International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 29-40 www.ijprajournal.com ISSN: 2456-4494

Absorbance Ratio Spectroscopic Method Development and Validation for Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Linagliptin

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Date of Submission: 27-06-2023 Date of Acceptance: 08-07-2023

ABSTRACT: Dapagliflozin Propanediol Monohydrate (DPM) and Linagliptin (LNG) 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. Absorbance ratio method were developed and validated for quantitative determination of Dapagliflozin Propanediol Monohydrate and Linagliptin in synthetic mixture. Absorbance ratio method were developed and validated by using distilled water and scan between 200 – 400 nm. All 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 Absorbance ratio method.

KEYWORDS: Dapagliflozin Propanediol Monohydrate, Linagliptin, Absorbance Ratio, Method validation.

I. INTRODUCTION

Abnormal insulin synthesis and increased blood glucose levels are characteristic of the metabolic disorder known as diabetes mellitus. When there is an absolute or relative absence of insulin, it is a disorder that causes irregularities in the metabolism and the blood vessels. People with diabetes mellitus usually refer to it as "sugar." It is the most common endocrine disorder and often shows up as an absence, deficiency, or impaired action of insulin. [1, 2, 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: The sodium-glucose cotransporter 2(SGLT2) which is largely found in the proximal tubule of the nephron is inhibited by dapagliflozin. Since 90% of resorption of glucose in the kidney is facilitated by SGLT2, its blockage enables glucose to be eliminated in the urine. Patients with type 2 diabetes can achieve better glucose control and possibly lose weight due to this excretion. **Adverse effect**: Felling Dizzy, Skin rash, Back pain.[5,6]

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.

Adverse effect: Itching Skin rash, hard skin blister, swelling on the face, throat, hands, legs, feet, joint pain. [7, 8]

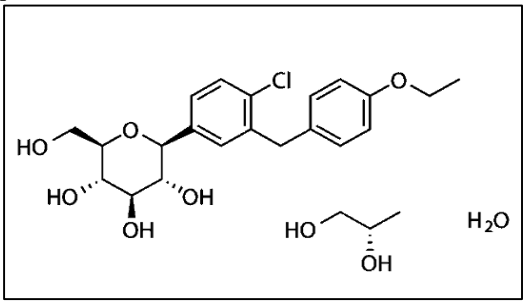

Figure 1.Chemical Structure of DPM

International Journal of Pharmaceutical research and Applications
Volume 8, Issue 4, Jul.-Aug. 2023, pp: 29-40 www.ijprajournal.com ISSN: 2456-4494

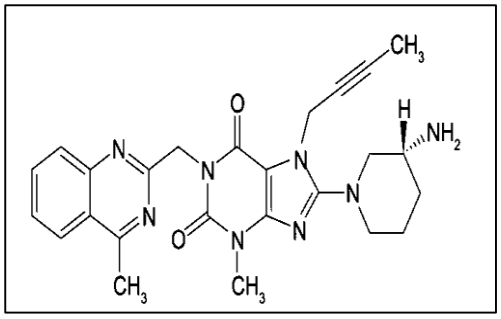

Figure 2.Chemical Structure of LNG

ANALYTICAL METHOD DEVELOPMENT

New methods are being created for the evaluation of the novel product when there are no alternative procedures.

These techniques have been thoroughly improved, and alternative approaches have been prepared and put into use to replace the current procedure with comparative laboratory data, with all of its benefits and drawbacks. [9]

Need for Method Development

Data on impurities, stability, bioavailability, and the impact of manufacturing parameters are generated during the drug's manufacture and development in order to support analytical techniques and confirm the safety of the process.

These new techniques for drug analysis were created for the following reasons:

1) When Pharmacopoeia fail to identify medication as being available on market.

2) There isn't a drug combination that is available in pharmacopoeias. [9]

3) When there are no analytical methods available for the drug's formulation or when such procedures are interfered with by formulation excipients.

4) The use of expensive reagents and solvents may be required as part of an analytical process, in addition to extraction and separation steps.

Introduction of UV-visible spectroscopy [10]

Ultraviolet-visible spectrophotometry is the most widely used spectroscopic technique in pharmaceutical assessment. The amount of ultraviolet (190-380 nm) or visible (380-800 nm) radiation absorbed by a substance in a solution is measured in UV-visible spectroscopy.

Absorbance Ratio Method (Q – Absorbance method) [11, 12, 13]

A modified form of the simultaneous equation approach called the Q – absorbance ratio method depends on the ratio of substances absorbance at two different wavelengths one is the greatest wavelength of any one of the two components and the Iso –Absorptive point's wavelength is the other.

This approach can be used for drug combinations that follow beer's law and have an absorbance ratio at two wavelength is a constant value which is independent of concentration and path length. This constant is termed as Q – Value or Hafner's Quotient.

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. [14]

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

II. MATERIALS 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, distilled 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.

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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 2ml from working solution of DPM (50 µg/ml) and 0.5ml from working solution of LNG (25 µg/ml) pipetted out into two separate 10 ml volumetric flask. Finally make volume up to mark with distilled water. This produced two analytical solution which is DPM 10 µg/ml and LNG 5 µg/ml. Each solution was scanned in range between 200 - 400 nm against Distilled water as a blank. Overlaying the both spectrum, it is observed that Iso – absorptive point was found at 214.5 nm and LNG had maximum absorbance at 227 nm. Therefore, based observation, wavelength of equal absorptivity of DPM and LNG was selected is 214.5 nm (λ2) and λmax of LNG was selected i.e., 227 nm (λ1).

PREPARATION OF CALIBRATION CURVE

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

Calibration curve for DPM consist concentration of standard solution of DPM ranging from 6 - 22 µg/ml. The solution was prepared by pipetting out 1.2ml, 2ml, 2.8ml, 3.6ml and 4.4ml of standard working solution of DPM (50 µg/ml) into series of 10 ml volumetric flask and the volume was made mark with distilled water to give 6- 22 µg/ml respectively. Each solution is scanned between 200 – 400 nm, using distilled water as blank and the spectrum was recorded.

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

Calibration curve for LNG consist concentration of standard solution of LNG ranging from 3 - 11 µg/ml. The solution was prepared by pipetting out 0.3ml, 0.5ml , 0.7ml, 0.9ml , 1.1ml of standard working solution of LNG (100 µg/ml) into series of 10 ml volumetric flask and the volume was made mark with distilled water to give 3 -11 µg/ml respectively. Each solution is scanned between 200 – 400 nm, using distilled water as blank and the spectrum was recorded.

VALIDATION OF PROPOSED METHOD

Parameters to be considered for the validation of method are:

1) Linearity

The linearity response was determined by evaluating 5 different levels of calibration curve in range of 6 - 22 µg/ml for DPM and 3 – 11 µg/ml for LNG (n=5).The calibration curve of absorbance vs. concentration was generated and correlation coefficient and regression line equation of DPM and LNG were Calculated.

2) Precision

A. Repeatability (n=6)

Aliquots of 2ml of working stock solution of DPM **(**50 µg/ml), 0.5ml of working stock solution of LNG (100 µg/ml) were taken into two separate 10 ml volumetric flask and volume was made mark with distilled water to give a solution 10 µg/ml, 5 µg/ml of DPM and LNG solution was analyzed with six times (n=6) and % RSD was calculated.

B. Intraday 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 (100 µg/ml) were taken in series of 10 ml volumetric flask. By using distilled water, volume was made mark to give a solution containing 10, 14, 18 µg/ml of DPM and 5, 7, 9 µg/ml of LNG solution were analyzed with 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 (100 µg/ml) were taken in series of 10 ml volumetric flask. By using distilled water, volume was made mark to give a solution containing 10, 14, 18 µg/ml of DPM and 5, 7, 9 µg/ml of LNG solution were analysed

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with three times (n=3) on three different day and % RSD was calculated.

3) Accuracy (n=3)

Accuracy % recovery was studied by addition of standard drug solution at three different levels of 80,100 and 120 % to pre - analysed solution. 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 01: 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 02: 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

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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

III. RESULT AND DISCUSSION

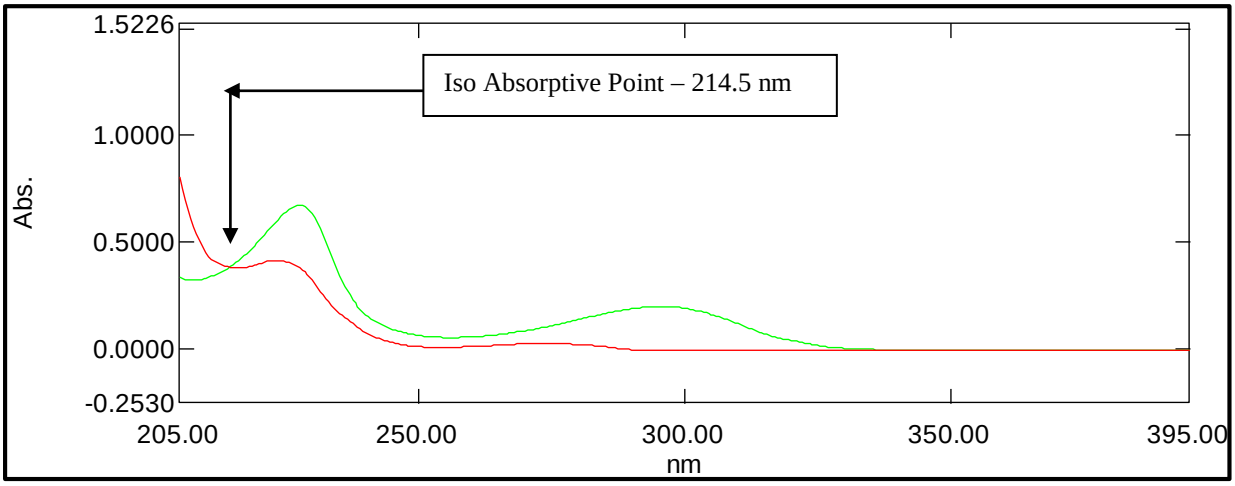

Fig 03: Overlain spectra of DPM (10 µg/ml, LNG (5 µg/ml)

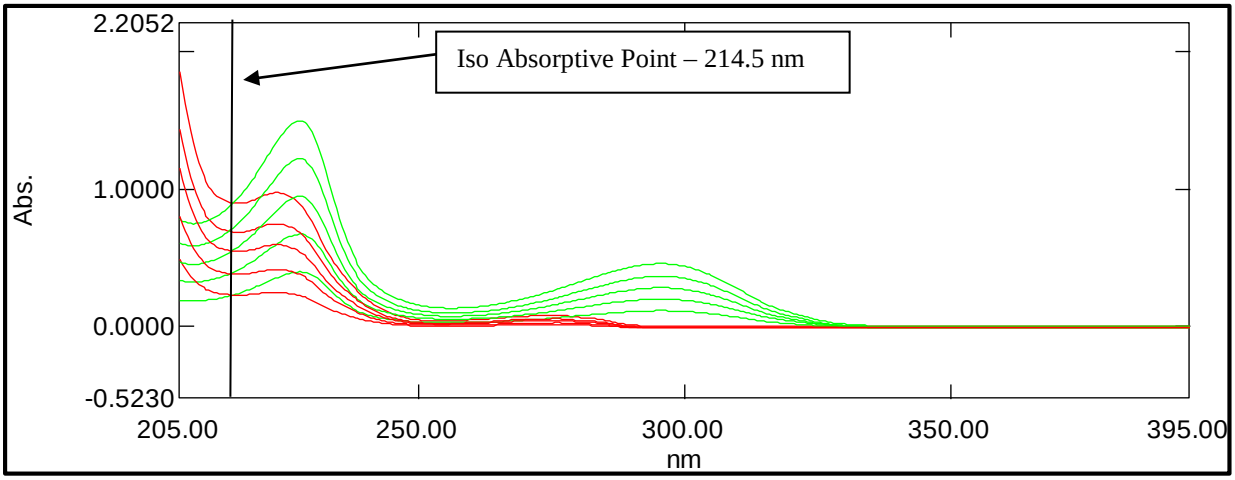

Fig 04: Overlain spectra of DPM (6 - 22 µg/ml) and LNG (3 - 11µg/ml)

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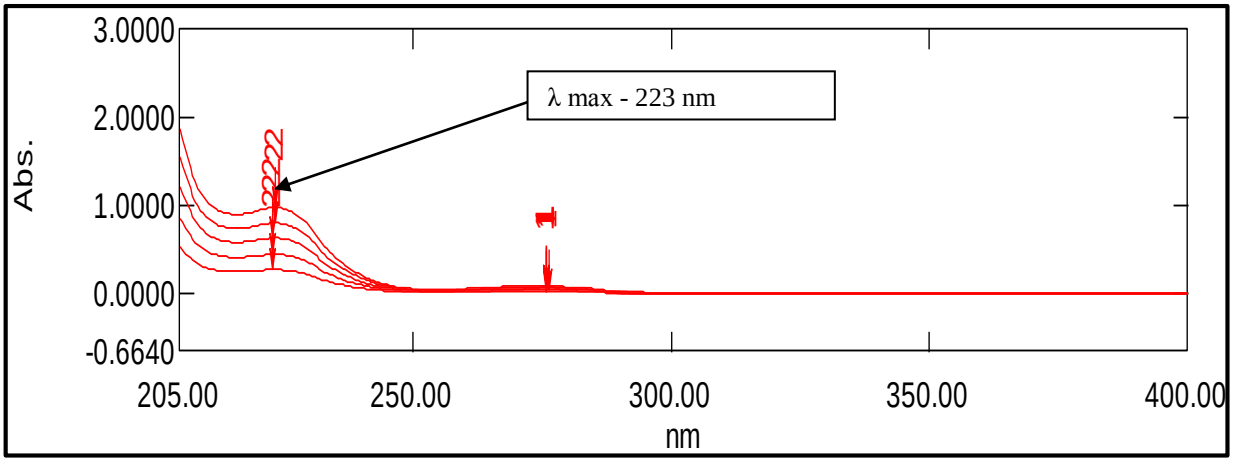

Fig 05: Overlain Spectra of DPM (6 - 22 µg/ml)

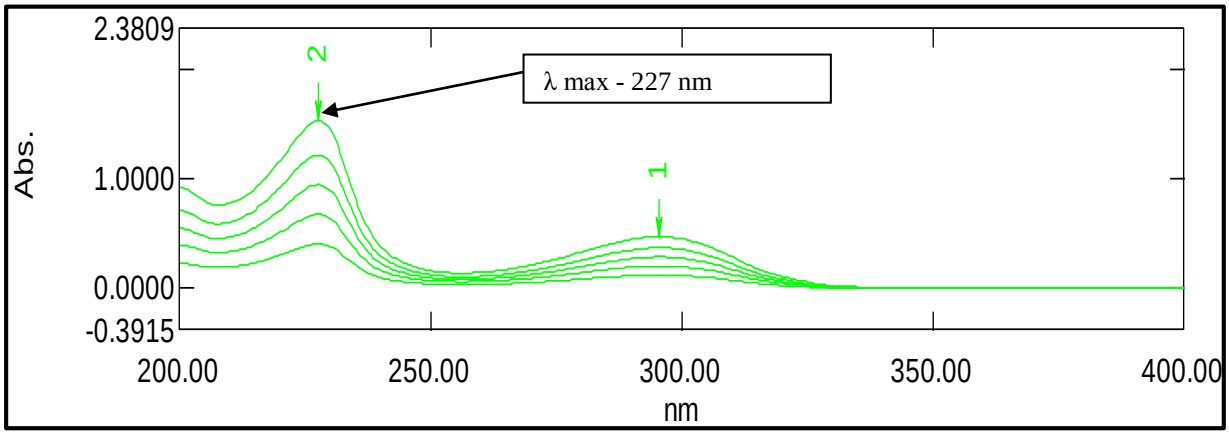

Fig 06: Overlain Spectra of LNG (3 - 11 µg/ml)

Validation of Proposed UV Method

1) Linearity

The Linearity range for DPM and LNG was found to be in the range of 6 – 22 µg/ml and 3 – 11 µg/ml.

Table 03: Linearity for DPM at 214.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=5)	% RSD
1.	6	0.2249 ± 0.0009	0.3971
2.	10	0.3820 ± 0.0011	0.2868
3.	14	0.5474 ± 0.0013	0.2430
4.	18	0.6916 ± 0.0010	0.1508
5.	22	0.8780 ± 0.0015	0.1755

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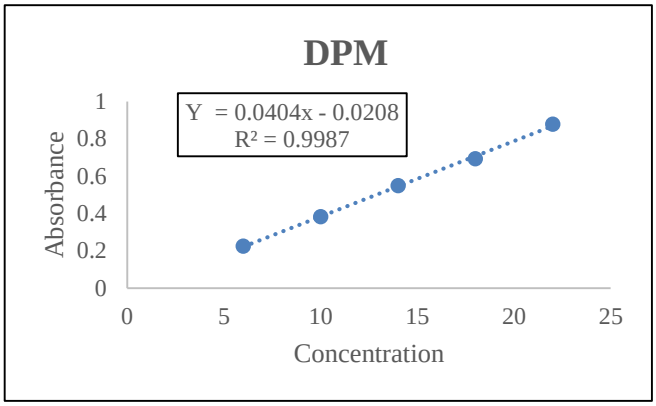

Fig 07: Calibration curve for DPM at 214.5 nm

Table 04: Linearity for LNG at 214.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=5)	% RSD
1.	3	0.2249± 0.0009	0.3971
2.	5	0.3820 ± 0.0011	0.2868
3.	7	0.5474 ± 0.0013	0.2432
4.	9	0.6915 ± 0.0010	0.1508
5.	11	0.8780 ± 0.0015	0.1755

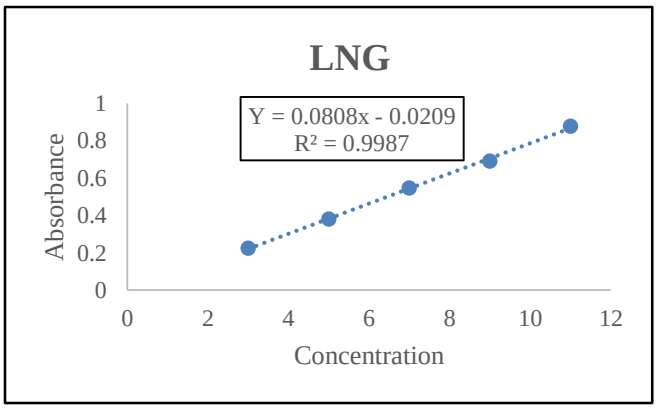

Fig 08: Calibration curve for LNG at 214.5 nm

Table 05: Linearity for DPM at 227 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=5)	% RSD
1.	6	0.2316 ± 0.0010	0.4355
2.	10	0.3875 ± 0.0012	0.3158
3.	14	0.5551 ± 0.0012	0.2236
4.	18	0.6979 ± 0.0017	0.2507
5.	22	0.9015 ± 0.0011	0.1167

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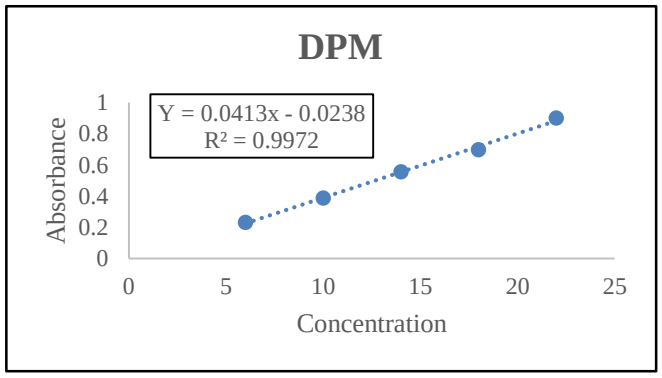

Fig 09: Calibration curve for DPM at 227 nm

Table 06: Linearity for LNG at 227 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=5)	% RSD
1.	3	0.3976 ± 0.0017	0.4161
2.	5	0.6721 ± 0.0015	0.2337
3.	7	0.9439 ± 0.0018	0.1915
4.	9	1.218 ± 0.0018	0.1500
5.	11	1.4739 ± 0.0027	0.1875

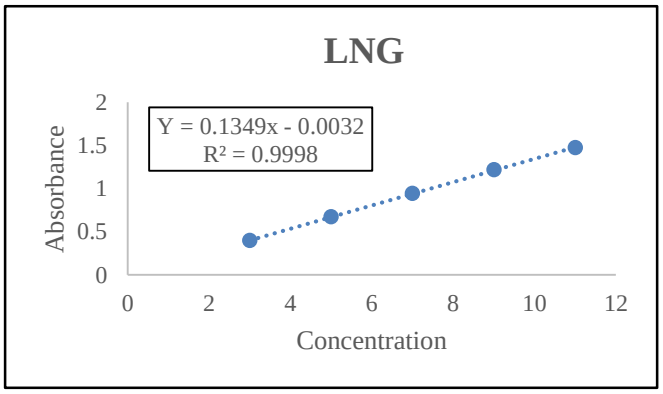

Fig 10: Calibration curve for LNG at 227 nm

Table 07: Correlation coefficient, regression coefficient, and regression line equation for DPM AND LNG

Sr.No.	Drugs	Regression line equation	Regression Coefficient (R^2)	Correlation coefficient (r)
1.	DPM (214.5 nm)	Y = 0.0404x - 0.0207	0.9987	0.9993
2.	LNG (214.5 nm)	Y = 0.0808x - 0.0207	0.9987	0.9993

Table 08: Correlation coefficient, regression coefficient, and regression line equation for DPM AND LNG

Sr.No.	Drugs	Regression line equation	Regression Coefficient(R^2)	Correlation coefficient (r)
1.	DPM (227 nm)	Y = 0.0413x - 0.0238	0.9972	0.9986
2.	LNG (227 nm)	Y = 0.1349x - 0.0032	0.9998	0.9999

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2) Precision

A. Repeatability

The data for repeatability for DPM and LNG at 214.5 nm and 227 nm. The result are shown in table

Table 09: Repeatability of DPM and LNG at 214.5 nm

Sr.No.	Drugs	Concentration (µg/ml)	Mean Abs ± S.D. (n=6)	% RSD
1.	DPM	10	0.3814 ± 0.0011	0.2918
2.	LNG	5	0.3814 ± 0.0011	0.2917

Table 10: Repeatability of DPM and LNG at 227 nm

Sr.No.	Drugs	Concentration (µg/ml)	Mean Abs ± S.D. (n=6)	% RSD
1.	DPM	10	0.3877 ± 0.0012	0.3238
2.	LNG	5	0.6720 ± 0.0016	0.2457

B. Intraday Precision

The data for intraday precision for DPM and LNG at 214.5 nm and 227 nm. The result are shown in table

Table 11: Intraday Precision of DPM at 214.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	10	0.3814 ± 0.0023	0.6054
2.	14	0.5464 ± 0.0027	0.5022
3.	18	0.6926 ± 0.0028	0.4097

Table 12: Intraday Precision of LNG at 214.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	5	0.3814 ± 0.0023	0.6053
2.	7	0.5464 ± 0.0027	0.5023
3.	9	0.6927 ± 0.0028	0.4095

Table 13: Intraday precession of DPM at 227 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	10	0.3868 ± 0.0024	0.6348
2.	14	0.5560 ± 0.0027	0.4895
3.	18	0.6977 ± 0.0029	0.4234

Table 14: Intraday precession of LNG at 227 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	5	0.6728 ± 0.0044	0.6568
2.	7	0.9451 ± 0.0046	0.4875
3.	9	1.2168 ± 0.0050	0.4131

C. Interday Precision

The data for Interday precision for DPM and LNG at 214.5 nm and 227 nm. The result are shown in tab

Table 15: Interday Precision of DPM at 214.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	10	0.3809 ± 0.0035	0.9413
2.	14	0.5452 ± 0.0041	0.7557
3.	18	0.6922 ± 0.0047	0.6760

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Table 16: Interday Precision of LNG at 214.5 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	5	0.3809 ± 0.0035	0.9411
2.	7	0.5452 ± 0.0041	0.7558
3.	9	0.6922 ± 0.0047	0.6762

Table 17: Interday Precision of DPM at 227 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	10	0.3861 ± 0.0038	0.9842
2.	14	0.5542 ± 0.0048	0.8699
3.	18	0.6953 ± 0.0049	0.7079

Table 18: Interday Precision of LNG at 227 nm

Sr.No.	Concentration (µg/ml)	Mean Abs ± S.D. (n=3)	% RSD
1.	5	0.6779 ± 0.0069	1.0135
2.	7	0.9457 ± 0.0080	0.8415
3.	9	1.2099 ± 0.0089	0.7315

3) Accuracy

Accuracy of proposed method was assured by performing recovery study from synthetic mixture at three levels using the standard addition method. The percentage recovery for DPM and LNG 214.5 nm and 227 nm were obtained respectively. The result is depicted in below table. Recovery was found to be in limit of 98 – 102 %.

Table 19: Determination of Accuracy of DPM and LNG at 214.5 nm

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	9.95	99.50
	80 %	10	8	18	18.15	100.84
	100 %	10	10	20	19.90	99.51
	120 %	10	12	22	21.94	99.76
LNG	0 %	5	0	5	4.99	99.94
	80 %	5	4	9	9.01	100.11
	100 %	5	5	10	9.98	99.84
	120 %	5	6	11	11.01	100.09

Table 20: Determination of Accuracy of DPM and LNG at 227 nm

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	9.96	99.60
	80 %	10	8	18	18.09	100.51
	100 %	10	10	20	19.91	99.55
	120 %	10	12	22	21.95	99.77
LNG	0 %	5	0	5	4.99	99.92
	80 %	5	4	9	9.01	100.11
	100 %	5	5	10	9.98	99.88
	120 %	5	6	11	10.99	99.97

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4) Analysis of Synthetic mixture: Analyzing the synthetic mixture was used method's suitability. The outcomes are show in table

Table 21: 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

IV. CONCLUSION

Based on result, obtained from the analysis of DPM and LNG in their synthetic mixture using Absorbance Ratio 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 at both wavelength 214.5nm and 227 nm 0.9987 and 0.9972 for DPM and 0.9987 and 0.9998 for LNG respectively and correlation coefficient (r) was found to be 0.9993 and 0.9986 for DPM and 0.9993 and 0.9999 for LNG at both wavelength 214.5 nm and 227 nm respectively. Limit of detection for DPM and LNG were found to be at 214.5 nm 0.1190 µg/ml and 0.1020 µg/ml and at 227 nm 0.1545 µg/ml and 0.1014 µg/ml. Limit of quantitation for DPM and LNG were found to be at 214.5 nm 0.3606 µg/ml and 0.3093 µg/ml and at 227 nm 0.4682 µg/ml and 0.3074 µg/ml respectively. The % assay was found to be 99.71 % and 99.15 % for DPM and LNG respectively. Further % R.S.D. was found to be less than 2 % for precision, intraday and Interday study.

ACKNOWLEDGEMENT

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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