



## Research Article

# Development And Validation of Stability Indicating HPLC Method For Simultaneous Estimation of Dapagliflozin, Linagliptin and Metformine Hydrochloride in Its Tablet Dosage Form

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## ABSTRACT

Dapagliflozin, Linagliptin and Metformine Hydrochloride belongs to the class of anti-diabetic drugs. A Simple, Precise and rapid stability indicating Reversed-Phase HPLC method was developed and validated for the simultaneous estimation of Dapagliflozin, Linagliptin and Metformine Hydrochloride combination in its tablet dosage form. The validation of this method was achieved as per ICH Q2 (R2) guidelines with the optimized experimental conditions. To achieve the proposed method on Kromasil C18 column (150 mm x 4.6 mm, 5  $\mu$ m) column as Stationary Phase and retention time of Dapagliflozin, Linagliptin and Metformine Hydrochloride was found to be 25.125 min., 15.115 min., and 3.113 min. respectively. The Mobile Phase consists of Acetonitrile: Phosphate buffer (60:40 v/v) and flow rate adjusted was 1 ml/min. Wavelength selected for detection was carried out at 272nm. The method is Linear over the range of 5-15  $\mu$ g/ml for Dapagliflozin, 2.5-7.5  $\mu$ g/ml for Linagliptin and 250-750  $\mu$ g/ml for Metformine Hydrochloride. The observed co-relation co-efficient for Dapagliflozin, Linagliptin and Metformine Hydrochloride are  $>0.999$ . The method was validated by determining its accuracy, linearity and precision.

## INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a prevalent metabolic disorder worldwide, characterized by chronic hyperglycemia resulting from impaired insulin action and/or secretion. T2DM affects multiple organ systems and presents with various

symptoms including excessive urination (polyuria), compensatory thirst (polydipsia), increased fluid intake, blurred vision, unexplained weight loss, lethargy, and altered energy metabolism. These manifestations result from the body's inability to maintain proper glucose homeostasis [1].

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## Anti-Diabetic Agents :

### Dapagliflozin :

Dapagliflozin (DAPA) is a member of a novel class of oral anti-diabetic agents known as Sodium-Glucose Co-Transporter 2 (SGLT2) inhibitors. It is indicated for the management of T2DM in conjunction with diet and exercise to improve glycemic control. Dapagliflozin acts by inhibiting SGLT2 in the renal proximal tubules, thereby preventing glucose reabsorption and promoting its excretion via urine [2].

- **Chemical Name:** (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6 (hydroxymethyl)oxane-3,4,5-triol
- **Molecular Formula:** C<sub>21</sub>H<sub>25</sub>ClO<sub>6</sub>
- **Molecular Weight:** 408.9 g/mol [3]

### Linagliptin :

Linagliptin is an oral anti-diabetic drug belonging to the class of Dipeptidyl Peptidase-4 (DPP-4) inhibitors. It enhances glycemic control by inhibiting DPP-4, an enzyme that degrades incretin hormones. This inhibition increases insulin secretion and suppresses glucagon release in a glucose-dependent manner, providing an anti-hyperglycemic effect [4,5].

- **Chemical Name:** 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-1H-purine-2,6-dione
- **Molecular Formula:** C<sub>25</sub>H<sub>26</sub>N<sub>8</sub>O<sub>2</sub>
- **Molecular Weight:** 472.5 g/mol [6]

### Metformin Hydrochloride :

Metformin Hydrochloride is a biguanide-class oral antihyperglycemic agent, commonly used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It exerts its effects by

decreasing hepatic glucose production, reducing intestinal glucose absorption, and enhancing insulin-mediated peripheral glucose uptake. Notably, metformin is associated with weight neutrality or modest weight loss and does not induce hypoglycemia when used alone. It is also indicated in the management of polycystic ovary syndrome and other insulin resistance-related disorders [7].

- **Chemical Name:** 1-carbamimidamido-N,N-dimethylmethanimidamide hydrochloride
- **Molecular Formula:** C<sub>4</sub>H<sub>11</sub>N<sub>5</sub>·HCl
- **Molecular Weight:** 165.62 g/mol [8]

### Purpose of the Study :

The objective of this study is to develop a simple, precise, and rapid stability-indicating High-Performance Liquid Chromatography (HPLC) method for the simultaneous estimation of Dapagliflozin, Linagliptin, and Metformin Hydrochloride in a combined tablet dosage form. This method aims to provide robust analytical performance and will be validated according to the International Council for Harmonisation (ICH) guidelines. To date, no stability-indicating HPLC methods have been reported for the simultaneous estimation of these three drugs in a single tablet formulation.

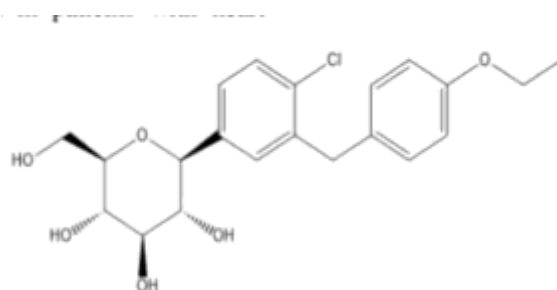


Fig : 1 Structure of Dapagliflozin

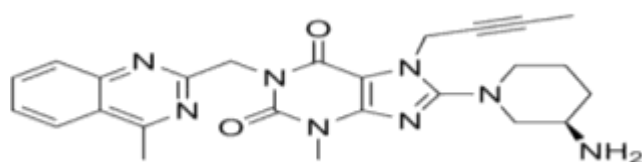


Fig : 2 Structure of Linagliptin

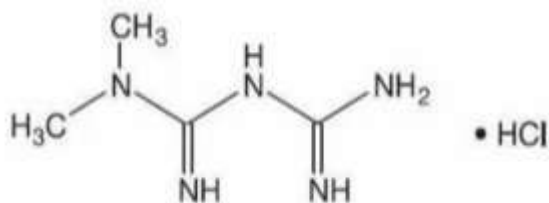


Fig : 3 Structure of Metformine Hydrochloride

## MATERIALS AND METHODS:

### Instrumentation:

- HPLC instrument with UV-visible detector – LC100
- Column - Kromasil C18 column (150mm x 4.6 mm, 5 µm)
- UV-visible Spectrophotometer – SHIMADZU-1800 Software-UV probe ,version-2.34
- Digital Analytical Balance - SCALE TEC, SAB 224 CL
- Ultra Sonicator - PHOENIX DSA – 50 SK2
- Digital pH meter - Chemiline ATC CL 120
- Controlled temperature water bath
- Hot air Oven - Lab Tech DST, Ahmadabad
- FTIR – SHIMADZU
- Volumetric flask - 10, 25, 50 and 100ml
- Pipette - 1, 2, 5 and 10ml.
- Measuring cylinder - 10, 100 and 500ml.
- Beakers - 250, 500 and 1000 ml

### Chemicals and Reagents:

- Metformin Hydrochloride (API) –certified supplier
- Dapagliflozin –(API) – certified supplier
- Linagliptin (API) –certified supplier
- Potassium dihydrogen phosphate – Thermo Fischer Scientific Pvt. Ltd.
- Triethylamine – SD Fine-Chem. Ltd., Mumbai, India
- Ortho Phosphoric acid HPLC Grade – Finar
- Acetonitril HPLC Grade – Finar
- Hydrochloric Acid – Rankem
- Methanol HPLC Grade – Life science Pvt Ltd
- Ethanol Analytical reagent – Fine Chemical Co.Ltd
- Sulphuric Acid HPLC Grade – Rankem
- Hydrogen Peroxide HPLC Grade – Rankem
- Sodium Hydroxide pellet – Rankem
- 1-Pantanesulphonic acid Sodium salt HPLC Grade – Finar
- Potassium bromide (KBr) – Rankem
- Water: Distill water, HPLC Grade water, - Rankem

## IDENTIFICATION AND CHARACTERIZATION :

### Solubility Stability Study :

For stability studies, the solubility of dapagliflozin, linagliptin and metformine hydrochloride were practically determined by adding 100mg of dapagliflozin, linagliptin and metformine hydrochloride to 100 ml volumetric flasks, then adding quantity of solvent (e.g., water or ethanol) at room temperature and shaking for a few minutes. The solubility was then classified based on the amount of solvent required to dissolve the solute.

Table : 1 Solubility table as per IP'2022 specification

DescriptionTerms	Relative Quantities of solvent for 1 parts of solute
Very Soluble	Less than 1 part
Freely Soluble	From 1 to 10 parts

Soluble	From 10 to 30 parts
Sparingly Soluble	From 30 to 100 parts
Slightly Soluble	From 100 to 1000 parts
Very Slightly Soluble	From 1000 to 10000 parts
Practically Insoluble	More than 10000 parts

**Table : 2 Solubility Data for Dapagliflozin, Linagliptin and Metformin**

Solvent	Dapagliflozin	Linagliptin	Metformin
Water	Sparingly Soluble	Very slightly Soluble	Soluble
Chloroform	Soluble	Practically Insoluble	Practically Insoluble
0.1 N HCL	Very Soluble	Freely soluble	Soluble
Acetonitrile	Soluble	Very slightly soluble	Very slightly soluble
Methanol	Soluble	Sparingly Soluble	Very Soluble
Ethanol	Freely Soluble	Sparingly Soluble	Slightly Soluble

### Identification By Melting Point Determination

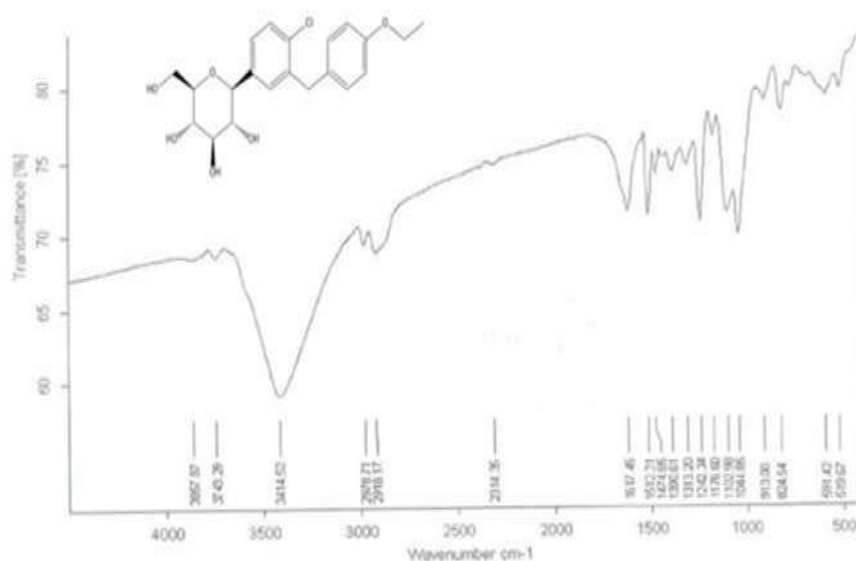
The melting point of Metformin, Dapagliflozin and Linagliptin were determined by using the open capillary method, a standard technique for this purpose. A small sample of dapagliflozin, Linagliptin and Metformine Hydrochloride are placed in an open capillary tube and heated gradually until it melts. The temperature at which the drug starts to melt is recorded as its melting point. Melting point for both the drug was observed and recorded in following table 3.

**Table : 3 Melting Point of Drugs**

Sr.No.	APIs	Melting Point	
		Reported	Measured
1	Dapagliflozin	75°C	74-78°C
2	Linagliptin	193.5°C	190-196°C
3	Metformin	223.5 °C	222-226 °C

### IR Spectra:

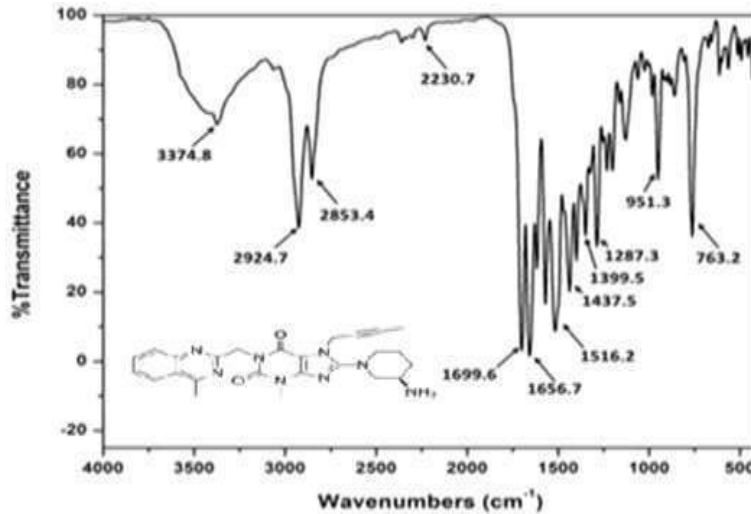
The IR Spectra of Metformin, Dapagliflozin and Linagliptin along with its functional group identification, were shown in the following graph.



**Fig : 4 IR Spectra of Dapagliflozin Sample**

**Table : 4 IR Spectra Interpretation for Dapagliflozin**

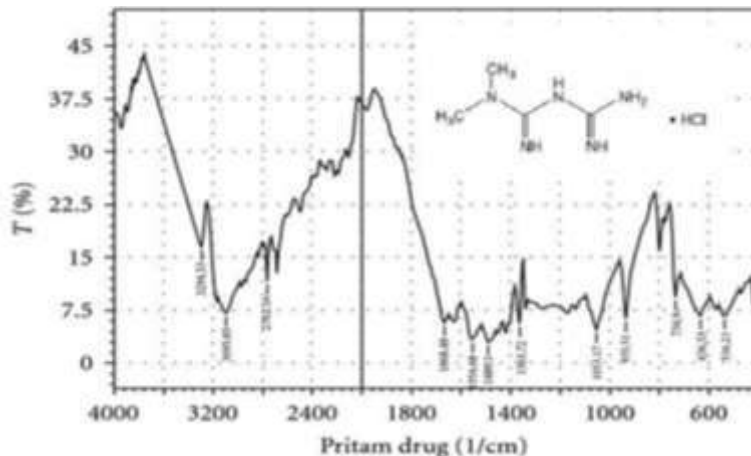
Groups	General Range(cm-1)	Observed Range(cm-1)
O-H (s)	3400-3200	3352.28
C-O (s)	1100-11050	1834.55
C-H (s)	2690-2850	2932
C-Cl(s)	850-550	980
C=C (s)	1300-800	1180.78
C-C(s)	1300-100	1250



**Fig : 5 IR Spectra of Linagliptin Sample**

**Table : 5 IR Spectra Interpretation for Linagliptin**

Groups	General Range(cm-1)	Observed Range(cm-1)
C=O (s)	1700-1100	1655
C-H (s)	2690-2850	2932
C=N (s)	2260-1240	1517
C-N (s)	1250-1000	1156.80
N-H (s)	3500-3300	3322.89
C≡C (s)	2250-2100	2234.22
C-C(s)	1300-1100	1155.89
C=C(s)	1300-800	1228.20



**Fig : 6 IR Spectra of Metformin Sample.**

**Table :6 IR Spectra Interpretation for Metformin**

Groups	General Range(cm-1)	Observed Range(cm-1)
C-H (s)	2690-2850	2932
C-N (s)	1350-1000	1665.4
N-H (s)	3500-3100	3372.2
C=N (b)	1690-1640	1624.5

## METHOD DEVELOPMENT :

### Preparation Of Buffer and Mobile Phase :

An amount of 1 mL of Methanol(85%) solution was taken in a 1000-mL volumetric flask; about 100 mL of milli-Q water was added and mixed well; then the final volume was made up to 1000 mL with milli-Q water, and the pH was adjusted to 3.0 with diluted Methanol(10 % v/v); 600 mL (0.1%) of phosphate buffer (pH 3.0) and 400 mL of acetonitrile were mixed in the ratio of 60:40 (% v/v) and degassed in an ultrasonic water bath for 15 min and then filtered through a 0.45- $\mu$ m membrane filter under vacuum.

## PREPARATION OF SOLUTIONS :

### Preparation Of Standard Solution :

#### Preparation Of Stock Solution of Metformin :

Accurately weighed Metformin (250 mg) was transferred into a 50-ml clean dry volumetric flask, 10 ml of a diluent was added, sonicated for 10 min, and made up to the final volume with diluents to give a stock solution of 5000  $\mu$ g/ml of Metformin.

#### Preparation Of Working Standard Solution of Metformin :

From above stock solution pipette out 1 ml of aliquot and diluted up to 10 ml to give a solution having strength of 500  $\mu$ g/ml of Metformin.

#### Preparation Of Stock Solution of Dapagliflozin

Accurately weighed Dapagliflozin (5 mg) was transferred into a 50-mL clean dry volumetric flask, 10 ml of a diluent was added, sonicated for 10 min, and made up to the final volume with diluents to give a stock solution of 100  $\mu$ g/ml of Dapagliflozin.

#### Preparation of Working Standard Solution of Dapagliflozin:

From above stock solution pipette out 1 ml of aliquot and diluted up to 10 ml to give a solution having strength of 10  $\mu$ g/ml of Dapagliflozin.

#### Preparation Of Stock Solution Of Linagliptin:

Accurately weighed Linagliptin (2.5 mg) was transferred into a 50-mL clean dry volumetric flask, 10 ml of a diluent was added, sonicated for 10 min, and made up to the final volume with diluents to give a stock solution of 50  $\mu$ g/ml of Linagliptin.

#### Preparation of Working Standard Solution of Linagliptin:

From above stock solution pipette out 1 ml of aliquot and diluted up to 10 ml to give a solution having strength of 5  $\mu$ g/ml of Linagliptin.

#### Preparation of Sample solution:

#### Preparation of Sample Stock Solution:

An accurate equivalent weight of the combination powder sample was transferred into a 100-ml volumetric flask; 50 ml of the diluent was added and sonicated for 25 min; further, the volume was made up with the diluent and filtered using HPLC filters (5000  $\mu$ g/ml of Metformin, 100  $\mu$ g/ml of Dapagliflozin, and 50  $\mu$ g/ml of Linagliptin).

#### Preparation of Working Sample Solution:

Take 1 ml of the filtered sample stock solution was transferred into a 10-mL volumetric flask and made up with the diluent. The solutions prepared comprised 500 µg/ml of Metformin, 10 µg/ml of Dapagliflozin, and 5 µg/ml of Linagliptin.

#### **VALIDATION OF PROPOSED METHOD :**

The proposed method was validated according to ICH guidelines (2005) for system suitability, specificity, recovery, precision, linearity, and robustness.

##### **System Suitability Test:**

System suitability test is an integral part of LC methods. This test is used to verify that the chromatographic system is adequate for the intended analysis. HPLC system suitability was optimized per United States of Pharmacopeia (USP) general chapter on chromatography <621>. About 10 µl of the standard solution of drugs was injected six replicate injections into the chromatographic system. To determine the system suitability of the proposed method, the parameters such as retention time, theoretical plates, and tailing factor were calculated.

##### **Specificity:**

The specificity of the method was carried out to check whether there is any interference of any impurities in the retention time of the analyte peaks. The specificity was performed by injecting blank, placebo, and standard solutions of drugs.

##### **Linearity:**

The standard stock solutions of Metformin, Dapagliflozin, and Linagliptin were suitably diluted with the mobile phase to obtain a series of solutions containing 250, 375, 500, 625, and 750 µg/ml of Metformin; 5, 7.5, 10, 12.5, and 15 µg/ml of Dapagliflozin, and 2.5, 3.75, 5, 6.25, and 7.5

µg/mL of Linagliptin. The linearity was determined by calculating a regression line from the plot of the peak area to the concentration of the drug. The method was evaluated by the determination of correlation coefficient and intercept values according to ICH guidelines.

##### **Precision:**

Precision is expressed as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Metformin (500 µg/ml), Dapagliflozin (10 µg/ml), and Linagliptin (5 µg/ml) were analyzed by injecting into a HPLC column on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on different days by different operators. The peak area of all injections was taken, and standard deviation and % relative standard deviation (RSD) were calculated.

##### **Accuracy:**

Accuracy is estimated using the standard addition method at different levels: 50, 100, and 150%. A known amount of the standard drug was added to the blank sample at each level. The mean recovery of Metformin, Dapagliflozin, and Linagliptin was calculated

##### **Robustness:**

HPLC conditions were slightly modified to evaluate the analytical method robustness. These changes included the flow rate, column temperature, and mobile phase.

##### **Forced Degradation Study :**

Alkaline, acidic, oxidation, thermal, water, and direct exposure to UV were carried out.



### **Alkali Hydrolysis :**

To 10 ml of the stock solution of Metformin, Dapagliflozin, and Linagliptin, 4 ml of 1.0 N sodium hydroxide was added and refluxed for 30 min at 60 °C. The solution was cooled to room temperature and neutralized with 4 mL of 1.0 N HCl, and finally, the solution was made up to the target concentration with the diluent.

### **Acid Hydrolysis :**

To 10 ml of the stock solution of Metformin, Dapagliflozin, and Linagliptin, 4 mL of 1.0 N hydrochloric acid was added and refluxed for 30 min at 60 °C. The solution was cooled to room temperature and neutralized with 4 ml of 1.0 N NaOH, and finally, the solution was made up to the target concentration with the diluent

### **Oxidative Stress :**

To 10 ml of the stock solution of Metformin, Dapagliflozin, and Linagliptin, 1 ml of 20% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added, and the solutions were kept for 30 min at 60 °C. The solution was cooled to room temperature and made up to the target concentration with the diluent.

### **Thermal Degradation :**

An amount of 10 mL of the standard stock solution of drugs was transferred into a 100 ml volumetric flask and placed in an oven at 80°C for 6 h to study dry heat degradation, and then the solution was

cooled and made up to the target concentration in a 100 ml volumetric flask with diluent.

### **Photolytic Degradation :**

Photo stability of the drugs was also studied by exposing 10 ml of the standard stock solution of Metformin, Dapagliflozin, and Linagliptin, to UV light in a glass beaker for 7 days, and then the solution was made up to the target concentration in a 100 ml volumetric flask with the diluent.

### **Hydrolytic Degradation :**

About 10 ml of the standard stock solution of drugs was transferred into a 100-ml volumetric flask; 10 ml of deionized water was added and heated on a water bath for 1 h. Finally, the solution was cooled and made up to the target concentration with the diluent.

## **RESULT AND DISCUSSION:**

### **Selection Of Wavelength :**

To determine wavelength for measurement, standard spectra of Dapagliflozin, Linagliptin and Metformin Hydrochloride were scanned between 200-400 nm against diluents. Absorbance maxima of Dapagliflozin, Linagliptin and Metformin Hydrochloride have detected at 272 nm. Chromatogram was taken at 272 nm, three drugs give good peak height and shape. So, 272 nm was selected for Simultaneous estimation of Dapagliflozin, Linagliptin and Metformin in their formulation.



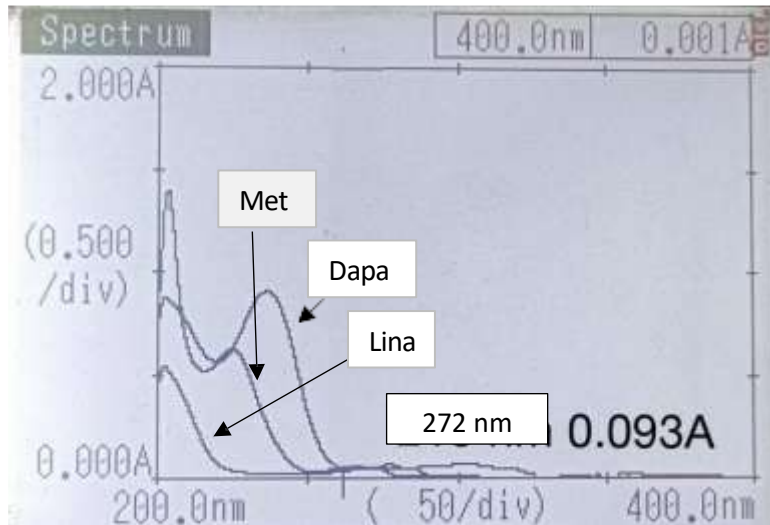


Fig : 7 UV Graph for Dapagliflozin, Linagliptin and Metformin

### Selection Of Column :

For HPLC Method various columns are available but our main aim is to resolve drug in the presence of excipients, so the Kromasil C18 column was selected for the estimation of Dapagliflozin, Linagliptin and Metformin. C-18 (id 4.6mm x 150 mm, 5  $\mu$ m) column was chosen to give good peak shape and high resolution, which also provides high peak symmetry, good retention to drug and facilitates the separation of the drug without the interference of excipients within short run time.

Fig : 8 Kromasil C<sub>18</sub> (id 4.6mm x 150 mm, 5  $\mu$ m) column

### Selection Of Mobile Phase :

#### Trail 1

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Methanol: Phosphate buffer(30:70v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30 minutes
- **Observations:** No peak detected.

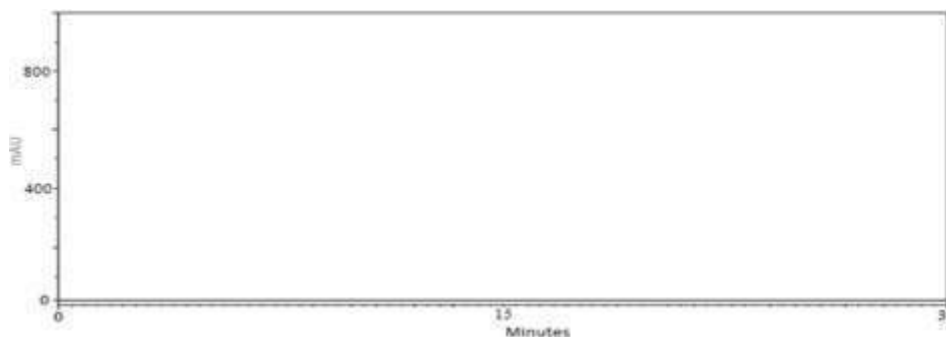


Fig : 9 Trial 1: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Methanol : Phosphate buffer(30:70v/v)

### Trail 2

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Methanol: Phosphate buffer(50:50v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30minutes
- **Observations:** only one peak detected.

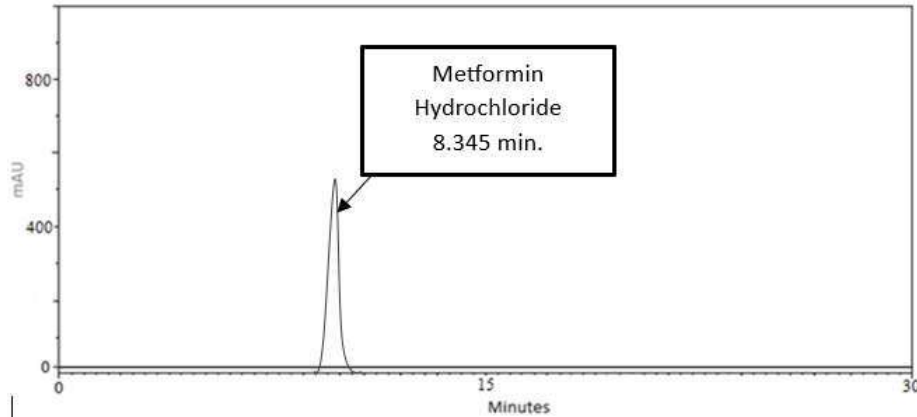


Fig : 10 Trial 2: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Methanol: Phosphate buffer(50:50v/v)

### Trail 3

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Methanol: Phosphate buffer(20:80v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30minutes
- **Observations:** Peaks detected and separated, but broad peaks observe.

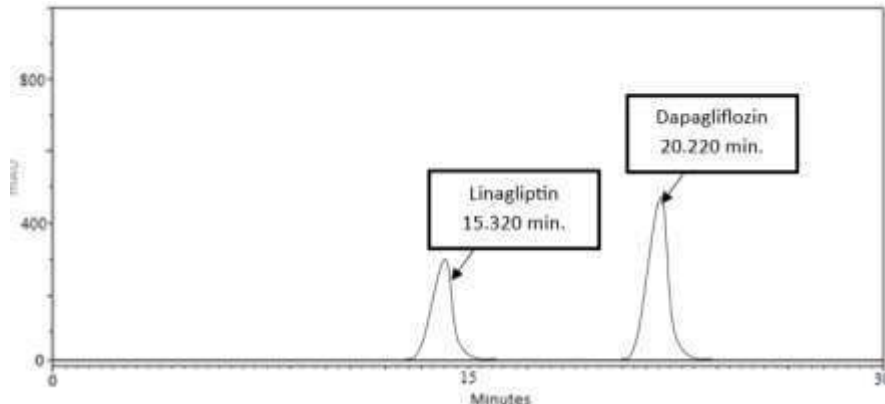


Fig :11 Trial 3: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Methanol: Phosphate buffer(20:80v/v)

### Trail 4

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Methanol: Water(30:70v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30 minutes
- **Observations:** No peak detected.

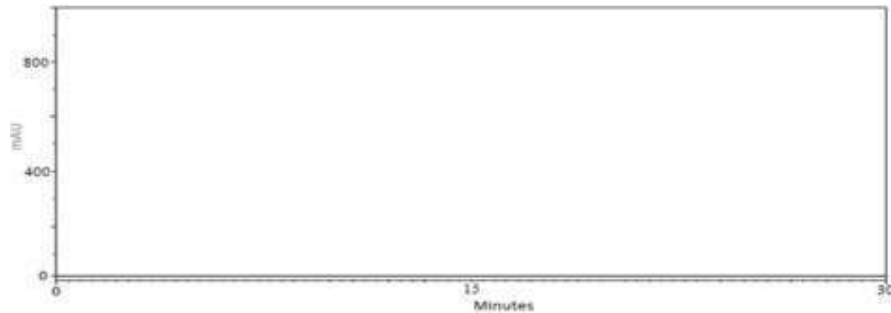


Fig :12 Trial 4: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Methanol: Water(30:70v/v)

**Trail 5**

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Methanol: Water (50:50v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30minutes
- **Observations:** only two peak detected with broad spectrum.

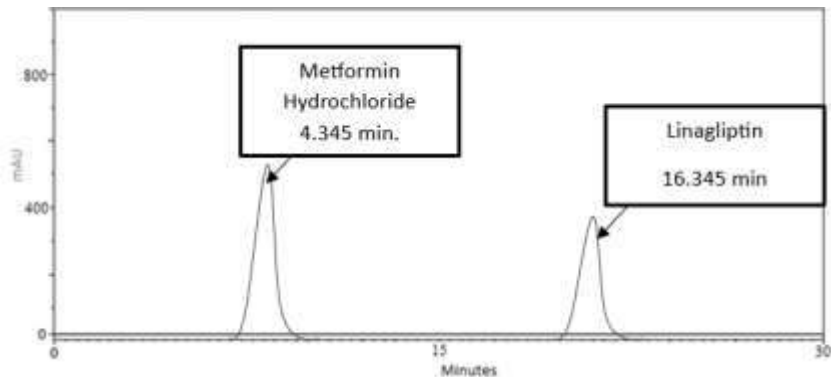


Fig : 13 Trial 5: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Methanol: Water (50:50v/v)

**Trail 6**

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Methanol: Water (20:80v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30minutes
- **Observations:** Two Peaks detected and separated, but broad peaks observe.

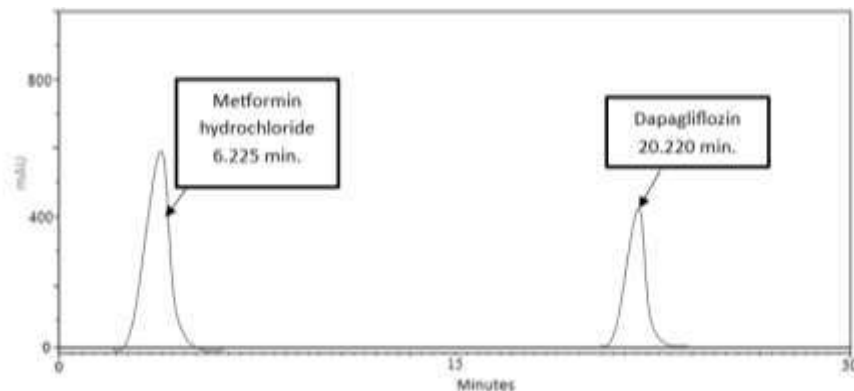


Fig : 14 Trial 6: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Methanol: Water (20:80v/v)

### Trail 7

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Acetonitrile: Phosphate buffer(30:70v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30 minutes
- **Observations:** No peak detected.

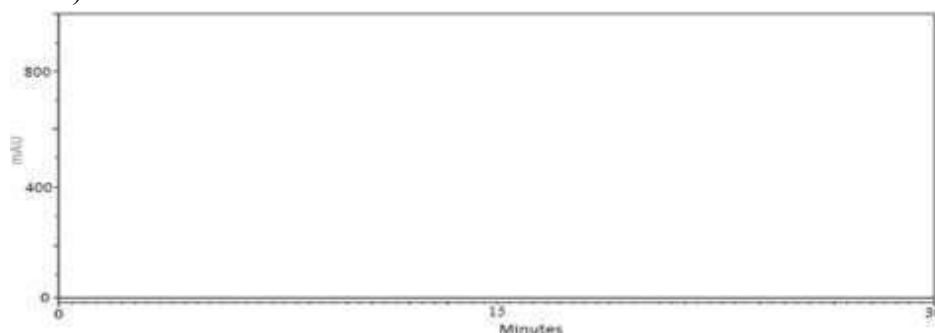


Fig : 15 Trial 7: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Acetonitrile: Phosphate buffer(30:70v/v)

### Trail 8

- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Acetonitrile: Phosphate buffer(50:50v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30minutes
- **Observations:** only one peak detected.

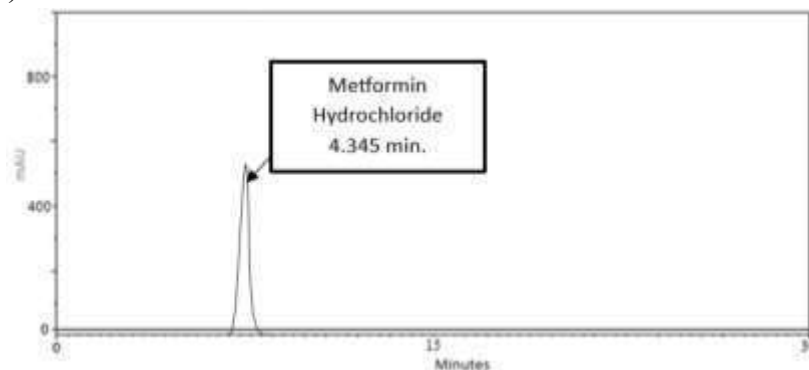
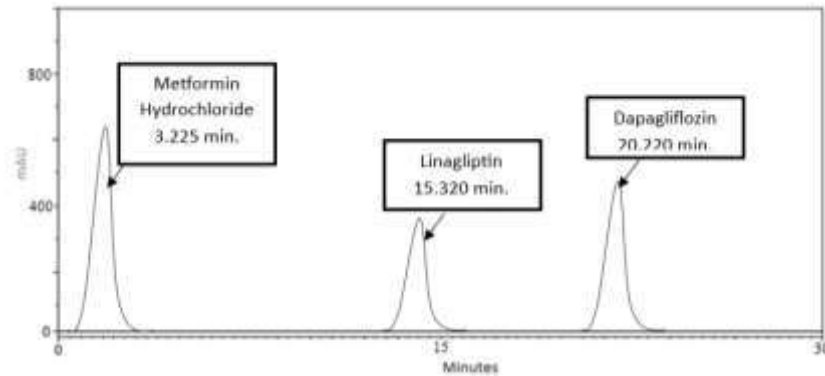


Fig : 16 Trial 8: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Acetonitrile : Phosphate buffer(50:50v/v)

### Trail 9

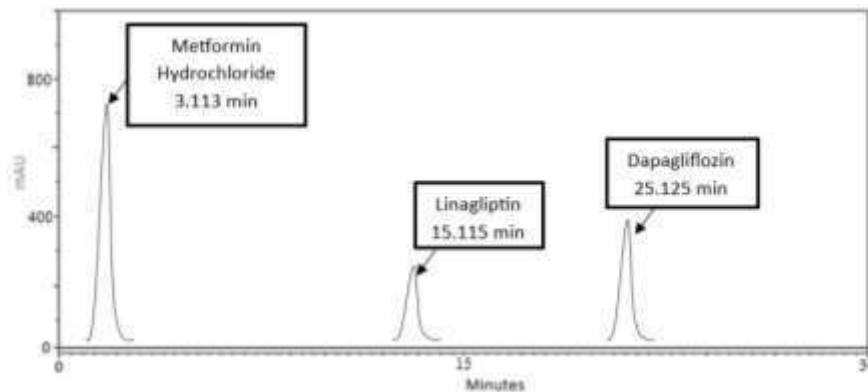
- Column: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Acetonitrile: Phosphate buffer(20:80v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30minutes
- **Observations:** Peaks detected and separated, but broad peaks observe.



**Fig : 17 Trial 9: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Ortho-phosphoric acid: Phosphate buffer(20:80v/v)**

**Trail 10**

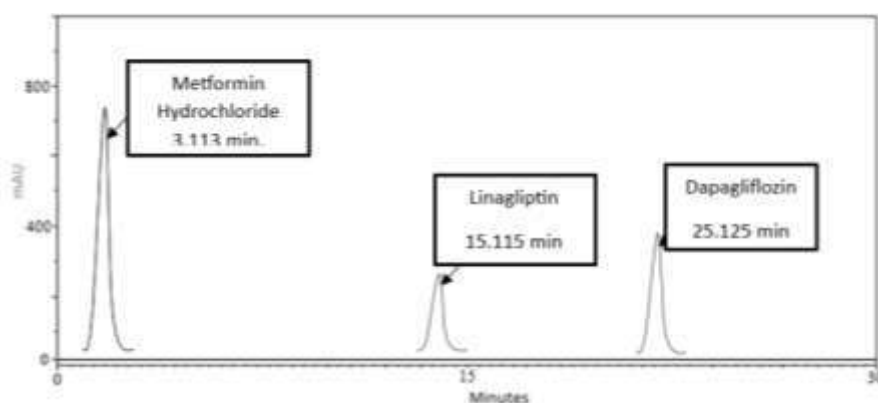
- Colum: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Acetonitrile: Phosphate buffer(60:40v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30minutes
- **Observations:** Good peaks with Adequate solution were observed.



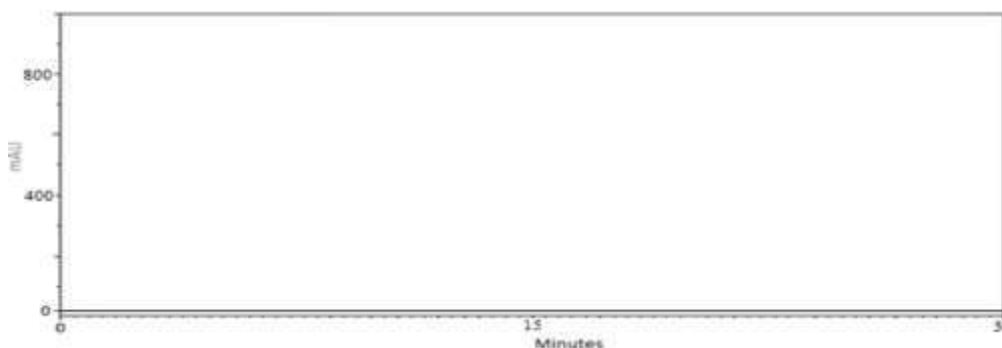
**Fig : 18 Trial 10: Chromatogram of Dapagliflozin, Linagliptin and Metformin Hydrochloride Acetonitrile: Phosphate buffer(60:40v/v)**

**Chromatographic conditions for optimized mobile phase trial :**

- Stationary phase: C-18 (id 4.6 x 150 mm, 5  $\mu$ m)
- Mobile Phase: Acetonitrile: Phosphate buffer (60:40v/v)
- Detection: 272 nm
- Flow rate:1 ml/min
- Run Time: 30 minutes
- Detector: UV detector
- Injection volume: 20  $\mu$ l
- Column Temperature: 40°C
- Mode: Isocratic



**Fig : 19 Optimized mobile phase trial for optimized chromatogram of Std Dapagliflozin:25.515 min, Linagliptin: 15.115 min, Metformin Hydrochloride: 3.113 min**



**Fig : 20 Chromatogram of blank Acetonitrile: Phosphate buffer (60:40v/v)**

**Method Validation :**

**Linearity :**

For the purpose of linearity, accurately weighed amount of Dapagliflozin (10 mg), and Linagliptin (5 mg) and Metformin Hydrochloride (500 mg) was taken into the volumetric flask (10 ml) and volume of the flask was raised to 10 ml with methyl alcohol to give stock solution containing 100 µg/ml of Ranitidine, and 100 µg/ml of Ondansetron. Various aliquots from this stock solution were transferred to another 10 ml volumetric flask and volume was raised to the mark with mobile phase to give final solutions containing 5+2.5+250, 7.5+3.75+375, 10+5+500, 12.5+6.25+625 and 15+7.5+750 µg/ml of Dapagliflozin, Linagliptin and Metformin Hydrochloride respectively.

**Table : 7 Linearity data for Dapagliflozin, Linagliptin and Metformin Hydrochloride**

Conc. (µg/ml)	Dapagliflozin		
	Mean Area	± SD (n=5)	% RSD
5	368163	368163± 2201.61	0.71
7.5	576253	576253 ± 2373.10	0.60
10	724860	724860 ± 1041.54	0.41
12.5	873147	873147 ± 2455.68	0.14
15	1086558	1086558 ± 2712.8	0.28

Conc. (µg/ml)	Linagliptin		
	Mean Area	± SD (n=5)	% RSD
2.5	187736	187736.7 ± 966.40	0.51
3.75	295117	295117.3 ± 4683.21	1.59
5	372946	372946.7 ± 3219.14	0.66

6.25	445179	445179.7 ± 1881.37	0.42
7.5	555102	555102.1 ± 3079.55	0.55

375	10595775	10595775.3 ± 1676.45	0.02
500	13743743	13743743.2 ± 1877.89	0.01
625	16893330	16893330.1 ± 1282.81	0.01
750	21154978.6	21154978.6 ± 1850.72	0.01

Metformin Hydrochloride			
Conc. (µg/ml)	Mean Area	± SD (n=5)	% RSD
250	6375404	6375404.4 ± 3620.871	0.06

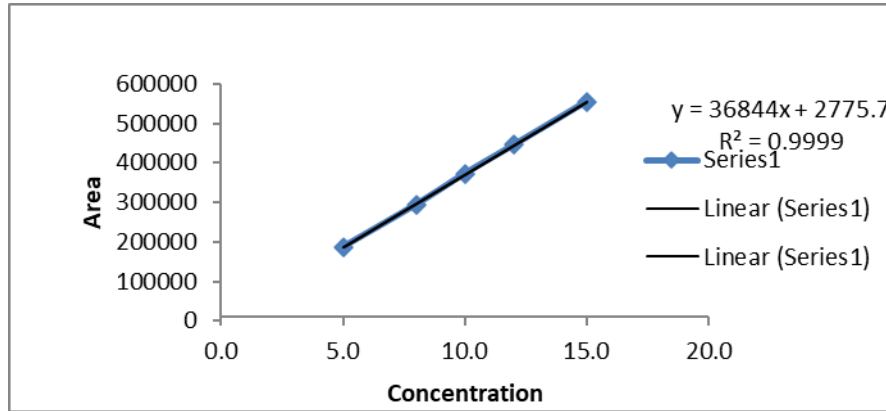


Fig : 21 Calibration curve of Dapagliflozin ( 5-15 µg/mL) R2 = 0.9999

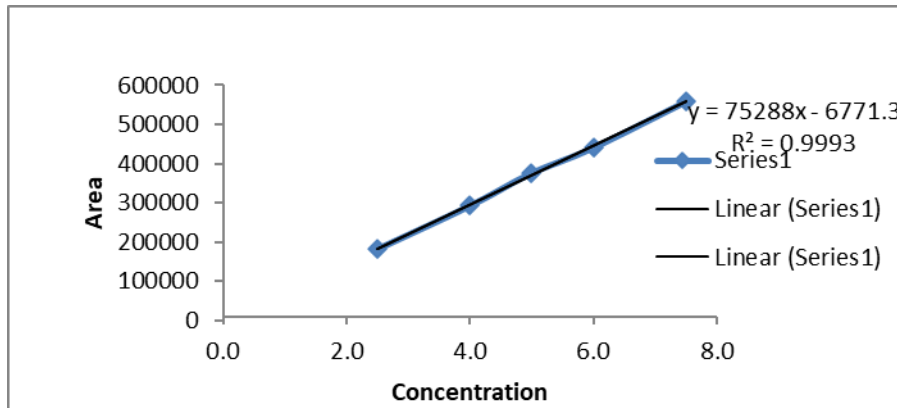


Fig : 22 Calibration curve of Linagliptin (2.5-7.5 µg/mL) R² = 0.9999

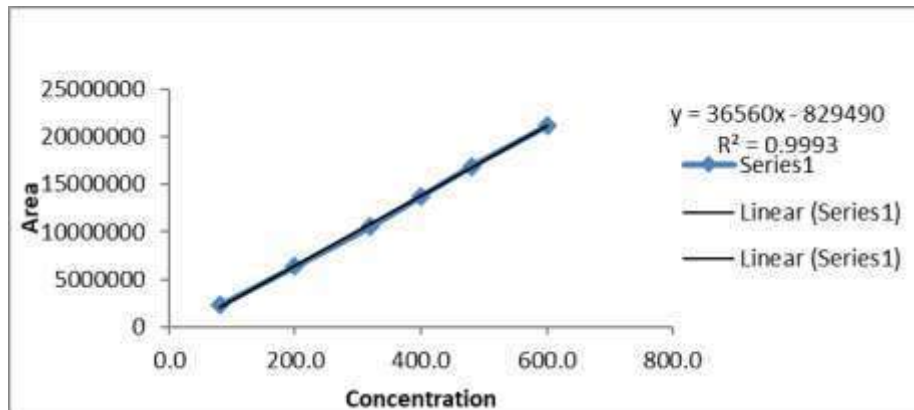


Fig : 23 Calibration curve of Metformin HCl (250-750 µg/mL) R² = 0.9993

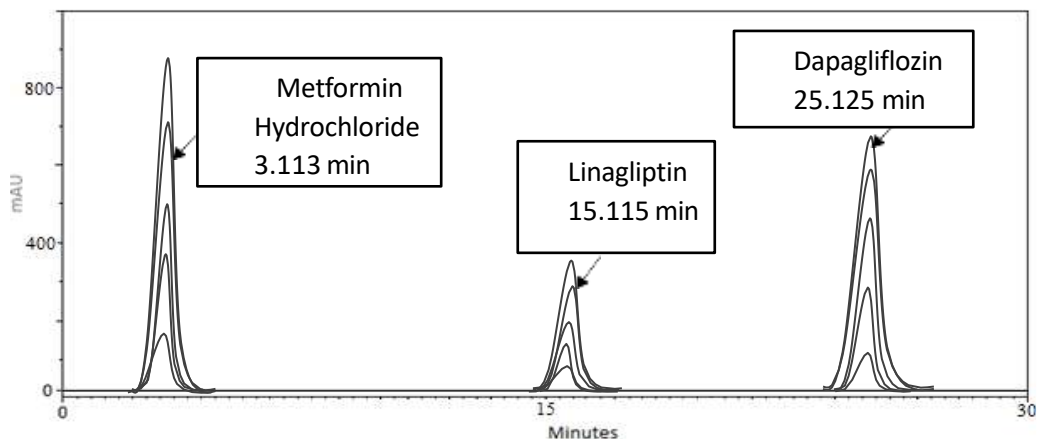


Fig : 24 Overlain Linearity Spectra of Dapagliflozin, Linagliptin and Metformin Hydrochloride

Table : 8 Linearity results for Dapagliflozin, Linagliptin and Metformin Hydrochloride

Regression Analysis	Dapagliflozin	Linagliptin	Metformin Hydrochloride
Concentration Range	5-15 µg/ml	2.5-7.5 µg/ml	250-750 µg/ml
Regression equation	$y = 36844x + 2775.7$	$y = 75288x - 6771.3$	$36560x - 829490$
Correlation co-efficient	0.9999	0.9993	0.9993

**Precision :**

shown in table 8.13. The % R.S.D For Repeatability data was found to be 0.50 % for Dapagliflozin , 0.38% for Linagliptin and 0.86% for Metformine Hydrochloride.

**Repeatability :**

The data for repeatability for Dapagliflozin, Linagliptin and Metformin Hydrochloride is

Table : 9 Repeatability data for Dapagliflozin, Linagliptin and Metformin Hydrochloride

Drugs	Conc. (µg/ml)	Mean Peak Area ± SD	%RSD
Dapagliflozin	10	3776324 ± 19111.14	0.50
Linagliptin	5	546511.7 ± 2097.61	0.38
Metformin Hydrochloride	500	13549340 ± 116906.38	0.86

**Inter-day precision :**

The % R.S.D for intraday precision was found to be 0.35-0.66 % for Dapagliflozin and 0.68-0.86 Linagliptin and 0.07-0.40 % for Metformin Hydrochloride.

The data for interday precision for Dapagliflozin, Linagliptin and Metformin is shown in table 8.14.

Table : 10 Inter-day precision data for estimation of Dapagliflozin, Linagliptin and Metformin Hydrochloride

Mcg/ml	Dapagliflozin		
	5	10	15
	375546	577682	725351
	370986	577763	725463
	374980	574090	729869
MEAN	373837.3333	576511.6667	726894.3333



± SD	2485.49	2097.61	2576.74
RSD	0.66	0.36	0.35

Linagliptin			
Mcg/ml	2.5	5	7.5
	188768	295461	374879
	185568	295564	378843
	187671	299890	373980
MEAN	187335.6667	296971.6667	375900.6667
± SD	1626.14	2527.87	2587.47
RSD	0.86	0.85	0.68

Metformin Hydrochloride			
Mcg/ml	250	500	750
	636732	10543511	13745631
	638879	10576671	13733551
	633456	10564781	13755416
MEAN	636355.66	10561654.3	13744866
± SD	2731.01	16799.66	10952.56
RSD	0.40	0.17	0.07

**Intra -day precision :**

The data for intra-day precision for Dapagliflozin, Linagliptin and Metformin Hydrochloride is

shown in table 11. The % R.S.D for intraday precision was found to be 0.35-0.66 % for Dapagliflozin, 0.68-0.86% for Linagliptin and 0.32 -0.93 % for Metformin Hydrochloride.

**Table : 11 Intra-day precision data for estimation of Dapagliflozin, Linagliptin and Metformin Hydrochloride**

Dapagliflozin			
Mcg/ml	5	10	15
	375546	577682	725351
	370986	577763	725463
	374980	574090	729869
MEAN	373837.3333	576511.6667	726894.3333
± SD	2485.49	2097.61	2576.74
RSD	0.66	0.36	0.35

Linagliptin			
Mcg/ml	2.5	5	7.5
	188768	295461	374879
	185568	295564	378843
	187671	299890	373980
MEAN	187335.6667	296971.6667	375900.6667
± SD	1626.14	2527.87	2587.47
RSD	0.86	0.85	0.68

Metformin Hydrochloride			
Mcg/ml	250	500	750



	6342511	10548792	13462811
	6398703	10533243	13241612
	6344510	10598783	13452610
MEAN	6361908	10560272.67	13385677.67
± SD	31881.08	34245.10	124868.74
RSD	0.50	0.32	0.93

**Accuracy :**

The difference between theoretically added amount and the practically achieved amount is called the accuracy of the analytical method. Accuracy of the method was performed by spiking

API to sample at specified levels. Accuracy was determined at three different levels 80%, 100%, and 120% of the target concentration in triplicate. The data show that the proposed method is accurate. Accuracy data is shown in the table. 12 :

**Table : 12 Data of recovery study of Dapagliflozin, Linagliptin and Metformin Hydrochloride**

<b>DAPAGLIFLOZIN</b>						
Level	Test (µg/mL)	Std Addition (µg/mL)	Total Conc. (µg/mL)	Mean Area ± SD (mV) (n=3)	Found Conc. (µg/mL)	%Recovery
80%	10	8	9	726132.3 ± 2272.022	15.76	99.62
100%	10	10	10	876799.67 ± 2379.07	20.24	101.05
120%	10	12	11	1058747.7 ± 16478.62	23.78	98.05
<b>Linagliptin</b>						
Level	Test (µg/mL)	Std Addition (µg/mL)	Total Conc. (µg/mL)	Mean Area ± SD (mV) (n=3)	Found Conc. (µg/mL)	%Recovery
80%	5	4	4.5	375873.33 ± 1231.85	9.41	102.10
100%	5	5	5	446238.33 ± 3447.78	10.23	101.09
120%	5	6	5.5	555659.33 ± 3846.98	11.29	99.96
<b>METFORMIN HCl</b>						
Level	Test (µg/mL)	Std Addition (µg/mL)	Total Conc. (µg/mL)	Mean Area ± SD (mV) (n=3)	Found Conc. (µg/mL)	%Recovery
80%	500	400	450	13657811 ± 33241.291	399.85	101.10
100%	500	500	500	16431414 ± 28896.75	512.95	102.40
120%	500	600	550	21536958 ± 140111.07	610.46	100.05

**LOD and LOQ :**

The limit of detection (LOD) and Limit of Quantification (LOQ) was found to be as per below:



**Table: 13 LOD and LOQ Limit for Dapagliflozin, Linagliptin and Metformin Hydrochloride**

Dapagliflozin		Linagliptin		Metformin Hydrochloride	
LOD( $\mu\text{g/ml}$ )	LOQ( $\mu\text{g/ml}$ )	LOD( $\mu\text{g/ml}$ )	LOQ( $\mu\text{g/ml}$ )	LOD( $\mu\text{g/ml}$ )	LOQ( $\mu\text{g/ml}$ )
0.20	0.68	0.34	1.15	0.15	0.51

**Limit of Detection (LOD)** is the lowest concentration of a substance that can be reliably distinguished from the absence of that substance (i.e., background noise).

**Equation for LOD:**

$$\text{LOD} = 3 \times \sigma / s$$

Where:

- $\sigma$  = Standard deviation of the blank (background noise)
- S = Slope of the calibration curve

**Limit of Quantification (LOQ)** is the lowest concentration of a substance that can be quantitatively measured with acceptable precision and accuracy.

**Equation for LOQ:**

$$\text{LOQ} = 10 \times \sigma / s$$

Where:

- $\sigma$  = Standard deviation of the blank (background noise)
- S = Slope of the calibration curve

**Selectivity :**

There is no interference in the mixture

**Robustness :**

The method is found to be robust as the results were not significantly affected by slight variation in Mobile Phase Composition and flow rate of mobile phase. The results are shown in table 14.

Variation seen was within the acceptable range respect to peak asymmetry and theoretical plates, so the method was found to be robust.

**Table :14 Robustness data for Dapagliflozin, Linagliptin and Metformin Hydrochloride**

Parameter	Level of Change	Effect on assay volume	
		Dapagliflozin	
		Assay $\pm$ SD	RSD
Flow rate	1.0 mL/min	98.70 $\pm$ 0.50	0.49
	0.9 mL/min	101.09 $\pm$ 0.72	0.72
	1.1 mL/min	99.80 $\pm$ 0.60	0.80
Mobile phase composition	58:38	98.47 $\pm$ 0.53	0.53
	60:40	98.39 $\pm$ 0.99	0.98
	62:42	99.51 $\pm$ 0.67	0.67

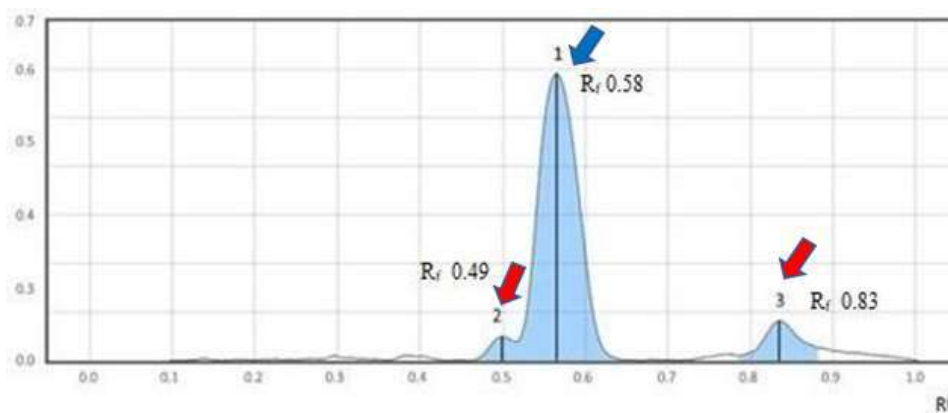
Parameter	Level of Change	Effect on assay volume	
		Linagliptin	
		Assay $\pm$ SD	RSD
Flow rate	1.0 mL/min	98.92 $\pm$ 0.48	0.48
	0.9 mL/min	55.92 $\pm$ 0.75	0.87
	1.1 mL/min	98.99 $\pm$ 0.83	0.83
Mobile phase composition	58:38	100.22 $\pm$ 1.43	1.43
	60:40	100.04 $\pm$ 1.06	1.06
	62:42	99.45 $\pm$ 0.77	0.78

Parameter	Level of Change	Effect on assay volume	
		Metformin Hydrochloride	
		Assay ± SD	RSD
Flow rate	1.0 mL/min	100.29 ±1.09	1.09
	0.9 mL/min	100.50 ±0.90	0.85
	1.1 mL/min	99.10 ±0.41	0.41
	58:38	98.98 ±0.30	0.30

Mobile phase composition	60:40	99.70 ±0.48	0.48
	62:42	99.30 ±0.12	0.12

**Alkali hydrolysis degradation :**

The densitogram showed peak at R<sub>f</sub> of 0.58 along with two degradation peaks at R<sub>f</sub> 0.49 (2) and 0.83 (3) indicating 15 % degradation Peak 1 (standard peak; R<sub>f</sub>: 0.58), peak 2 (degradant 2;R<sub>f</sub>: 0.49), peak 3(deradant3; R<sub>f</sub>: 0.83).

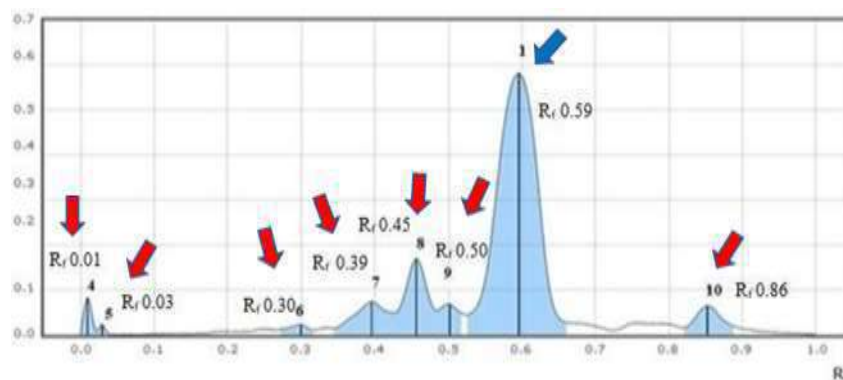


**Fig: 25 Densitogram of Alkali hydrolysis degradation with two degradants**

Peak 1 (standard peak; R<sub>f</sub>: 0.58), peak 2 (degradant 2;R<sub>f</sub>: 0.49), peak 3(deradant3; R<sub>f</sub>: 0.83).

The densitogram showed peak at R<sub>f</sub> of 0.59 along with seven peaks of degradation products at R<sub>f</sub> 0.01 (4), 0.03(5), 0.30 (6), 0.39 (7), 0.45(8), 0.50 (9) and 0.86 (10) resulting in 20.20 degradation (Figure 6.6 and Table 6.7).

**Acid Hydrolysis degradation :**



**Fig : 26 Densitogram of Acid Hydrolysis degradation with seven degradants**

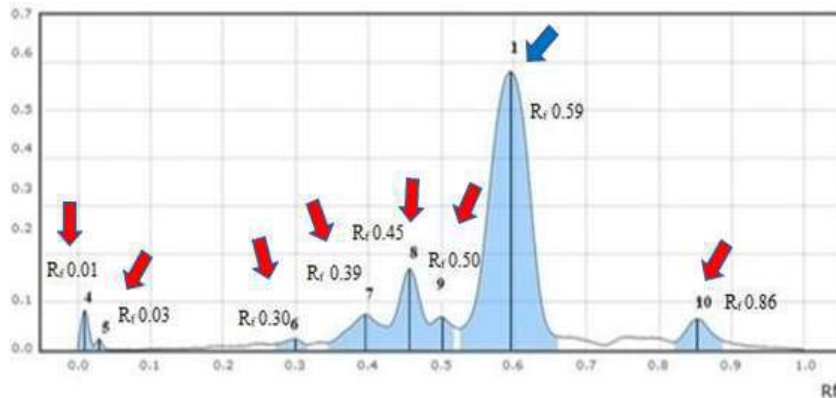
Peak 1 (standard peak; R<sub>f</sub> : 0.59), peak 4 (degradant 4; R<sub>f</sub>: 0.01),peak 5 (degradant 5; R<sub>f</sub>:

0.03), Peak 6 (degradant 6; R<sub>f</sub>: 0.30), peak 7 (degradant 7; R<sub>f</sub> : 0.39), peak 8 (degradant 8; R<sub>f</sub>:

0.45), peak 9 (degradant 9; Rf: 0.50), peak 10 (degradant 10; Rf: 0.86).

**Hydrolytic degradation :**

The densitogram showed peak at Rf of 0.59 along with seven peaks of degradation products at Rf 0.01 (4), 0.03(5), 0.30 (6), 0.39 (7), 0.45(8), 0.50 (9) and 0.86 (10) resulting in 20.20 % degradation.

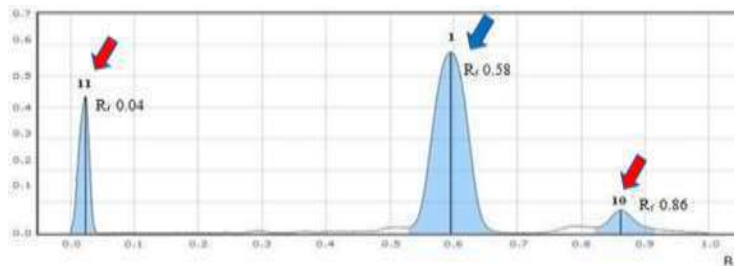


**Fig : 27 Densitogram of Hydrolytic degradation with seven degradants**

Peak 1 (standard peak; Rf : 0.59), peak 4 (degradant 4; Rf : 0.01), peak 5 (degradant 5; Rf : 0.03), Peak 6 (degradant 6; Rf : 0.30), peak 7 (degradant 7; Rf : 0.39), peak 8 (degradant 8; Rf : 0.45), peak 9 (degradant 9; Rf : 0.50), peak 10 (degradant 10; Rf: 0.86).

**Oxidative degradation :**

The densitogram of subjected to oxidative stress showed a peak of UPA at Rf of 0.58 along with two degradation peaks at Rf 0.86 (10) and 0.04 (11) indicating 17.25 % degradation.

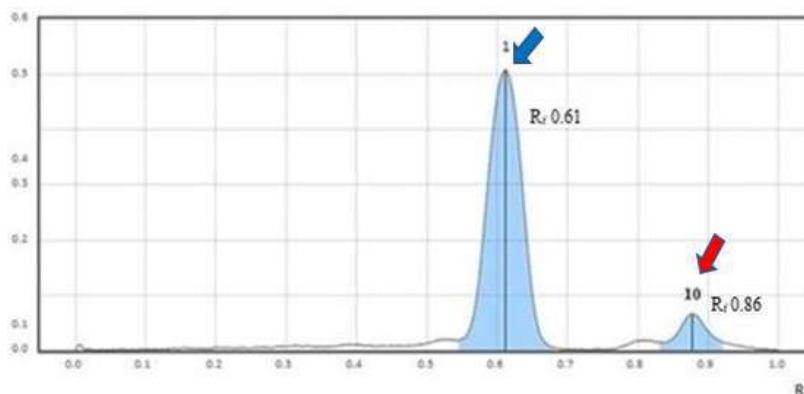


**Fig : 28 Densitogram of oxidative degradation with two degradants**

Peak 1 (standard peak; Rf : 0.58), peak 10 (degradant 10; Rf: 0.86), peak 11; (degradant 11; Rf: 0.04)

The densitogram showed peak of at Rf of 0.61 along with one degradation peak at Rf 0.86 (10) resulting in 10.02 % degradation.

**Thermal degradation :**

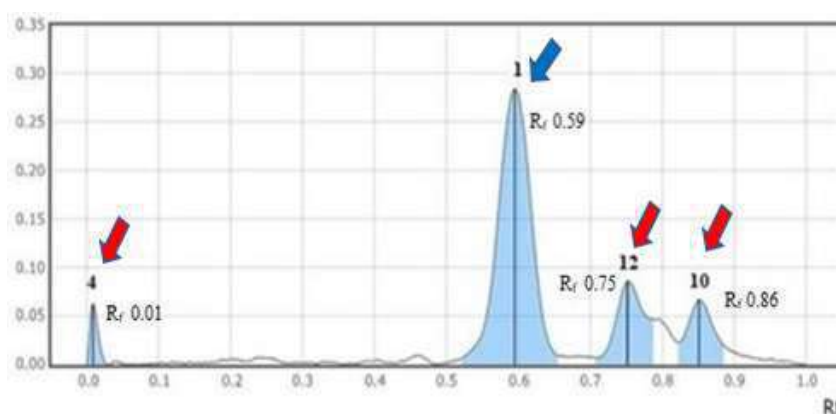


**Fig : 29 Densitogram of thermal degradation with one degradant**

Peak 1 (standard peak; R<sub>f</sub> : 0.61), peak 10 (degradant 10; R<sub>f</sub> : 0.86)

In the photodegradation, the peak of drug was observed at R<sub>f</sub> of 0.59 along with three degradation peaks at R<sub>f</sub> of 0.01 (4), 0.86 (10) and 0.75 (12)

**Photodegradation :**



**Fig : 30 Densitogram of photodegradation with three degradants**

Peak 1 standard peak; R<sub>f</sub>: 0.59), peak 4 (degradant peak 4; R<sub>f</sub> : 0.01), peak 10 (degradant 10; R<sub>f</sub>: 0.86), peak 12 (degradant 12; R<sub>f</sub>: 0.75)

**Analysis of marketed product :**

The proposed method was successfully applied Solid dosage form (Dapagliflozin 10mg, Linagliptin 5mg, Metformine hydrochloride 500mg) to analysis of the commercially available tablet formulation. The % drugs were found satisfactory, which is comparable with the corresponding label claim.

**Table : 15 Analysis of marketed formulations**

Drug	Amount taken (µg/mL)	Amount found (µg/mL)	% Assy
Dapagliflozin	10	2.93±0.04	99.80 ±1.20
Linagliptin	5	3.03 ±0.10	100.70 ±1.07
Metformin Hydrochloride	500	2.80±0.01	99.96 ±1.30

**SUMMARY OF METHOD VALIDATION :**

**Table : 16 Summary of validation parameter of RP-HPLC method**

Optimized chromatographic Condition	
Stationary Phase	Kromasil C-18 (id 4.6 mm x 150 mm, 5 $\mu$ m)
Mobile Phase	Acetonitrile: Phosphate buffer (60:40v/v)
Detection wave Length	272 nm
Flow rate	1 ml/minute
Run time	30 minutes
Retention Time	Dapagliflozin: 25.125 min, Linagliptin: 15.115 min, Metformin Hydrochloride: 3.113

Validation parameters					
Parameter	Limit	Result			Conclusion
		Dapagliflozin	Linagliptin	Metformin Hydrochloride	
Linearity and Range	R2> 0.995	0.999 (5-15 $\mu$ g/mL)	0.9993 (2.5-7.5 $\mu$ g/mL)	0.9993 (200-600 $\mu$ g/mL)	Method was linear
Repeatability	RSD<2	0.50	0.38	0.86	Method was repeatable
LOD	-	0.20	0.34	0.15	-
LOQ	-	0.68	1.15	0.51	-
Intra-day Precision	RSD<2	0.68-0.66%	0.32-0.93%	0.32-0.93%	Method was precise
Inter-Day Precision	RSD<2	0.35-0.66%	0.68-0.86%	0.07-0.40%	Method was precise
%Recovery	98-102%	98.22%	101.32 %	101.56 %	Method was accurate
Robustness	RSD<2	0.41– 0.63	0.40-0.91	100.04-1.06	Method was robust
Assay%		99.80 $\pm$ 1.20	100.70 $\pm$ 1.07	99.96 $\pm$ 1.30	-

## CONCLUSION :

A simple, economic, specific, accurate and precise Stability indicating HPLC methods have been developed and validated for the estimation of Dapagliflozin, Linagliptin and Metformine Hydrochloride in Tablet dosage form. All method validation parameters lie within its acceptance criteria as per ICH Q2(R2) guideline so we can conclude that methods are specific, linear, accurate and precise. In HPLC method, Linearity was observed in the concentration rang of Dapagliflozin is 5-15  $\mu$ g/mL , Linagliptin is 2.5-7.5  $\mu$ g/mL and Metformine Hydrochloride is 250-750  $\mu$ g/mL with correlation coefficient of correlation co-efficient for Dapagliflozin, Linagliptin and Metformine Hydrochloride are >0.999. The proposed method was successfully applied for the simultaneous estimation of three drugs in

combined dosage form. The assay value of Dapagliflozin was found to be 99.80  $\pm$ 1.20, Linagliptin was found to be 100.70 $\pm$ 1.07 and Metformin Hydrochloride was found to be 99.96 $\pm$ 1.30. The Mean recovery were found to be in the range 98-102% . LOD and LOQ were found to be 0.20  $\mu$ g/ml and 0.68  $\mu$ g/ml for Dapagliflozin, 0.34  $\mu$ g/ml and 1.15  $\mu$ g/ml for Linagliptin and 0.15  $\mu$ g/ml and 0.51  $\mu$ g/ml for Metformin Hydrochloride. Hence, proposed method is well suited for assay of Dapagliflozin, Linagliptin and Metformine Hydrochloride in its Tablet dosage form. it can be easily and conveniently adopted for routine analysis of Tablet dosage form.

**CONFLICTS OF INTEREST:** Authors have no conflict of interest.



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