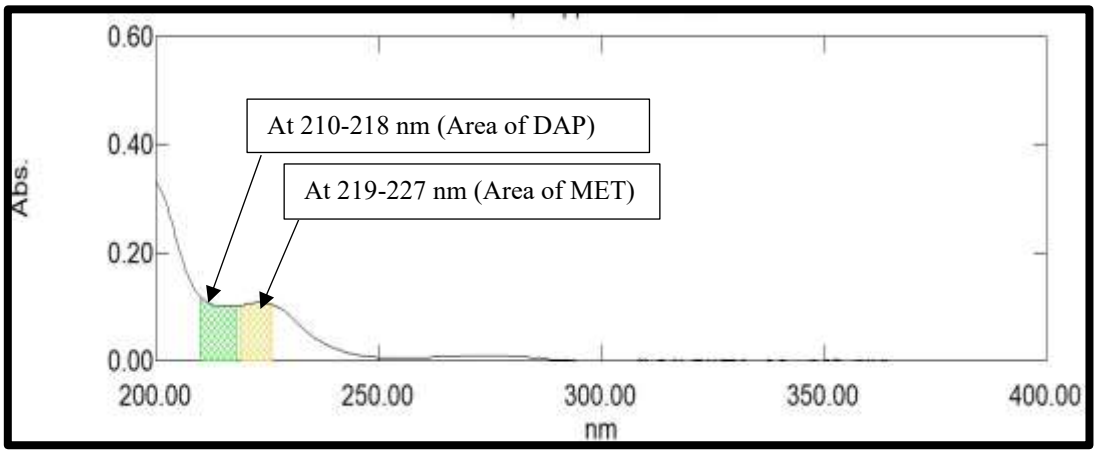


Fig. 3: AUC Spectra of Dapagliflozin Propanediol Monohydrate 2 µg/ml in wavelength range 210-218 nm and 219-227 nm

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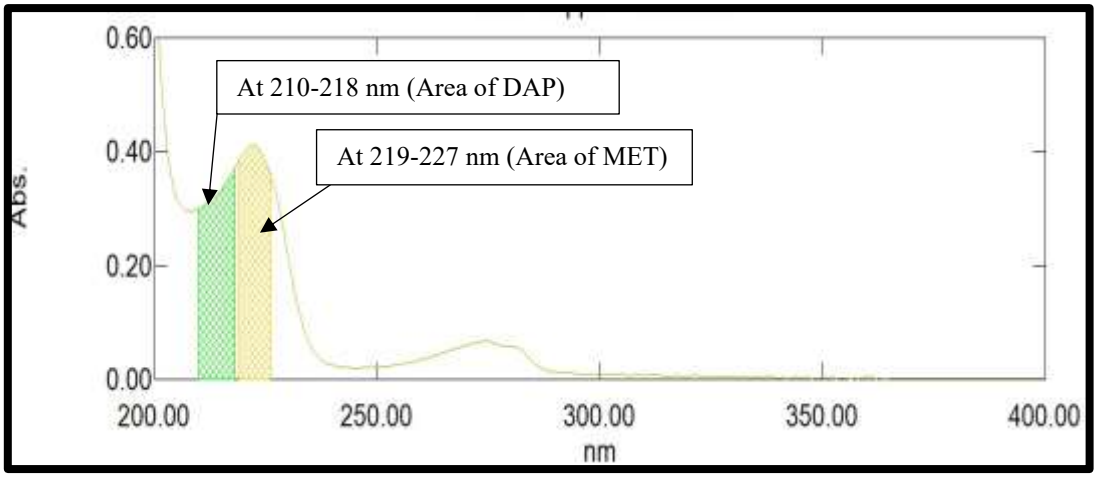


Fig. 4: AUC Spectra of Metoprolol Succinate 10 µg/ml in wavelength range 210-218 nm and 219-227 nm

Result and Calibration Reading

Table 1: Linearity data for DAP at 210-218 nm (Area of DAP)

Sr.No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	2	0.8322 ± 0.006030	0.7241
2.	3	1.3863 ± 0.009210	0.6642
3.	4	1.9424 ± 0.011650	0.5997
4.	5	2.3892 ± 0.011870	0.4968
5.	6	2.8232 ± 0.009880	0.3502

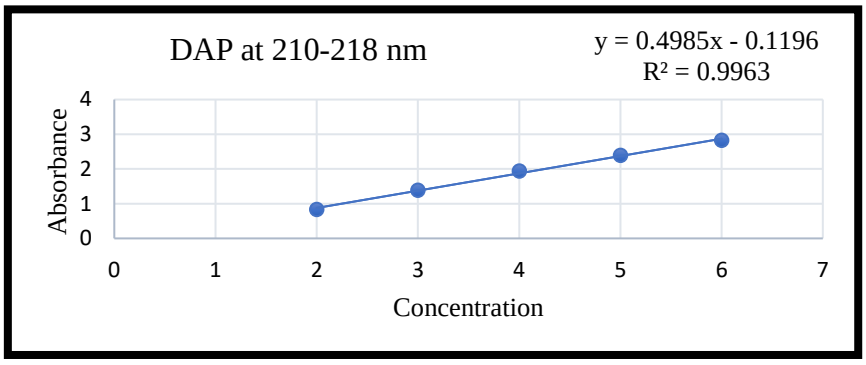


Fig. 5: Calibration curve of DAP at 210-218 nm (Area of DAP)

Table 2: Linearity of DAP at 210-218 nm (Area of MET)

Sr.No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	2	0.7432 ± 0.006200	0.8344
2.	3	1.1632 ± 0.008870	0.7626
3.	4	1.6421 ± 0.011290	0.6875
4.	5	2.1427 ± 0.011790	0.5504
5.	6	2.5332 ± 0.011020	0.4354

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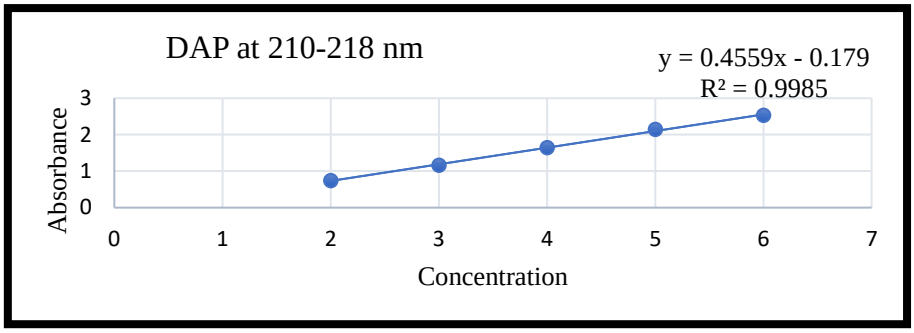


Fig. 6: Calibration curve of DAP at 210-218 nm (Area of MET)

Table 3: Linearity of MET at 219-227 nm (Area of DAP)

Sr.No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	10	2.6331 ± 0.022500	0.8545
2.	15	3.3572 ± 0.025500	0.7596
3.	20	4.0941 ± 0.027900	0.6814
4.	25	4.9502 ± 0.025700	0.5191
5.	30	5.8952 ± 0.027300	0.4631

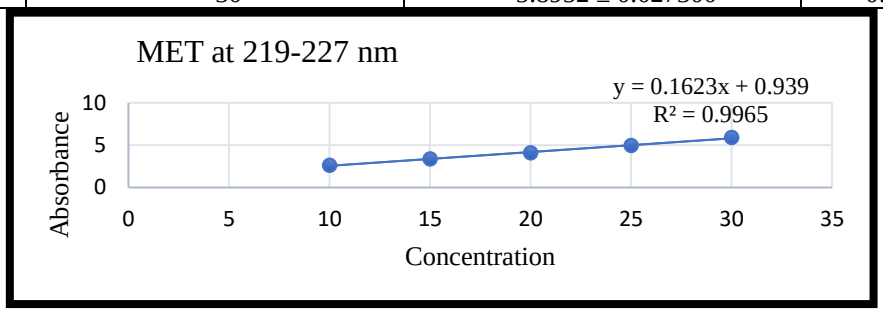


Fig. 7: Calibration curve of MET at 219-227 nm (Area of DAP)

Table 4: Linearity of MET at 219-227 nm (Area of MET)

Sr.No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1.	10	2.7773 ± 0.019670	0.7082
2.	15	3.5353 ± 0.024010	0.6791
3.	20	4.4233 ± 0.027130	0.6133
4.	25	5.3533 ± 0.025470	0.4758
5.	30	6.3741 ± 0.024210	0.3796

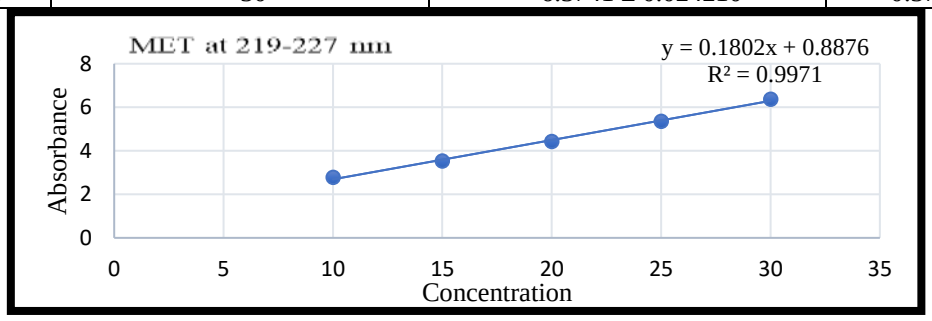


Fig. 8: Calibration curve of MET at 219-227 nm (Area of MET)

Table 5: Assay Result of Synthetic Mixture

Synthetic Mixture (Tablet)	Actual conc. (%w/w)		Amt. obtained Mean ± S.D. (n=5) (%w/w)		% Purity ± S.D. (n=5)	
	DAP	MET	DAP	MET	DAP	MET
	4	20	3.983 ± 0.00016	19.96 ±0.00791	99.58 ± 0.00395	99.80 ± 0.03953

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METHOD VALIDATION

PRECISION

A. Repeatability Study

Table 6: Repeatability data for DAP and MET

Conc. (µg/ml)	DAP 210-218 nm (Area of DAP)		Conc. (µg/ml)	MET 219-227 nm (Area of MET)	
	Mean Abs. ± S.D.	%R.S.D.		Mean Abs. ± S.D.	%R.S.D.
4	1.9422 ± 0.011620	0.5982	20	4.4245 ± 0.026890	0.6076

B. Intraday Study

Table 7: Intraday Study data for DAP and MET

Conc. (µg/ml)	DAP 210-218 nm (Area of DAP)		Conc. (µg/ml)	MET 219-227 nm (Area of MET)	
	Mean Abs. ± S.D.	%R.S.D.		Mean Abs. ± S.D.	%R.S.D.
3	1.3867 ± 0.009050	0.6526	15	3.5362 ± 0.023640	0.6685
4	1.9436 ± 0.011560	0.5947	20	4.4253 ± 0.026450	0.5976
5	2.3904 ± 0.011630	0.4865	25	5.3556 ± 0.024980	0.4663

C. Interday Study

Table 8: Interday Study data for DAP and MET

Conc. (µg/ml)	DAP 210-218 nm (Area of DAP)		Conc. (µg/ml)	MET 219-227 nm (Area of MET)	
	Mean Abs. ± S.D.	%R.S.D.		Mean Abs. ± S.D.	%R.S.D.
3	1.3882 ± 0.008920	0.6424	15	3.5383 ± 0.023280	0.6579
4	1.9462 ± 0.011430	0.5872	20	4.4268 ± 0.026310	0.5943
5	2.3914 ± 0.010860	0.4541	25	5.3606 ± 0.023950	0.4468

Accuracy

Table 9: Method 2 Accuracy Table of DAP and MET

Drugs	Level	Amount of sample (µg/ml)	Amount of Std. spiked(µg/ml)	Total amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
DAP	0%	2	0	2	1.986	99.33
	80%	2	1.6	3.6	3.581	99.47
	100%	2	2	4	3.982	99.55
	120%	2	2.4	4.4	4.389	99.75
MET	0%	10	0	10	9.905	99.05
	80%	10	8	18	17.868	99.27
	100%	10	10	20	19.930	99.65
	120%	10	12	22	22.044	100.20

Limit of Detection and Limit of Quantitation

Table 10: L.O.D. and L.O.Q data for DAP and MET

Drugs	L.O.D. (µg/ml)	L.O.Q. (µg/ml)
DAP	0.1326	0.3963
MET	0.2072	0.6256

SUMMARY AND CONCLUSION

The proposed Area Under Curve Methods 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.

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