Research Article

RP – HPLC Method Development and Validation for Simultaneous Estimation of Dapagliflozin Propanediol Monohydrate and Linagliptin

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ABSTRACT

Dapagliflozin Propanediol Monohydrate (DPM) and Linagliptin (LNG) are used in 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 RP – HPLC. RP – HPLC method were developed and validated for quantitative determination of Dapagliflozin Propanediol Monohydrate and Linagliptin in synthetic mixture. RP – HPLC was developed and selection and optimization of mobile phase. The development of the RP – HPLC method included selecting and optimizing the mobile phase. By using shim – pack solar C18 (250mm × 4.6mm, 5 µm) separation was achieved. By utilizing Acetonitrile: Phosphate buffer (pH -3 adjusted with 0.1 % OPA) in ratio of (60:40 v/v) detection was carried out at 225 nm. RP – HPLC method was found to be linear over the concentration range of 6 – 22 µg/ml and 3 – 11 µg/ml for DPM and LNG respectively and Co – relation coefficient for DPM and LNG 0.9998 and 0.9943 respectively. All the methods were validated according to ICH guideline.

Keywords: RP – HPLC, Method validation, Dapagliflozin Propanediol Monohydrate, Linagliptin.

INTRODUCTION

A metabolic condition called Diabetes Mellitus is characterized by abnormal insulin production and elevated blood glucose levels. It is a condition that results in abnormalities in the metabolism and the blood vessels when there is an absolute or relative lack of insulin[1-3].

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. Patients with type 2 diabetes can achieve better glucose control and possibly lose weight due to this excretion. The IUPAC name of Dapagliflozin Propanediol Monohydrate is (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[4-5].

Linagliptin (LNG)

Mechanism of Action:

A reversible competitive DPP- 4 inhibitor. Linagliptin is an inhibitor. Enzyme Inhibition delays the breakdown of GLP – 1 a 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. The IUPAC name is 8-[(3*R*)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl) methyl] purine-2, 6-dione[6-7].

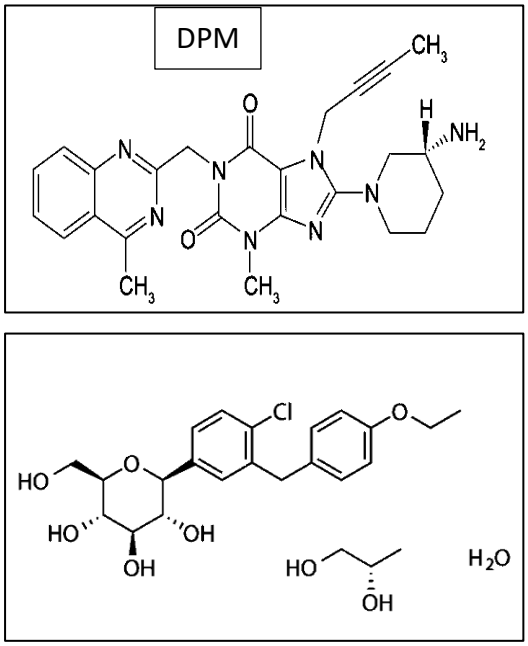

Figure 1: Structure of DPM and LNG

CDSCO given permission to Alkem Health Science to conduct Phase – III Clinical trial with the FDC of Dapagliflozin Propanediol Monohydrate 10 mg and Linagliptin 5 mg film coated tablet on 06 - 07-2022. After conducting Literature review on Analytical method development and validation for Dapagliflozin Propanediol Monohydrate and Linagliptin, it was found that till date no UV, HPLC, HPTLC method has been reported for this combination. After conducting extensive Literature review

on Development and Validation of Analytical methods for Dapagliflozin Propanediol Monohydrate and Linagliptin it was found that there were various UV, RP-HPLC, HPTLC,UPLC and stability indicating assay methods were reported for both drugs individually and in combination with other drugs.

Thus, there is a scope to develop simple, economic, accurate and precise Spectroscopic and RP – HPLC methods for the simultaneous estimation of Dapagliflozin Propanediol Monohydrate and Linagliptin in combination and validation of developed methods as per ICH guideline.

Introduction to HPLC Method

A basic HPLC principle is adsorption. The components of a mixture will move in accordance with their respective affinities against the stationary phase if they are placed into an HPLC column. Components with a higher affinity move more slowly whereas those with a lower affinity move more quickly away from the stationary phase. When no two components have the same affinity against the stationary phase, the components are separated[8-16].

It is the preferred approach for the analysis of:

- Non-volatile substance, as well as those that are thermally unstable and decomposable
- Ionic samples or substances with high polarity
- High molecular weight compounds,

MATERIALS AND METHODS

Apparatus and Instrument

Model - SHIMADZU LC – 2010 CHT

Column - Shim – pack solar C18 (250 mm× 4.6 mm, 5 µm)

Injector - Auto injector

Detector - UV detector

Software - LC Solution

Electronic analytical balance (REPTECH)

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

MATERIALS

Dapagliflozin Propanediol Monohydrate (DPM) was received as gift sample from CTX Lifescience Pvt. Ltd, Surat. Linagliptin (LNG) was received as a gift sample from Mehta API Pvt. Ltd, Maharashtra. All reagents and chemicals used were of analytical grade of Rankem, India.

Preparation of Stock Solution:

Standard Stock solution of Dapagliflozin Propanediol Monohydrate (DPM) was prepared by dissolving 10 mg of drug in 10 ml water and volume was made up to mark with water (1000 µg/ml).

Standard Stock solution of Linagliptin (LNG) was prepared by dissolving 10 mg of drug in 100 ml water and volume was made up to mark with water (100 µg/ml).

Preparation of Working Solution for DPM

Aliquot 5 ml from standard stock solution was pipetted out into 25 ml volumetric flask and volume was made up to mark with water (200 µg/ml). From this pipette out 12.5 ml from stock solution and diluted it up to 50 ml with water and volume was made up to mark with water (50 µg/ml).

Preparation of Dapagliflozin Propanediol Monohydrate (DPM) 10 µg/ml and Linagliptin (LNG) 5 µg/ml solution

Aliquot of 2 ml from working solution of DPM (50 µg/ml) and 0.5 ml from standard solution of LNG (100 µg/ml) were taken in to two volumetric flask and diluted it up to 10 ml with mobile phase to make final concentration of DPM (10 µg/ml) and LNG (5 µg/ml).

METHOD VALIDATION

The proposed method validated as per ICH guidelines.

Selection of Wavelength

Aliquots of 2.0 ml from working solution of DPM (50 µg/ml) and 0.5 ml from standard solution of LNG (100 µg/ml) were pipette out into two different 10 ml of volumetric flask and volume was made mark with water to get 10 µg/ml of DPM and 5 µg/ml of LNG. Each solution of DPM and LNG is scanned between 200 – 400 nm using UV – Visible spectrophotometer. The DPM and LNG overlay spectra were used to select the wavelength.

Preparation of Buffer

Dissolve about 3.4 gm of Potassium dihydrogen ortho phosphate in 500 ml and volume was made up to mark with water.

Linearity

The linearity response was evaluate by analyzing 5 different levels of concentration in the range of 6 - 22 µg/ml and 3 - 11 µg/ml for Dapagliflozin Propanediol Monohydrate and Linagliptin respectively.

Preparation of Calibration curves

1. Calibration curve for Dapagliflozin Propanediol Monohydrate (DPM)

Calibration curve for Dapagliflozin Propanediol Monohydrate consisted of five distinct levels of concentrations solution Ranging from 6 – 22 µg/ml. Calibration curve of peak area vs. Conc. was plotted and regression equation was found.

2. Calibration curve for Linagliptin (LNG)

Calibration curve for Linagliptin consisted of five distinct levels of concentrations solution Ranging from 3 – 11

µg/ml. Calibration curve of peak area vs. Conc. was Plotted and regression equation was found.

Precision

A. Repeatability

Repeatability of the developed method was determined by analyzing samples from the same batch 6 time with standard solution containing concentration 10 µg/ml for Dapagliflozin Propanediol Monohydrate and 5 µg/ml for Linagliptin and %R.S.D. was determined.

B. Intraday Precision

It was evaluated by analyzing samples from the same batch with three standard solution containing concentration of 10, 14, 18 µg/ml for Dapagliflozin Propanediol Monohydrate and 5, 7, 9 µg/ml for Linagliptin. % R.S.D. was determined.

C. Interday Precision

It was evaluated by analyzing samples from the same batch with three standard solution containing concentration 10, 14, 18 µg/ml for Dapagliflozin Propanediol Monohydrate and 5, 7, 9 µg/ml for Linagliptin. Solution were analyzed thrice (n=3) on the three different day and % R.S.D. was determined.

Accuracy

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 mobile phase 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.

LOD and LOQ

The set of five calibration curves that were used to determine 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 5 Calibration curve

The set of five calibration curves that were used to determine the method's linearity were used to estimate the LOQ (Limit of Quantitation). The following formula 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 5 Calibration curve

Robustness

The robustness of the method was established by making deliberate minor variation in the following method parameter.

Mobile phase Ratio: ± 2.0 ml

Flow rate: ± 0.2 units

System suitability studies

Evaluation of system suitability is done by analyzing five replicate of Dapagliflozin Propanediol Monohydrate (DPM) and Linagliptin (LNG) in a mixture at concentration of 10 µg/ml and 5 µg/ml respectively. Each replicate's column efficiency, peak asymmetry and resolution were calculated.

Specificity

Specificity involves quantitative detection of analyte in presence of those components that may be expected to be part of sample matrix. Specificity of developed method was demonstrated by spiking of Dapagliflozin Propanediol Monohydrate and Linagliptin in hypothetical placebo (i.e. might be expected to be present) and expressing that analytes peak were not obstructed from excipients.

Simultaneous estimation of DPM and LNG synthetic mixture

A synthetic mixture containing 10 mg equivalent of DPM and 5 mg of LNG were taken into 100 ml volumetric flask. Distilled water was added and sonicated for 2 – 3 minutes, and the volume was make up to the mark with water. Whatmann filter paper no.42 were used to filter the mixture. As a result, the final solution prepared was 100 µg/ml DPM and 50 µg/ml LNG. From above solution , 1.0 ml was pipette out and transferred in 10 ml volumetric flask and volume was made up to the mark with mobile phase to give a solution containing DPM (10 µg/ml) + LNG (5 µg/ml). Resulting solution containing DPM + LNG solution was used for assay. Estimation of DPM and LNG in synthetic mixture chromatogram is recorded and concentration of DPM and LNG was obtained by solving regression equation.

RESULTS AND DISCUSSION

Optimized Chromatographic Conditions:

1. Column: Shim - pack solar C18 (250 mm× 4.6 mm, 5 µm)

2. Mobile Phase: Acetonitrile: 0.05 M Phosphate Buffer pH – 3.0 (60: 40 v/v) Phosphate buffer pH – 3.0 adjusted with 0.1% OPA

3. Flow rate: 1.0 ml/min

4. Run time: 10 min

5. Volume of Injection: 10 µl

6. Detection Wavelength: 225 nm

7. Diluent: Mobile phase

8. Column Temperature: 40 – 65 °C

Chromatogram in Optimized Chromatographic Condition

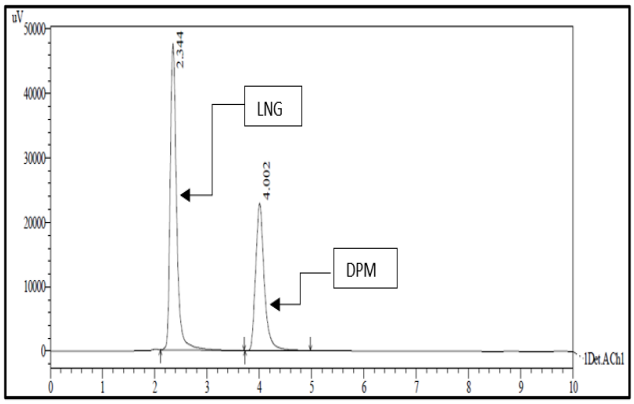

Figure 2: Chromatogram of DPM and LNG in Acetonitrile: Phosphate buffer (pH – 3.0), (60:40 v/v) pH – 3.0 adjusted with 0.1% OPA

METHOD VALIDATION

System Suitability

System suitability parameters shown in table 1.

Linearity

At five different concentrations levels for each drug linearity study was performed. The linearity range of Dapagliflozin Propanediol Monohydrate 6 - 22 µg/ml and Linagliptin 5 - 11 µg/ml for both drug and data are shown in table 2.

Table 1: System Suitability

Drug	Parameters	Mean ± S.D. (n=5)	% R.S.D
Dapagliflozin Propanediol Monohydrate	Retention time	4.003 ± 0.0045	0.1131
	Theoretical plate	18722.7 ± 23.50	0.1255
	Tailing Factor	1.342 ± 0.0018	0.1353
Linagliptin	Retention time	2.343 ± 0.0033	0.1434
	Theoretical plate	12290.1 ± 15.227	0.1239
	Tailing Factor	1.402 ± 0.0015	0.1127
	Resolution	6.369 ± 0.0080	0.1268

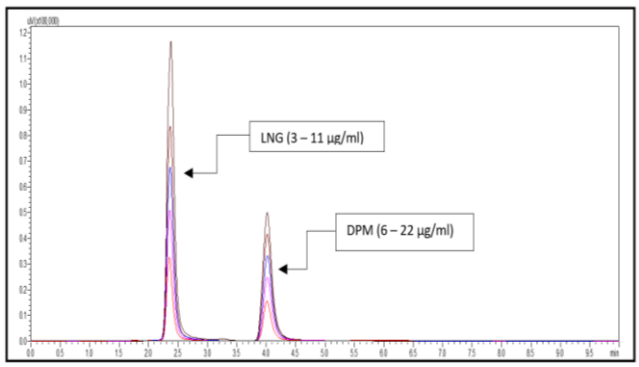

Figure 3: Overlay Chromatogram of DPM (6 – 22 µg/ml) and LNG (3 – 11 µg/ml)

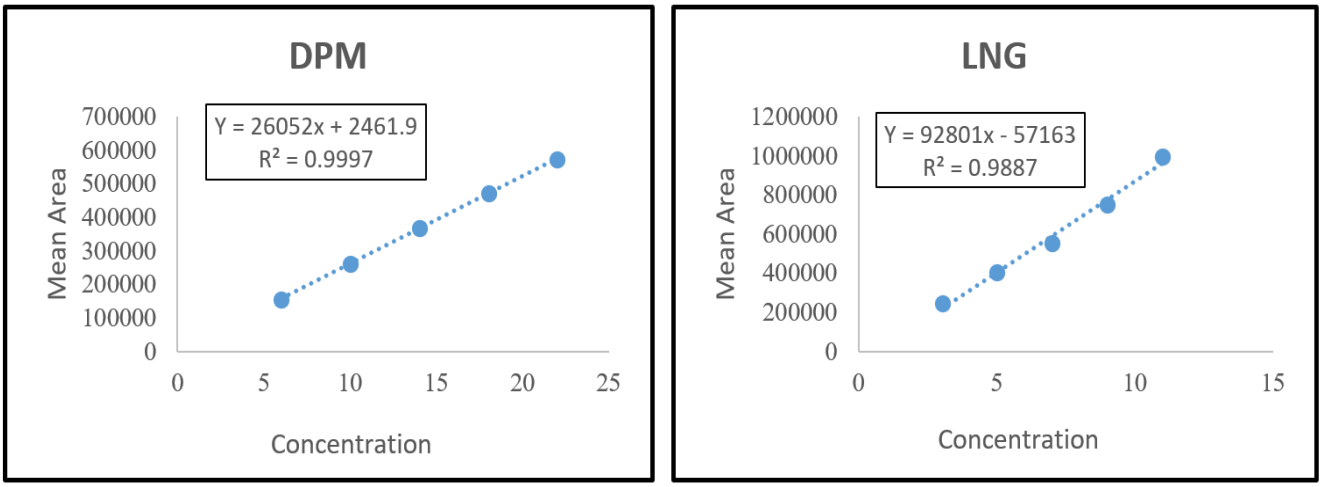

Figure 4: Calibration Curve of DPM and LNG

Table 2: Linearity data of Dapagliflozin Propanediol Monohydrate and Linagliptin

Drugs	Concentration (µg/ml)	Mean peak area ± S.D.	% R.S.D
DPM	6	155761.8 ± 663.99	0.4262
	10	264961.6±1009.01	0.3808
	14	369601.8 ± 691.79	0.1871
	18	472657.2 ± 690.98	0.1461
	22	572948.8±1844.32	0.3219
LNG	3	245893.4 ± 923.02	0.3753
	5	406194.2±1336.31	0.3289
	7	557303.4 ± 932.90	0.1674
	9	751640.6 ± 937.15	0.1246
	11	1001176±1054.88	0.1053

Precision

A) Repeatability

The data of repeatability for Dapagliflozin Propanediol Monohydrate and Linagliptin are shown in below table

B) Intraday Precision

The Intraday Precision for Dapagliflozin Propanediol Monohydrate and Linagliptin are shown in below table

C) Interday Precision

The Interday Precision for Dapagliflozin Propanediol Monohydrate and Linagliptin are shown in table 3.

Accuracy

The standard addition method (conventional method) was used to conduct the percentage recovery analysis. To pre – quantified sample solution of Dapagliflozin Propanediol Monohydrate (10 µg/ml) and Linagliptin (5 µg/ml) known quantities of regular Dapagliflozin Propanediol Monohydrate and Linagliptin were added at 80 %, 100 % and 120 %. The quantities of Dapagliflozin Propanediol Monohydrate and Linagliptin were calculated using calibration curve's regression equation. The smaller standard deviation, the more accurate the proposal approach is the results of recovery studies are shown in table 4.

Table 3: Precision data for Dapagliflozin Propanediol Monohydrate and Linagliptin

Repeatability data for DPM and LNG(n=6)			
Drugs	**Concentration (µg/ml)**	**Mean peak Area ± S.D**	**% R.S.D**
DPM	10	265199.5±1074.27	0.4050
LNG	5	406686.6±1393.48	0.3428
Intraday Precision for DPM and LNG (n=3)			
DPM	10	264075 ± 1520.58	0.5758
	14	367856.3 1705.91	0.4637
	18	471715.3±1858.03	0.3938
LNG	5	405434 ± 2149.05	0.5300
	7	556583 ± 2070.65	0.3720
	9	752482.6±2298.16	0.3054
Interday Precision for DPM and LNG(n=3)			
DPM	10	262898.3±2111.93	0.8033
	14	366541.6±2310.97	0.6304
	18	470613 ± 2400.65	0.5101
LNG	5	406455 ± 3072.25	0.7558
	7	553379 ± 3178.11	0.5743
	9	754530 ± 3192.89	0.4231

LOD and LOQ

- LOD for Dapagliflozin Propanediol Monohydrate and Linagliptin was found to be 0.1014 µg/ml and 0.1000 µg/ml respectively.

- LOQ for Dapagliflozin Propanediol Monohydrate and Linagliptin was found to be 0.3075 µg/ml and 0.3031 µg/ml respectively.

Robustness

The robustness of the method was established by making deliberate minor variation in the following method parameter.

- Mobile phase Ratio: ± 2.0 ml
- Flow rate: ± 0.2 units

Table 4: Accuracy data for Dapagliflozin Propanediol Monohydrate and Linagliptin

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	9.93	99.30
	80%	10	8	18	17.97	99.83
	100%	10	10	20	20.07	100.19
	120%	10	12	22	21.92	99.63
LNG	0%	5	0	5	4.99	99.97
	80%	5	4	9	8.98	99.85
	100%	5	5	10	10.01	100.14
	120%	5	6	11	11.03	100.27

Table 5: Robustness data for Dapagliflozin Propanediol Monohydrate and Linagliptin

Parameter	Level	Mean Peak Area ± S.D. (n=3)	% R.S.D.	Rt ± S.D. (n=3)	% R.S.D.
Robustness data for DPM					
Mobile phase (60:40%v/v)	62:38%v/v	264985.6 ± 699.91	0.2641	3.861 ± 0.007	0.1837
	58:42%v/v	264991.3 ± 543.41	0.2050	3.922 ± 0.006	0.1671
Flow rate (1.0 ml/min)	0.8 ml/min	264839.6 ± 378.23	0.1428	4.103 ± 0.006	0.1547
	1.2 ml/min	264984 ± 450.56	0.1700	3.886 ± 0.004	0.1269
Robustness data for LNG					
Mobile phase (60:40%v/v)	62:38%v/v	406133 ± 807.75	0.1988	2.278 ± 0.004	0.1856
	58:42%v/v	406105 ± 1052.65	0.2592	2.355 ± 0.004	0.1914
Flow rate (1.0 ml/min)	0.8 ml/min	406216 ± 652.39	0.1606	2.475 ± 0.004	0.1681
	1.2 ml/min	406100.3 ± 653.60	0.1609	2.148 ± 0.002	0.1171

Applicability of Proposed method

Table 6: Analysis of synthetic mixture

Synthetic Mixture	Concentration (µg/ml)		Amount obtained (µg/ml)		% Assay of DPM ± S.D. (n=3)	% Assay of LNG ± S.D. (n=3)
	DPM	LNG	DPM	LNG		
	10	5	9.98	4.98	99.81 ± 0.030	99.62 ± 0.035

Summary of RP - HPLC Method

Table 7: Summary of RP HPLC Method

Parameters	DPM	LNG
Linearity (µg/ml)	6 - 22 µg/ml	3 - 11 µg/ml
Regression Equation (Y = mx + c)	Y = 26052x + 2461.9	Y = 92801x - 57163
Regression Coefficient (R^2)	0.9997	0.9887
Correlation Coefficient (r)	0.9998	0.9943
Repeatability (% R.S.D.) (n=6)	0.4050	0.3428
Intraday Precision (% R.S.D.) (n=3)	0.3938 – 0.5758	0.3054 – 0.5300
Interday Precision (% R.S.D.) (n=3)	0.5101 – 0.8033	0.4231 – 0.7558
LOD (µg/ml)	0.1014	0.1000
LOQ (µg/ml)	0.3075	0.3031
% Recovery (n=3)	99.30 – 100.19	99.85 – 100.27
Assay (%) ± S.D.	99.70 ± 0.2203	99.46 ± 0.3066

CONCLUSION

Based on results, obtained from the analysis of Dapagliflozin Propanediol Monohydrate and Linagliptin in synthetic mixture using RP – HPLC Method, it can be concluded that the method has linearity in the range of 6 – 22 µg/ml for Dapagliflozin Propanediol Monohydrate and 3 – 11 µg/ml for Linagliptin. The regression coefficient (R^2) was found to be 0.9997 for Dapagliflozin Propanediol Monohydrate and 0.9887 Linagliptin. The correlation coefficient (r) was found to be 0.9998 and 0.9943 for Dapagliflozin Propanediol Monohydrate and Linagliptin at 225 nm respectively.

Limit of detection for Dapagliflozin Propanediol Monohydrate and Linagliptin were found to be 0.1014 and 0.1000 µg/ml and limit of Quantitation for Dapagliflozin Propanediol Monohydrate and Linagliptin were found to be 0.3075 µg/ml and 0.3031 µg/ml respectively. The % assay was found to be 99.70% and 99.46% for Dapagliflozin Propanediol Monohydrate and Linagliptin respectively. Further % R.S.D. was found to be less than 2 % precision, intraday and Interday study.

Methods were developed and validated as per ICH guideline Q2(R1). The Precision of the developed methods was confirmed by intra – day and inter day analysis and 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.

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