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DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE UV SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE AND METOPROLOL SUCCINATE

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ABSTRACT
In the present study First Order Derivative Spectroscopic Method was developed for the estimation 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 method for DAP and MET was found to be linear over the range of 2-6 µg/ml and 10-30 µg/ml respectively. The estimation of DAP was done at ZCP of MET (209 nm) and MET was done at the ZCP of DAP (216 nm). 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, First Order Derivative Spectroscopic Method, Validation, ICH.*

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
6.

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

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output by producing negative chronotropic and inotropic effects without presenting activity towards membrane stabilization nor intrinsic sympathomimetics. [8,9]

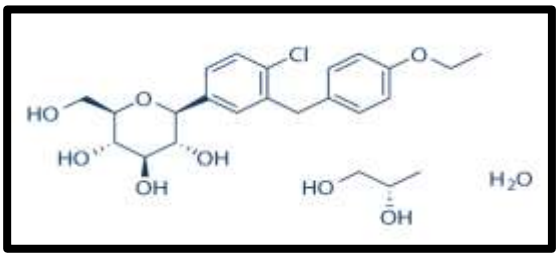

Fig. 1: Chemical Structure of DAP

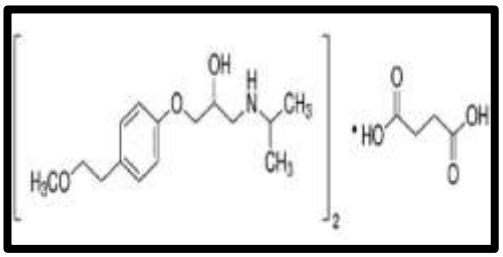

Fig. 2: Chemical Structure of MET

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 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.

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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 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).

Absorbance of resulting solution was recorded by converting zero order spectra into first order at 209 nm for DAP (ZCP of MET) and 216 nm for MET (ZCP of DAP). The concentration ofDAP and MET obtained by solving the regression equation:

1. y = 0.0053x - 0.0048 for DAP at 209 nm (ZCP of MET)
2. y = 0.0009x + 0.0027 for MET at 216 nm (ZCP of DAP

METHOD DEVELOPMENT

Calibration Curve and Determination of the Zero Crossing point

Calibration curve for DAP and MET consisted of five different concentrations of standard solution of DAP and MET ranging from 2-6 µg/ml and 10-30 µg/ml respectively. Each solution was scanned against distilled water as blank and corresponding spectra was recorded. The First derivative (dA/dλ) spectra of all these solutions were obtained by transformation of zero order spectra of every solution. dA/dλ absorbance at 216 nm (Zero Crossing Point of DAP) and 209 nm (Zero Crossing Point of MET) respectively was computed and the plot of dA/dλ absorbance vs. concentration was plotted and regression equation was obtained.

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/dλ absorbance vs. concentration was plotted and correlation coefficient and regression line equation for DAP and MET were calculated.

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.

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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

First Order Derivative Method Selection of Detection Wavelengths

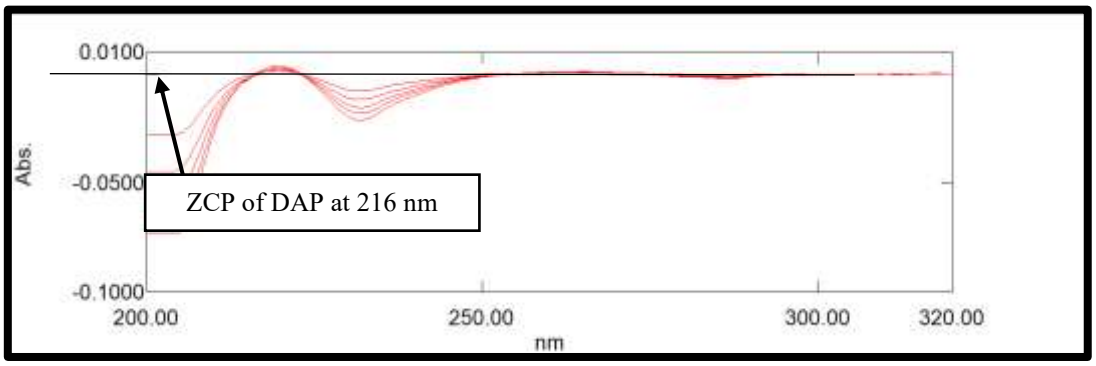

Fig. 3: Overlain First Order Derivative Spectra of DAP

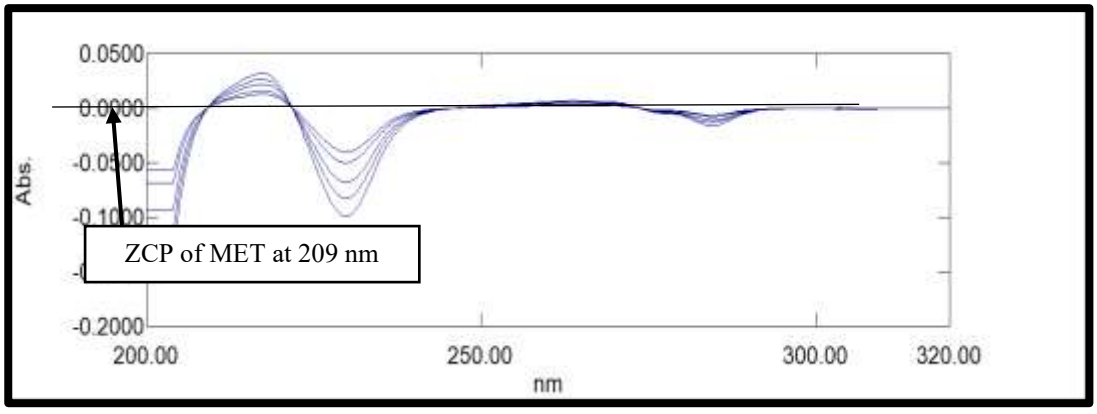

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Fig. 4: Overlain First Order Derivative Spectra of MET

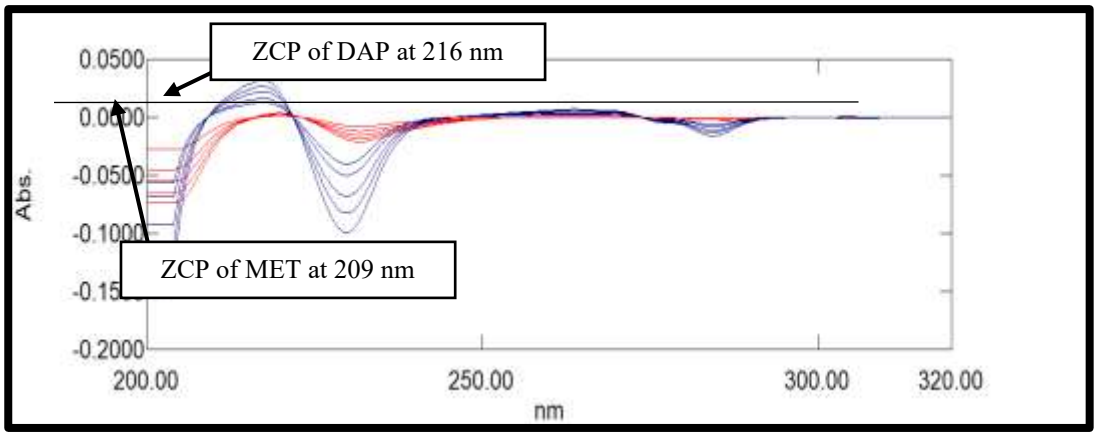

Fig. 5: Overlain First Order Derivative Spectra of DAP and MET

Result and Calibration Reading

Table 1: Linearity data for DAP at 209 nm (ZCP of MET)

Sr.No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1.	2	0.0152 ± 0.000126	0.8289
2.	3	0.0206 ± 0.000155	0.7542
3.	4	0.0261 ± 0.000172	0.6590
4.	5	0.0316 ± 0.000169	0.5348
5.	6	0.0365 ± 0.000180	0.4931

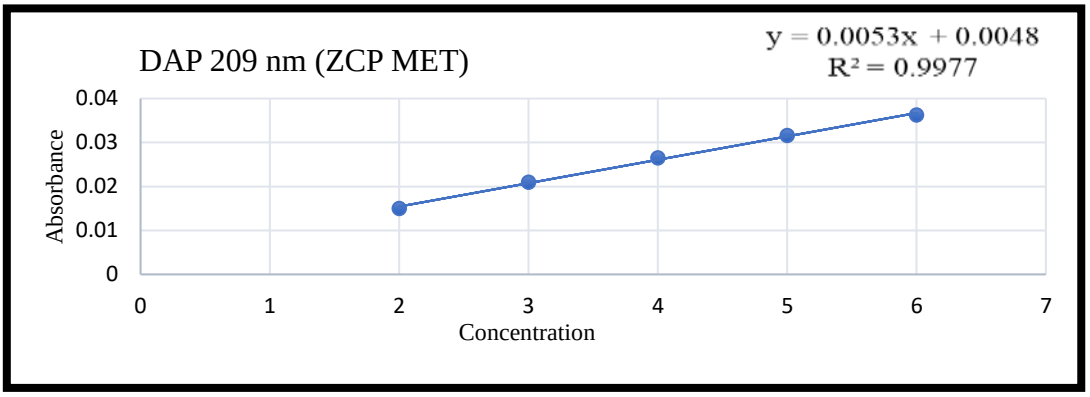

Fig. 6: Calibration Curve of DAP at 209 nm (ZCP of MET)

Table 2: Linearity data for MET at 216 nm (ZCP of DAP)

Sr.No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1.	10	0.0120 ± 0.000089	0.7417
2.	15	0.0165 ± 0.000110	0.6667
3.	20	0.0205 ± 0.000122	0.5951
4.	25	0.0253 ± 0.000146	0.5375
5.	30	0.0301 ± 0.000150	0.4814

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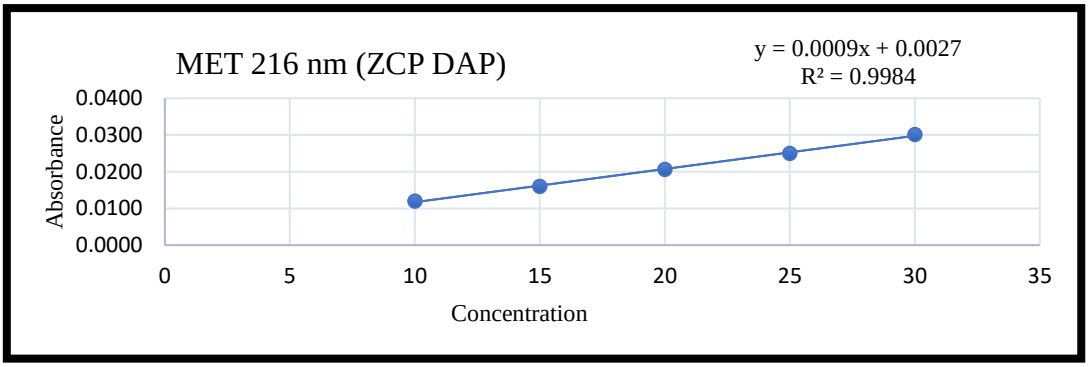

Fig. 7: Calibration Curve of MET at 216 nm (ZCP of DAP)

Table 3: 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	3.982 ± 0.00102	19.896 ±0.00158	99.57 ± 0.02550	99.48 ± 0.00791

METHOD VALIDATION

PRECISION

A. Repeatability Study

Table 4: Repeatability data for DAP and MET

Conc. (µg/ml)	DAP 209 nm (ZCP of MET)		Conc. (µg/ml)	MET 216 nm (ZCP of DAP)	
	Mean Abs. ± S.D.	%R.S.D.		Mean Abs. ± S.D.	%R.S.D.
4	0.0265 ± 0.000180	0.6792	20	0.0208 ± 0.000120	0.5769

B. Intraday Study

Table 5: Intraday Study data for DAP and MET

Conc. (µg/ml)	DAP 209 nm (ZCP of MET)		Conc. (µg/ml)	MET 216 nm (ZCP of DAP)	
	Mean Abs. ± S.D.	%R.S.D.		Mean Abs. ± S.D.	%R.S.D.
3	0.0211 ± 0.000160	0.7547	15	0.0172 ± 0.000130	0.7441
4	0.0269 ± 0.000183	0.6691	20	0.0212 ± 0.000127	0.5660
5	0.0322 ± 0.000175	0.5372	25	0.0256 ± 0.000136	0.5329

C. Interday Study

Table 6: Interday Study data for DAP and MET

Conc. (µg/ml)	DAP 209 nm (ZCP of MET)		Conc. (µg/ml)	MET 216 nm (ZCP of DAP)	
	Mean Abs. ± S.D.	%R.S.D.		Mean Abs. ± S.D.	%R.S.D.
3	0.0217 ± 0.000170	0.7692	15	0.0183 ± 0.000138	0.7541
4	0.0273 ± 0.000184	0.6727	20	0.0217 ± 0.000120	0.5529
5	0.0329 ± 0.000180	0.5319	25	0.0263 ± 0.000134	0.5095

Accuracy

Table 7: Accuracy Table of DAP and MET

Drugs	Level	Amount of sample(µg/ml)	Amount ofStd. spiked(µg/ml)	Total amount (µg/ml)	Amount ofsample found (µg/ml)	% Recovery
DAP	0%	2	0	2	1.984	99.22
	80%	2	1.6	3.6	3.582	99.51
	100%	2	2	4	3.982	99.56
	120%	2	2.4	4.4	4.405	100.13
MET	0%	10	0	10	9.925	99.25
	80%	10	8	18	17.879	99.33
	100%	10	10	20	19.884	99.42
	120%	10	12	22	21.903	99.56

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Limit of Detection (L.O.D.) and Limit of Quantitation (L.O.Q.):

Table 8: L.O.D. and L.O.Q data for DAP and MET

Drugs	L.O.D. (µg/ml)	L.O.Q. (µg/ml)
DAP	0.1288	0.3904
MET	0.2061	0.6245

SUMMARY AND CONCLUSION

The proposed First Order Derivative Spectrophotometric Method is 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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