JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN PROPANEDIOL MONOHYDRATE AND LINAGLIPTIN

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Abstract: Dapagliflozin Propanediol Monohydrate and Linagliptin are used in ratio (10:5) for treatment of patient suffering with Type – 2 Diabetes mellitus. The objective of work is to develop simple, economic, accurate, precise methods for simultaneous estimation of both drugs by using spectrophotometric. First order derivative methods were developed and validated for quantitative determination of Dapagliflozin Propanediol Monohydrate and Linagliptin in synthetic mixture. First order derivative method were developed and validated by using distilled water and scan between 200 – 400 nm. UV spectrophotometric method for DPM and LNG were found to be linear over range of 6 – 22 µg/ml and 3 – 11 µg/ml respectively for First order derivative. The regression coefficient (R^2) was found to be 0.9982 and 0.9999 for DPM and LNG respectively.

***Keywords*:** Dapagliflozin Propanediol Monohydrate, Linagliptin, First order derivative, Method validation.

I. INTRODUCTION

Diabetes Mellitus is a metabolic disorder characterized by malfunctioning insulin synthesis and increased blood glucose levels. It is a disorder that happens when there is a complete or partial shortage of insulin and leads to anomalies in the metabolism and blood vessels. Diabetes mellitus is often referred to as "sugar." It is the most common endocrine disorder and often shows up as a lack or deficiency of insulin or, less frequently, as a dysfunction of insulin activity [1 - 3].

Introduction to Diabetes Mellitus
Types of Diabetes Mellitus [4]
Mainly four types of Diabetes Mellitus are mentioned below:-
a) Insulin dependent Diabetes Mellitus (Type 1 IDDM)
b) Non-insulin dependent Diabetes Mellitus (Type 2 NIDDM)
c) Gestational Diabetes Mellitus (GDM)
d) Other specific type (Monogenic type)

Dapagliflozin Propanediol Monohydrate [DPM]
Mechanism of Action: Dapagliflozin inhibits the sodium-glucose cotransporter 2 (SGLT2), which is primarily present in the proximal tubule of the nephron. Since SGLT2 facilitates 90% of the resorption of glucose in the kidney, its blockage allows glucose to be excreted in the urine. Due to this excretion, patients with type 2 diabetes may experience improved glucose control and possibly lose weight. [5, 6]
Adverse effect: Felling Dizzy, Skin rash, Back pain.

Linagliptin [LNG]
Mechanism of Action: A reversible competitive DPP-4 inhibitor. Linagliptin is an inhibitor. Enzyme Inhibition delays the breakdown of GLP – 1 and glucose dependent insulin tropic polypeptide (GLP). GLP-1 and GIP promote the release of insulin from pancreatic beta cell while inhibiting the synthesis of glucagon by pancreatic beta cells. [7, 8]

Adverse effect: Itching Skin rash, hard skin blister, swelling on the face, throat, hands, legs, feet, joint pain.

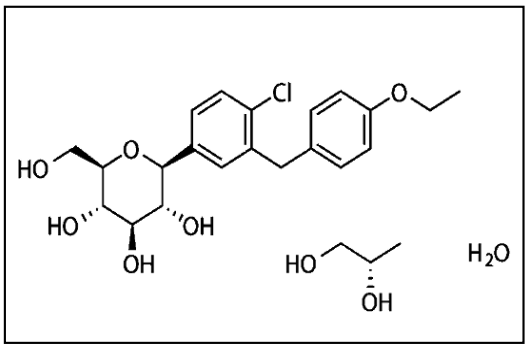

Figure 1: Chemical Structure of DPM

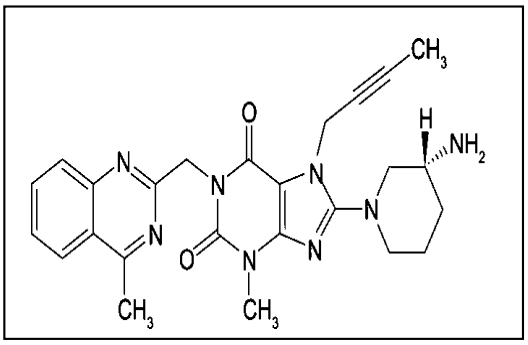

Figure 2: Chemical Structure of LNG

Introduction to UV – Visible Spectroscopy

The most popular spectroscopic method in pharmaceutical analysis is ultraviolet-visible spectrophotometry. In UV-visible spectroscopy, the amount of ultraviolet (190-380 nm) or visible (380-800 nm) radiation absorbed by a substance in a solution is measured [9].

Principle

A Certain portion of the energy associated with the specific radiation is absorbed by a molecule when it is exposed to electromagnetic radiation (EMR). The molecule receive energy from the radiant energy beam. This is known as absorption, and the study of it is known as absorption spectrophotometry [10].

Derivative Spectrophotometry

The idea of derivative spectroscopy involves converting a simple absorption spectrum into its derivative spectrum (first, second or higher) depend on wavelength. It is used to describe any chemical conformation in spectrum analysis [11, 12, 14].

In comparison to derivative spectra, zero – order spectra are simpler. The rate of change of absorbance against wavelength is graphically represented in a first – order derivative spectrum. A first – order derivative begins and ends at zero point, passing through zero Points as λmax of the absorbance band. This point is known as the inflection point because, at the same wavelength, one side of it exhibits a positive band and the other a negative band with both maxima and minima values. The maximum positive and maximum negative slopes of the Zero – order spectrum correspond the maximum and minimum of the first – order spectrum, respectively. Since the wavelength of zero slope in a zero – order spectrum is λmax, the value of dA/d λ in a first – order spectrum is zero [11, 12, 14].

Analytical Method Validation

Validation:

Validation is establishing documented evidence which provide a high degree of assurance that a specific process will consistently produce product meeting its pre- determined specifications and quality attributes [13].

ICH Guideline (ICH Q2, R1) for Analytical Procedure and Validation.

MATERIAL AND METHODS

Procurement of Drug

Dapagliflozin Propanediol Monohydrate was received as gift sample from CTX Lifescience Pvt. Ltd, Surat. Linagliptin was received as a gift sample from Mehta API Pvt. Ltd, Maharashtra.

Apparatus and Instrument

Model - UV - Visible spectrophotometer (Double Beam) (Shimadzu – 1900i)
Software - Lab solution (2.03 Version)
Electronic Analytical Balance (REPTECH) (0.1 mg)
Digital Melting point apparatus
Ultrasonic cleaner (Athena Technology)
Pipette (Borosil)
Volumetric Flask (Borosil)

Spectrophotometric Condition

Mode: Scan
Scan speed: Medium
Wavelength range: 200 - 400 nm
Scale of Absorbance: 0.00 - 2.00 A
Baseline Correction: Distilled water

Selection of Solvent

According to solubility study, distilled water was found to be the most common solvent for this drug. For UV techniques, dist illed water was selected as the solvent. Drugs like Dapagliflozin propanediol monohydrate and Linagliptin give linear spectra in distilled water at the measured wavelength. Therefore, the preferred solvent is distilled water.

Preparation of Standard Solution

1. Preparation of DPM Standard stock solution (1000 µg/ml)

Accurately weighed 10 mg of DPM and place into 10 ml volumetric flask and it was dissolved in distilled water and volume was make up to 10 ml with distilled water to produce stock solution (1000µg/ml).

2. Preparation of DPM Working stock solution (200µg/ml)

Aliquot of 5 ml from above standard stock solution was pipetted out in 25 ml of volumetric flask and volume was make up to 25 ml with distilled water to get a working solution 200 µg/ml.

3. Preparation of DPM working stock solution (50µg/ml)

Aliquot of 12.5 ml from above standard stock solution was pipetted out in 50 ml of volumetric flask and volume up to 50 ml with distilled water to get a working solution 50 µg/ml.

4. Preparation of LNG Standard stock solution (100 µg/ml)

Accurately weighed 10 mg of LNG and take in 100 ml of volumetric flask and it was dissolved in distilled water and volume was make up to 100 ml with distilled water to produce stock solution (100 µg/ml).

Selection of Wavelength

Aliquots of 2 ml from working stock solution of DPM (50µg/ml) and 0.5 ml from working stock solution of LNG (25 µg/ml) and were pipetted 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 10 µg/ml and 5 µg/ml of DPM and LNG. Each and every solution was scanned between 200 – 400 nm against distilled water as blank. The zero order spectra data was processed to produce a first order derivative spectrum between in the range of 400 – 200 nm. In first order spectra Zero crossing point at 215.5 nm, 223 nm and after 310.0 nm for ZCP of DPM and ZCP of LNG at 207.5 nm , and 227.0 nm. Wavelength selected for Quantitation were 223.0 nm for LNG (ZCP of DPM) and 207.5 nm for DPM (ZCP of LNG).

Preparation of Calibration Curve

1. Calibration curve for DPM (6 - 22 µg/ml)

Calibration curve for DPM consists of different concentration of standard DPM solution ranging from 6 – 22 µg/ml. The solution were produced by withdrawing 1.2 ml , 2ml, 2.8ml, 3.6ml, and 4.4ml of working standard solution of DPM (50µg/ml) into series of 10 ml volumetric flask and the volume was adjusted to mark with distilled water. The absorbance of each measured at 223 nm against distilled water as blank. The first order derivative spectra of all these solutions were obtained by transformation of zero order spectra of every solution. dA/dλ absorbance at 207.5 nm (Zero crossing point of LNG) was computed and the plot of dA/dλ absorbance vs. Concentration was plotted and regression equation was obtained.

2. Calibration curve for LNG (3 -11 µg/ml)

Calibration curve for LNG consists of different concentration of standard LNG solution ranging from 3-11 µg/ml. The solution were produced by withdrawing 0.3ml,0.5ml,0.7ml,0.9ml and 1.1ml of working standard solution of LNG (25 µg/ml) into series of 10 ml volumetric flask and the volume was adjusted to mark with distilled water. The absorbance of each measured at 227 nm against distilled water as blank. The first order derivative spectra of all these solutions were obtained by transformation of zero order spectra of every solution. dA/dλ absorbance at 223.0 nm (Zero crossing point of DPM) was computed and the plot of dA/dλ absorbance vs. Concentration was plotted and regression equation was obtained.

Validation of Proposed Method

Parameters to be considered for the validation of method are:

1) Linearity

The linearity response was determination by analyzing 5 independent levels of calibration curve in the range of 6 -22 µg/ml for DPM, 3 -11 µg/ml for LNG (n=5).In order to calculate the correlation coefficient and regression line equation for DPM and LNG, the calibration curve of dA/dλ absorbance vs. concentration was computed.

2) Precision

A. Repeatability

Aliquots of 2ml of working stock solution of DPM (50 µg/ml), 0.5ml of working stock solution of LNG (25 µg/ml) were taken in two separate 10ml of volumetric flask and volume was made up to mark with distilled water to give a solution containing 10 µg/ml, 5 µg/ml of DPM and LNG solution was analyzed six times (n=6) and % RSD was calculated.

B. Intraday Precision

Aliquots of 2ml, 2.8ml, 3.6ml of working solution of DPM (50 µg/ml) were taken into series of 10 ml volumetric flask. Aliquots of 0.5ml, 0.7ml, 0.9ml of working stock solution of LNG (50 µg/ml) were taken in series of 10 ml volumetric flask. Using the distilled water, volume was made up to mark with distilled water to give a solution containing 10, 14, 18 µg/ml of DPM and 5, 7, 9 µg/ml of LNG solution were analyzed for three times (n=3) on same day within short interval of time and % RSD was calculated.

C. Interday Precision (n=3)

Aliquots of 2ml, 2.8ml, 3.6ml of working solution of DPM (50 µg/ml) were taken into series of 10 ml volumetric flask. Aliquots of 0.5ml, 0.7ml, 0.9ml of working stock solution of LNG (50 µg/ml) were taken in series of 10 ml volumetric flask. Using

distilled water, volume was made up to mark with distilled water give a solution containing 10, 14, 18 µg/ml of DPM and 5, 7, 9 µg/ml of LNG solution were analyzed for three times (n=3) on three different day and % RSD was calculated.

3) Accuracy (n=3)

Synthetic Mixture of 10 mg equivalent of powder was taken in to 10 ml volumetric flask. Distilled water was added and sonicated for 2- 3 mins and volume was made up mark with distilled water. Solution was filtered through Whatmann filter paper no.42 and first few ml of solution was discarded. Thus, resulting solution contains 1000 µg/ml of DPM and 1000 µg/ml of LNG.

From the above Solution, 1.0 ml was pipette out and transferred to 10 ml of volumetric flask and volume was made up to mark with distilled water in order to get 100 µg/ml solution. From the above Solution, 1.0 ml was pipette out and transferred to 10 ml of volumetric flask and volume was made up to mark with distilled water in order to get 10 µg/ml solution. Each solution was scanned from 200 – 400 nm against distilled water as a blank. Absorbance of solution was measured at selected wavelength for DPM and LNG. The amount of DPM and LNG was calculated at each level (80 %, 100 % and 120 %) and % recovery were calculated.

The percentage recovery was calculated from respective linearity calibration curve. Preparation of sample solution for DPM and LNG.

Synthetic mixture solution X: DPM (100 µg/ml) + LNG (50 µg/ml)

Solution Y: DPM (100 µg/ml)

Solution Z: LNG (100 µg/ml)

Table 1. Steps for Accuracy measurement for DPM

Sr.No.	Step 1	Step 2	Step 3	Total DPM conc. (µg/ml)
1.	Take 1 ml of Solution X	-	Make up volume to 10 ml with distilled water	10
2.	Take 1 ml of Solution X	Add 0.8 ml of solution Y	Make up volume to 10 ml with distilled water	18
3.	Take 1 ml of Solution X	Add 1.0 ml of solution Y	Make up volume to 10 ml with distilled water	20
4.	Take 1 ml of Solution X	Add 1.2 ml of solution Y	Make up volume to 10 ml with distilled water	22

Table 2. Steps for Accuracy measurement for LNG

Sr.No.	Step 1	Step 2	Step 3	Total LNG conc. (µg/ml)
1.	Take 0.5 ml of Solution X	-	Make up volume to 10 ml with distilled water	5
2.	Take 0.5 ml of Solution X	Add 0.4 ml of solution Z	Make up volume to 10 ml with distilled water	9
3.	Take 0.5 ml of Solution X	Add 0.5 ml of solution Z	Make up volume to 10 ml with distilled water	10
4.	Take 0.5 ml of Solution X	Add 0.6 ml of solution Z	Make up volume to 10 ml with distilled water	11

4) LOD and LOQ

The set of 5 calibration curve that were used to assess the method's linearity were used to estimate the LOD (Limit of Detection). The following formula was used to determine the LOD:

LOD = 3.3 × S.D. / Slope

Where,

S.D. = Standard deviation of the Y – intercept of 5 calibration curves

Slope = Mean slope of the 5 Calibration curve

The set of 5 calibration curve that were used to assess the method's linearity were used to estimate the LOQ (Limit of Quantitation).The following was used to determine the LOQ:
LOQ = 10 × S.D. / Slope
Where,
S.D. = Standard deviation of the Y – intercept of 5 calibration curves
Slope = Mean slope of the 5 Calibration curve

RESULT AND DISCUSSION

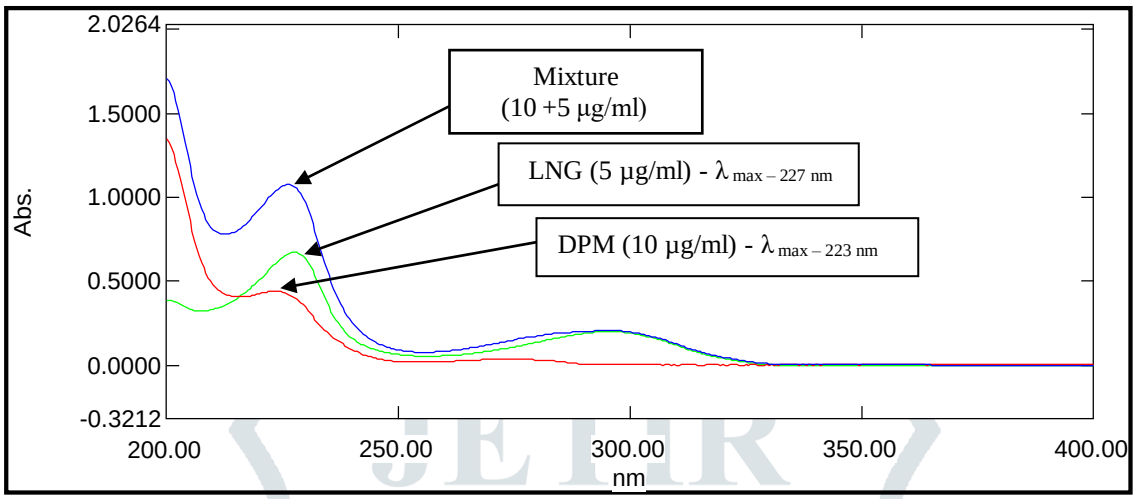

Figure 3: Overlain spectra of DPM (10 µg/ml), LNG (5 µg/ml) and Mixture (10 +5 µg/ml)

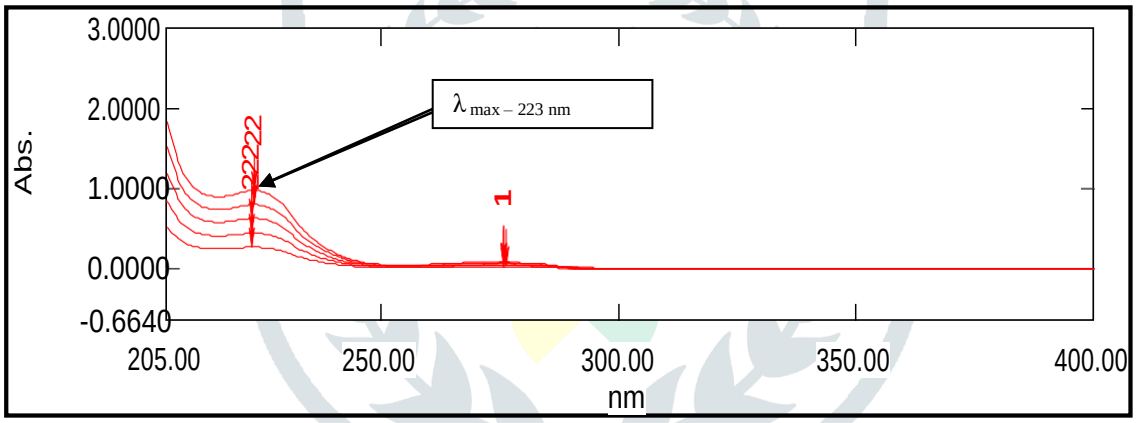

Figure 4: Zero order spectra of DPM (6 – 22 µg/ml)

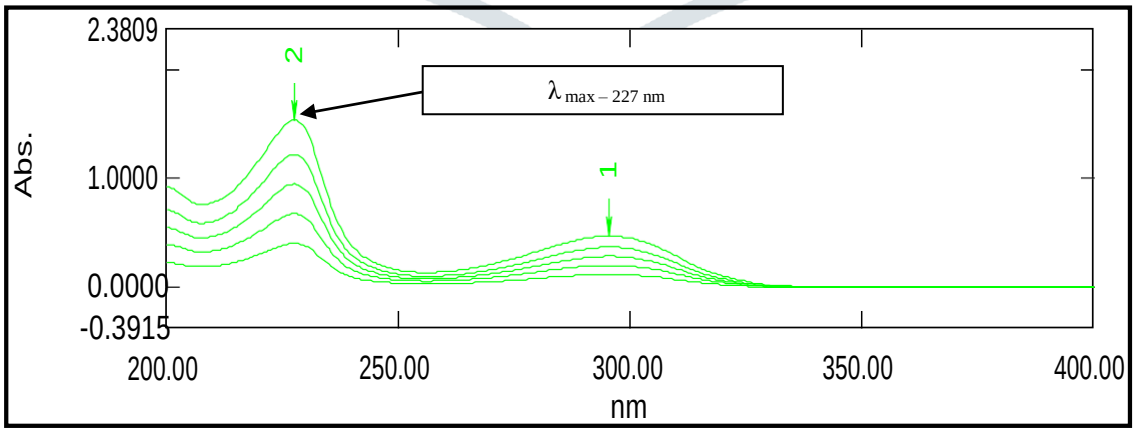

Figure 5: Zero order spectra of LNG (3 -11 µg/ml)

Selection of Wavelength for estimation of DPM and LNG
Standard spectra of DPM and LNG were scanned between 200 and 400 nm with distilled water used as references to determine the wavelength for estimation. For the estimation of LNG and DPM were found at 223.0 nm and 207.5 nm. Since these wavelengths produce adequate absorbance. Overlay first order spectra of DPM and LNG are presented in figure

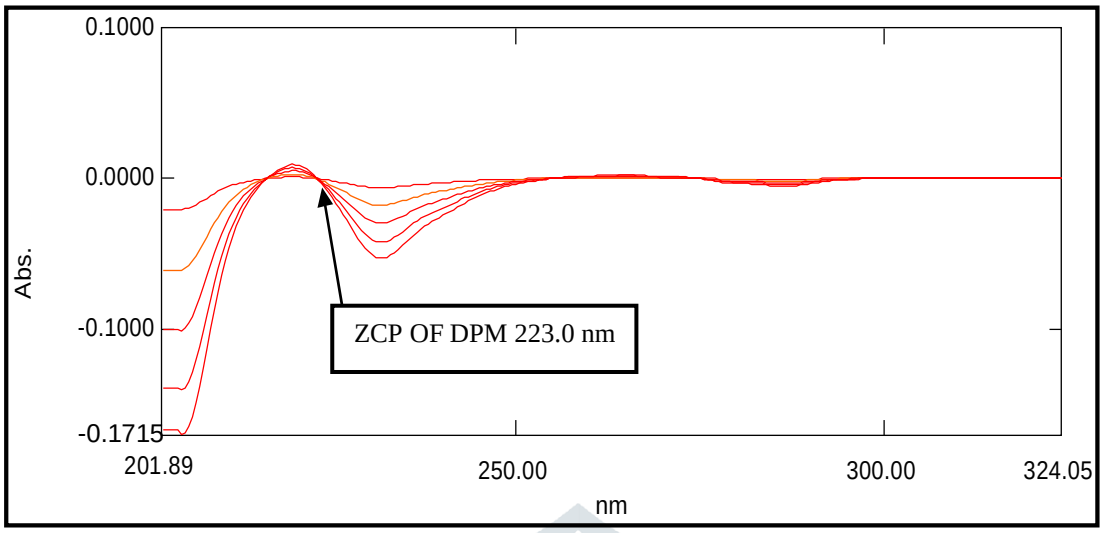

Figure 6: Overlain first order spectra of DPM (6 – 22 µg/ml)

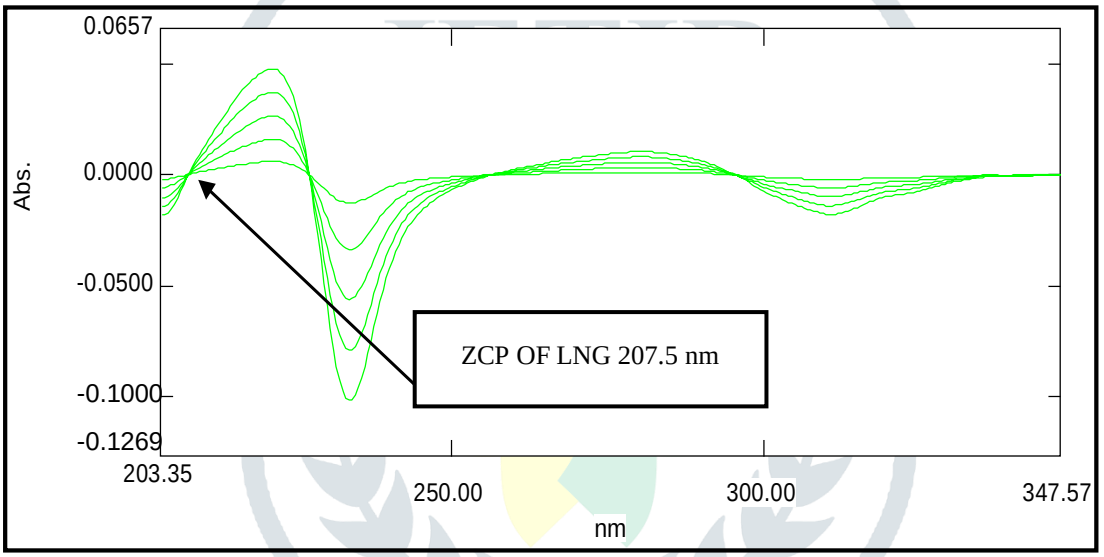

Figure 7: Overlain First order spectra of LNG (3 – 11 µg/ml)

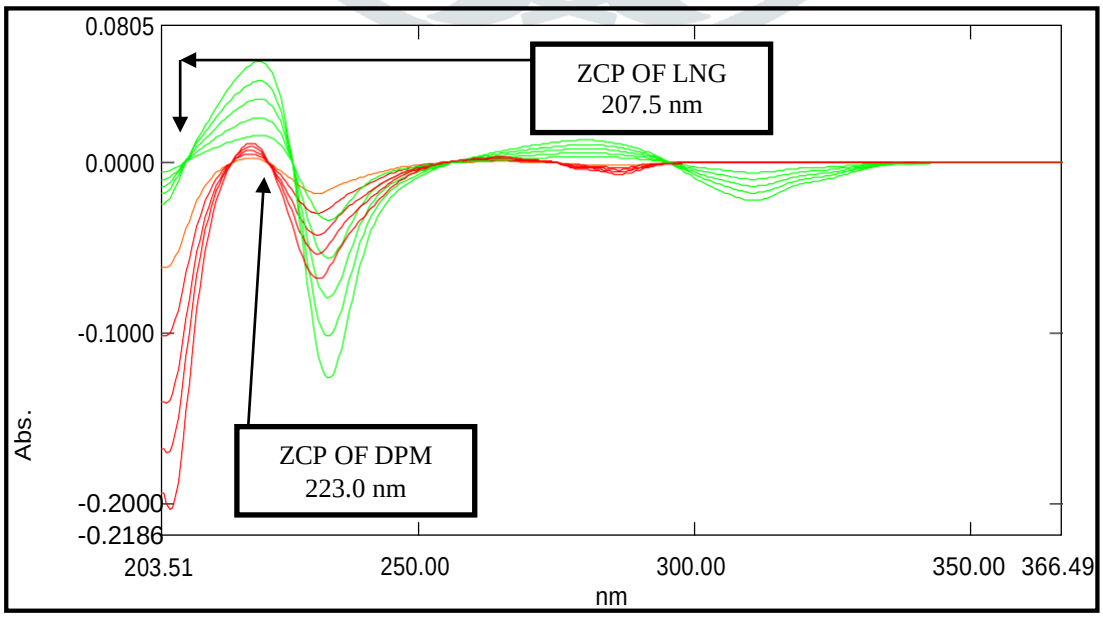

Figure 8: Overlain first order spectra of DPM (6 -22 µg/ml) and LNG (3 -11 µg/ml)

Result and Discussion

1) Linearity

The linearity range for DPM has been found to be in the range of 6 - 22 µg/ml and for LNG has been found to be in range of 3 - 11 µg/ml. Linearity data for DPM at 207.5 nm and LNG at 223 nm.

Table 3: Linearity data for DPM at 207.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=5)	% RSD
1.	6	0.0433 ± 0.00013	0.3005
2.	10	0.0722 ± 0.00019	0.2661
3.	14	0.1014 ± 0.00021	0.2044
4.	18	0.1265 ± 0.00020	0.1619
5.	22	0.1605 ± 0.00031	0.1939

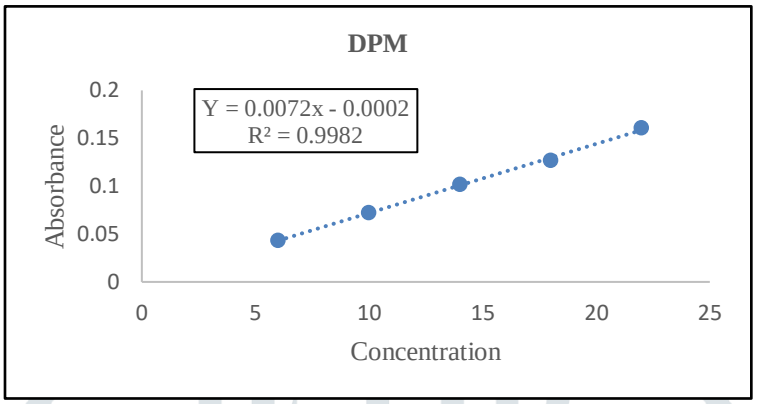

Figure 9: Calibration curve for DPM at 207.5 nm

Table 4: Linearity data for LNG at 223 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=5)	% RSD
1.	3	0.0156 ± 0.00013	0.8347
2.	5	0.0254 ± 0.00019	0.7549
3.	7	0.0354 ± 0.00023	0.6492
4.	9	0.0453 ± 0.00027	0.5959
5.	11	0.0556 ± 0.00028	0.5087

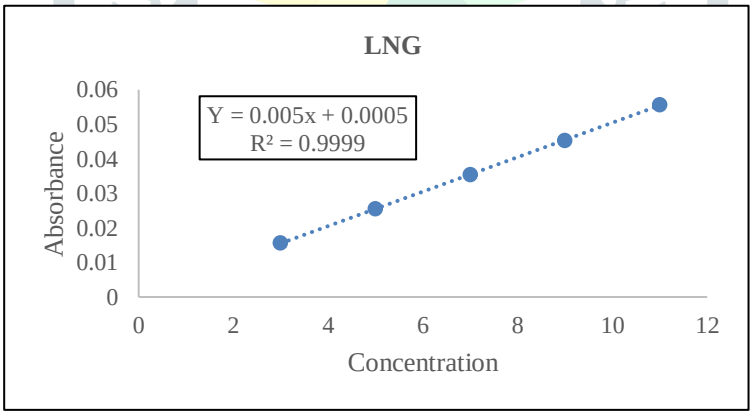

Figure 10: Calibration curve for LNG at 223 nm

Table 5: Regression line equation, Regression, coefficient and correlation coefficient for DPM and LNG

Sr.No.	Drugs	Regression line equation	Regression Coefficient (R^2)	Correlation coefficient (r)
1.	DPM	Y = 0.0072x - 0.0002	0.9982	0.9991
2.	LNG	Y = 0.005x + 0.0005	0.9999	0.9999

2) Precision

A. Repeatability

The data for repeatability of DPM and LNG at 207.5 nm and 223 nm respectively are depicted in table

Table 6: Repeatability data for DPM and LNG

Sr.No.	Drugs	Concentration (µg/ml)	Mean Abs ± S.D. (n=6)	% RSD
1.	DPM	10	0.0722 ± 0.00020	0.2713
2.	LNG	5	0.0252 ± 0.00019	0.7676

B Intraday Precision

The data for intraday precision for DPM at 207.5 nm and LNG at 223 nm are depicted in table

Table 7: Intraday Precision data for DPM at 207.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	10	0.0728 ± 0.00037	0.5191
2.	14	0.1013 ± 0.00042	0.4169
3.	18	0.1263 ± 0.00040	0.3167

Table 8: Intraday Precision data for LNG at 223 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	5	0.0255 ± 0.00023	0.9061
2.	7	0.0356 ± 0.00027	0.7603
3.	9	0.0455 ± 0.00031	0.6873

C. Interday Precision

The data for Interday precision for DPM at 207.5 nm and LNG at 223 nm are depicted in table

Table 9: Interday Precision data for DPM at 207.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	10	0.0712 ± 0.00065	0.9209
2.	14	0.1006 ± 0.00083	0.8333
3.	18	0.1254 ± 0.00087	0.6979

Table 10: Interday Precision data for LNG at 223 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	5	0.0239 ± 0.00025	1.0515
2.	7	0.0345 ± 0.00034	0.9721
3.	9	0.0446 ± 0.00036	0.8128

3) Accuracy

Accuracy of proposed method was assured by performing recovery study from synthetic mixture at three levels by standard addition method. Percentage recovery of DPM and LNG 207.5 nm and 223 nm were obtained respectively. The result is shown in table. Recovery was found to be in the limit of 98 – 102 %.

Table 11: Determination of Accuracy of DPM and LNG

Drugs	Level	Amount of Sample (µg/ml)	Amount of Std. Spiked (µg/ml)	Total Amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
DPM	0 %	10	0	10	10.01	100.13
	80 %	10	8	18	17.99	99.94
	100 %	10	10	20	20.03	100.15
	120 %	10	12	22	21.84	99.31
LNG	0 %	5	0	5	5.02	100.04
	80 %	5	4	9	9.05	100.55
	100 %	5	5	10	10.01	100.01
	120 %	5	6	11	11.04	100.37

4) Analysis of Synthetic mixture

Analyzing the synthetic mixture was used the method's suitability. The result are shown in table.

Table 12: Determination of Assay of DPM and LNG

Synthetic Mixture	Concentration (µg/ml)		Amount obtain mean ± S.D. (µg/ml)		% Assay of DPM ± S.D. (n=3)	%Assay of LNG ± S.D. (n=3)
	DPM	LNG	DPM	LNG		
	10	5	9.95 ± 0.0006	4.98 ± 0.0004	99.56 ± 0.025	99.64 ± 0.045

Table 13: Summary of First Order Derivative Method

Parameters	DPM	LNG
Zero Crossing Point	207.5 nm	223 nm
Linearity (µg/ml)	6 – 22 µg/ml	5 – 11 µg/ml
Regression Equation (Y = mx +c)	Y = 0.0072x - 0.0002	Y = 0.005x + 0.0005
Regression Coefficient (R^2)	0.9982	0.9999
Correlation Coefficient (r)	0.9991	0.9999
Repeatability (% R.S.D.) (n=6)	0.2713	0.7676
Intraday Precision (% R.S.D.)(n=3)	0.3167 – 0.5191	0.6873 – 0.9061
Interday Precision (% R.S.D.) (n=3)	0.6979 – 0.9209	0.8128 – 1.0515
LOD (µg/ml)	0.1288	0.1061
LOQ (µg/ml)	0.3904	0.3217
% Recovery (n=3)	99.31 - 100.15	100.01 - 100.55
Assay (%) ± S.D. (n=3)	99.56 ± 0.025	99.64 ± 0.045

CONCLUSION

Based on the result obtained from the analysis of DPM and LNG in their synthetic mixture using first order derivative method, it can be concluded that the method has linearity in the range of 6 – 22 µg/ml for DPM and 3 – 11 µg/ml for LNG. The regression coefficient (R^2) was found to be 0.9982 and 0.9999 for DPM and LNG respectively. The correlation coefficient (r) was found to be 0.9991 and 0.9999 for DPM and LNG at 207.5nm (ZCP of LNG) 223nm (ZCP of DPM) respectively.

Limit of Detection for DPM and LNG were found to be 0.1288 µg/ml and 0.1061 µg/ml and limit of quantification for DPM and LNG were found to be 0.3904 µg/ml and 0.3217 µg/ml respectively. The % assay was found to be 99.56 %w/w and 99.64 %w/w for DPM and LNG respectively. Further % RSD was found to be 2 % for precision, intraday and Interday study.

ACKNOWLEDGMENTS

The authors are thankful CTX Lifescience Pvt. Ltd Surat and Mehta API Pvt. Ltd Maharashtra for providing gift samples and author are also thankful to ROFEL Shri G.M. Bilakhia College of pharmacy for providing necessary equipment, facility and chemicals to complete research work.

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