

# Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Glimpiride and Linagliptin in Their Synthetic Mixture

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**ABSTRACT:** To determine Glimpiride and Linagliptin in their synthetic mixture, A Simple, Rapid and Precise UV Spectroscopic (Absorbance Correction Method) method was developed and validated in accordance with ICH Q2(R2) guidelines. Methanol has been used as a solvent in the development of UV Spectroscopic Method for Glimpiride and Linagliptin at 244nm and 296nm respectively. Linearity was observed in the concentration ranges of 2-10µg/ml for GLIM and 5-25µg/ml for LINA using absorbance correction method with Correlation coefficient of 0.999.

**KEYWORDS:** Glimpiride, Linagliptin, Absorbance Correction Method, Simultaneous Estimation, Analytical method validation.

the sulfonylurea receptors of the channel to promote insulin secretion from the beta cell.

**Adverse Effects:** Severe Hypoglycemia, Low Blood Sugar, GIT disturbances, Headache, Dizziness. [6,7,8]

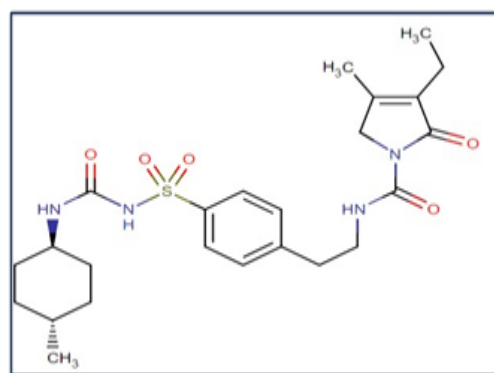


Figure 1: Chemical Structure of GLIM LINAGLIPTIN(LINA)

## I. INTRODUCTION

### Introduction to Diabetes:

Diabetes is a long-lasting condition in which the body's metabolism is affected, resulting in high levels of glucose (or sugar) in the blood. This can cause severe harm to the heart, blood vessels, eyes, kidneys, and nerves over a period of time.

### Types of Diabetes:

Two types of Diabetes are there:

#### A. Diabetes Mellitus:

1. Type-1 Diabetes or Insulin-dependent Diabetes Mellitus
2. Type-2 Diabetes or Non-Insulin-dependent Diabetes Mellitus
3. Gestational Diabetes

#### B. Diabetes Insipidus [1,2,3]

### GLIMEPIRIDE(GLIM)

**Mechanism of Action:** It is a Second- generation Sulfonylurea. It lowers blood sugar by stimulating the release of insulin from pancreatic beta cells and by inducing increased activity of Intracellular insulin receptors. It blocks the ATP-sensitive potassium channel by binding non-specifically to

**Mechanism of Action:** Linagliptin is a competitive, reversible DPP-4 inhibitor. Inhibition of this enzyme slows the breakdown of GLP-1 and Glucose- dependent Insulinotropic Polypeptide (GIP). GLP-1 and GIP stimulate the release of insulin from beta cells in the pancreas while inhibiting release of glucagon from pancreatic beta cells. These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose.

**Adverse Effects:** Itching or skin rash, Pain in stomach, Severe joint pain. [9]

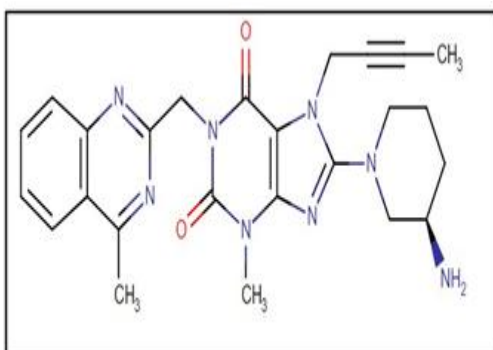


Figure 2: Chemical Structure of LINA

### ANALYTICAL METHOD DEVELOPMENT

The Analytical method involves the application of specific techniques and thorough step-by-step instructions utilized in examining a sample for one or more analytes in areas such as qualitative, quantitative, and structural analysis.

#### Need for Method Development

1. When there are no approved medications or combinations of medications listed in the pharmacopoeias.
2. The current analytical methods might necessitate expensive chemicals and solvents.

### ANALYTICAL METHOD VALIDATION

Method Validation is the process of establishing documented evidence which provides a high degree of assurance that a specific process or equipment will consistently produce a product meeting its predetermined specification and quality attributes. ICH Q2(R2) guideline for Validation of analytical procedures. [4,5]

## II. MATERIALS AND METHODS

- **Synthetic Mixture Formula Per Tablet (100 mg):**

Table 1: Synthetic Mixture Formula

Ingredients	Qty. (mg)	Uses
Glimepiride	2.00	API
Linagliptin	5.00	API
Microcrystalline Cellulose	35.00	Diluent
Mannitol	45.00	Diluent
Sodium Starch Glycolate	8.00	Disintegrant

Hydroxypropyl Methyl Cellulose	3.00	Binder
Magnesium Stearate	2.00	Lubricant

- **UV SPECTROSCOPIC METHOD DEVELOPMENT:**

- **ABSORBANCE CORRECTION METHOD:**

- **Apparatus and Instruments:**

- Double beam UV-Visible Spectrophotometer (Jasco V-530)
- Analytical Balance: Contech
- Ultra Sonicator: Citizen
- Filtration Assembly
- Volumetric Flasks: 10ml, 50ml, 100ml
- Pipettes: 1ml, 2ml, 5ml
- Measuring Cylinder: 10ml, 50ml, 100ml
- Beaker: 50ml, 100ml, 250ml, 500ml

- **Reagent and Chemicals:**

- Glimepiride: Exemed Pharmaceuticals Ltd.
- Linagliptin: CTX Lifescience Ltd.
- Methanol was used as a solvent throughout the experimentation.

- **UV Spectroscopic Conditions:**

- Solvent: Methanol
- Mode: Spectrum
- Scanning range: 200-400nm
- Scale of absorbance: 0.00-2.00
- Baseline correction: Methanol
- Diluent: Methanol

#### Preparation of Solutions for UV Spectroscopic Method:

- **Preparation of Standard Stock Solution:**

Accurately weighed 100mg of Glimepiride and 100mg of Linagliptin and transferred to individual 100ml volumetric flasks separately. 50 ml methanol was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with methanol and labelled as Standard stock solution 1 and 2. (1000µg/ml of Glimepiride and 1000µg/ml of Linagliptin).

- **Preparation of Standard Working Solution:**

10 ml from each stock solution was pipet out and taken into a 100ml volumetric flask and made up with methanol and labelled as Standard working solution 1 and 2. (100µg/ml of Glimepiride and 100µg/ml of Linagliptin).

- **Preparation of Sample Stock Solution-I:**

Accurately weighed the weight equivalent to 20mg of Glimepiride and 50mg of Linagliptin was transferred into a 10ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with methanol and filtered through Whatmann filter paper. (2000µg/ml of Glimepiride and 5000µg/ml of Linagliptin).

• **Preparation of Sample Working Solution-II:**

1 ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up volume with diluent (200µg/ml of Glimepiride and 500µg/ml of Linagliptin).

• **Selection of Wavelength:**

0.2 ml from the standard working solution of Glimepiride and 0.5 ml from the standard working solution of Linagliptin were transferred in separate 10 ml volumetric flask and dilute upto the mark with methanol to give a solution containing 2 µg/ml of GLIM and 5 µg/ml of LINA. Each solution was scanned in the UV range of 200-400 nm against methanol as blank. It was observed that GLIM was estimated at 230 nm where there was interference of LINA which was deducted from absorbance of GLIM at 244 nm. LINA was estimated at 296 nm where Glimepiride shows zero absorbance.

• **Preparation of Calibration Curve**

**1. Calibration Curve for Glimepiride:**

Calibration curve for Glimepiride consisted of Five different concentrations of standard solution of GLIM ranging from 2-10 µg/ml. The solution was prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and 1.0 ml from standard working solution-1 of GLIM (100µg/ml) into series of 10 ml volumetric flasks and the volume was made upto mark with methanol to get 2, 4, 6, 8 and 10 µg/ml respectively. Each solution was scanned against methanol as blank and corresponding spectra was recorded and GLIM

was estimated by taking Absorbance Correction at 244 nm.

**2. Calibration Curve for Linagliptin:**

Calibration curve for Linagliptin consisted of Five different concentrations of standard solution of LINA ranging from 5-25 µg/ml. The solution was prepared by pipetting out 0.5, 1.0, 1.5, 2.0 and 2.5 ml from standard working solution-2 of LINA (100µg/ml) into series of 10 ml volumetric flasks and the volume was made upto mark with methanol to get 5, 10, 15, 20 and 25 µg/ml respectively. Each solution was scanned against methanol as blank and corresponding spectra was recorded and LINA was estimated by taking absorbance difference at 296nm.

• **UV SPECTROSCOPIC METHOD VALIDATION:**

**1. Specificity:** Specificity was determined by examining standard drugs and sample of Glimepiride and Linagliptin. The results suggested that proposed method is specific, and the excipients present in the formulation does not affect the result.

**2. Linearity and Range:** The Linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2-10 µg/ml and 5-25 µg/ml for GLIM and LINA respectively (n=3). %RSD value less than 2% clearly indicate that the developed method is linear in range of 2-10µg/ml of GLIM and 5-25µg/ml of LINA.

**3. Accuracy:** Accuracy of the method was confirmed by recovery study from synthetic mixture at three levels of standard addition. Preparation of Sample solution for GLIM and LINA.

**Synthetic Mixture Solution X:** 200 µg/ml Glimepiride and 500µg/ml Linagliptin

**Working Standard Solution Y:** Glimepiride (100µg/ml)

**Working Standard Solution Z:** Linagliptin (100µg/ml)

**Table 2: Steps for Accuracy Measurement for Glimepiride**

Step1	Step2	Step3	Total Conc.
Take 0.4ml from solution X	-	Make upto 10 ml with Methanol	4 µg/ml
Take 0.4ml from solution X	Add 0.2 ml solution Y	Make upto 10 ml with Methanol	6 µg/ml
Take 0.4ml from solution X	Add 0.4 ml solution Y	Make upto 10 ml with Methanol	8 µg/ml
Take 0.4ml from solution X	Add 0.6 ml solution Y	Make upto 10 ml with Methanol	10 µg/ml

**Table 3: Steps for Accuracy Measurement for Linagliptin**

Step 1	Step 2	Step 3	Total Conc.
Take 0.4 ml from solution X	-	Make upto 10 ml with Methanol	10 µg/ml
Take 0.4 ml from solution X	Add 0.5 ml solution Z	Make upto 10 ml with Methanol	15 µg/ml
Take 0.4 ml from solution X	Add 1 ml solution Z	Make upto 10 ml with Methanol	20 µg/ml
Take 0.4 ml from solution X	Add 1.5 ml solution Z	Make upto 10 ml with Methanol	25 µg/ml

**4. Precision:**

**A. Repeatability:** Precision of the instrument was checked by repeated scan of the 3 concentrations (4, 6 and 8 µg/ml of GLIM and 10, 15 and 20 µg/ml of LINA) for 3 times without changing the spectroscopic conditions.

**B. Intraday Precision:** 3 concentrations (4, 6 and 8 µg/ml of GLIM and 10, 15 and 20 µg/ml of LINA) for 3 times on the same day without changing the spectroscopic conditions.

**C. Interday Precision:** 3 concentrations (4, 6 and 8 µg/ml of GLIM and 10, 15 and 20 µg/ml of LINA) for 3 times on three consecutive days without changing the spectroscopic conditions.

**5. Detection Limit (DL) and Quantitation Limit (QL):** The Detection Limit and Quantitation Limit was assessed from the set of three calibration curves that were used to

determine linearity of the method. DL was determined using the following formula:

$$DL = 3.3 \times S.D./Slope$$

QL was determined using the following formula:  
 $QL = 10 \times S.D./Slope$

Where, S.D. = Standard deviation of the Y-intercepts of three calibration curves

Slope = Mean slope of 3 calibration curves

**6. Robustness:** To study the robustness of method, analytical method was evaluated by changing the wavelength. The response of the method remains unchanged as a result of such modifications.

**7. Assay:** The proposed method was applied to analyze the combined synthetic mixture of Glimepiride and Linagliptin.

### III. RESULTS AND DISCUSSION

#### • Selection of Wavelength

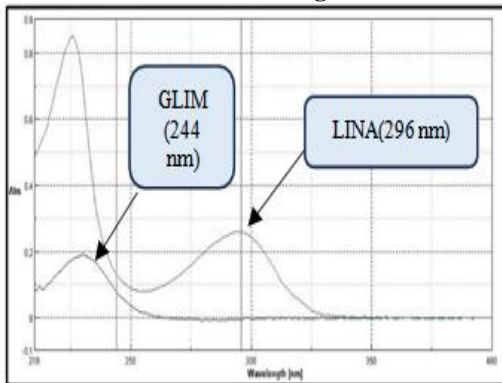


Figure 3: Selection of Wavelength

#### • Calibration Curve:

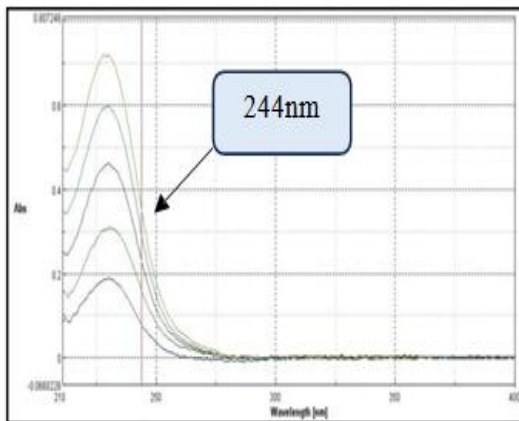


Figure 4: Overlain Spectra of Glimepiride

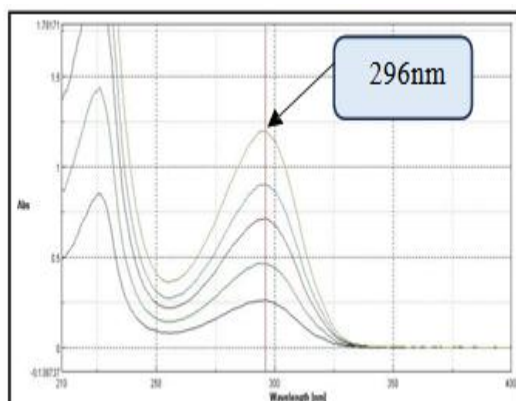


Figure 5: Overlain Spectra of Linagliptin

#### • UV SPECTROSCOPIC METHOD VALIDATION:

##### 1. Specificity:

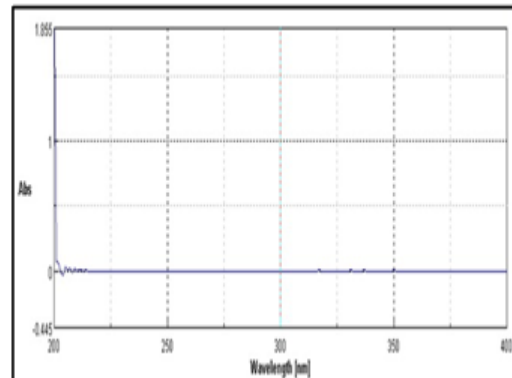


Figure 6: Spectra of Solvent- Methanol (Blank)

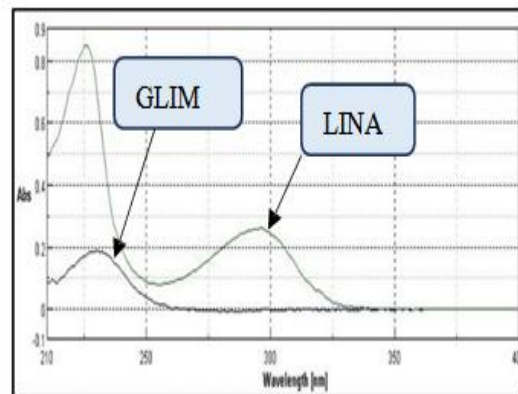


Figure 7: Spectra of GLIM and LINA

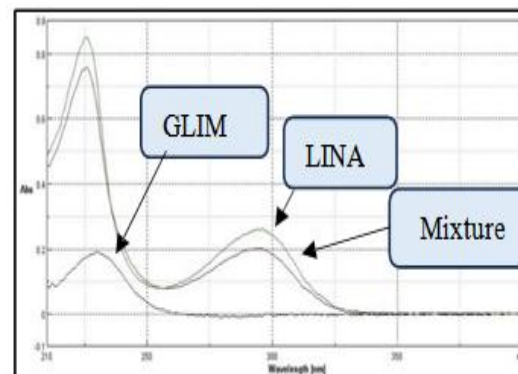


Figure 8: Spectra of Glimepiride, Linagliptin and Mixture

2. **Linearity and Range:** The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 2-10  $\mu\text{g/ml}$  and 5-25  $\mu\text{g/ml}$  for Glimepiride and Linagliptin respectively ( $n=3$ ) which is shown in Figure 9.



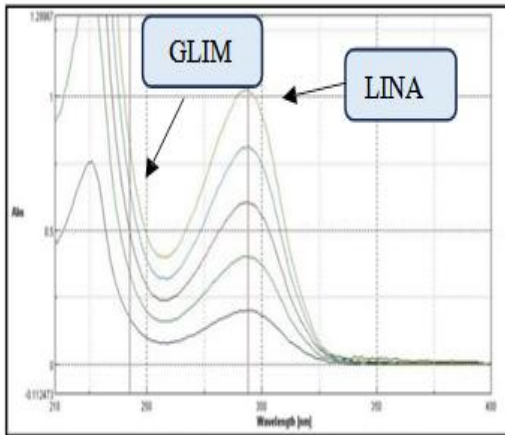


Figure 9: Overlain Spectra of GLIM and LINA (Synthetic mixture)

Table 4: Linearity of Glimepiride

Conc. (µg/ml)	Mean Absorbance± SD	%RSD
2	0.1975±0.00094	0.47
4	0.3724±0.00096	0.25
6	0.5633±0.00097	0.17
8	0.7427±0.00165	0.22
10	0.9235±0.00110	0.11
<b>Correlation Coefficient</b>	0.9996	
<b>Regression Equation</b>	$y = 0.0906x + 0.0202$	

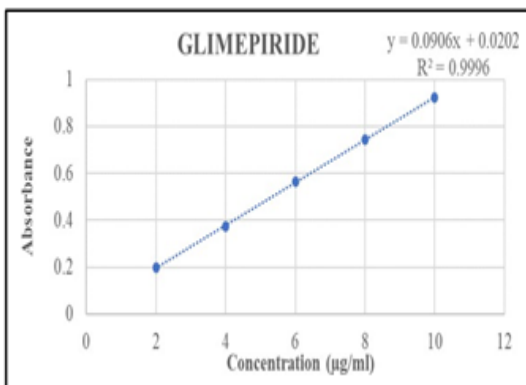


Figure 10: Calibration Curve of GLIM

Table 5: Linearity of Linagliptin

Conc. (µg/ml)	Mean Absorbance± SD	%RSD
5	0.2627±0.00145	0.55
10	0.4329±0.00115	0.26
15	0.6219±0.00176	0.28
20	0.8135±0.00376	0.46
25	1.0144±0.00225	0.22
<b>Correlation Coefficient</b>	0.9991	
<b>Regression Equation</b>	$y = 0.0377x + 0.0639$	

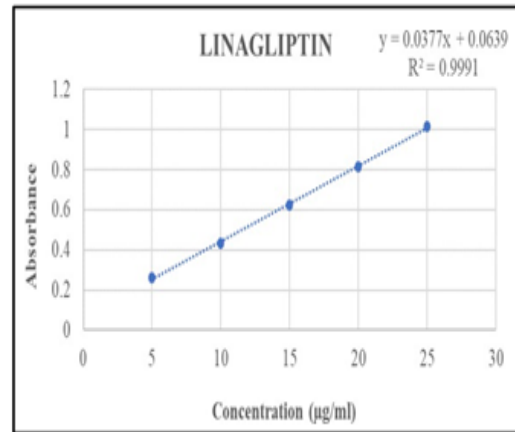


Figure 11: Calibration Curve of LINA

3. **Accuracy:** %Recovery for Glimepiride was found to be in range of 99.06-99.58%, while for Linagliptin it was found to be in range of 98.90-99.25%.

**Table 6: Accuracy Data of Glimepiride**

% Level	Target Conc. (µg/ml)	Std. Spiked conc. (µg/ml)	Total conc. (µg/ml)	Standard conc. (µg/ml)	% Recovery	Mean %Recovery ± SD	% RSD
0%	4	0	4	-	-	-	-
50%	4	2	6	1.99	99.55	99.1333± 0.59651	0.60
	4	2	6	1.96	98.45		
	4	2	6	1.98	99.40		
100%	4	4	8	3.97	99.30	99.0666± 0.58594	0.59
	4	4	8	3.98	99.50		
	4	4	8	3.93	98.40		
150%	4	6	10	5.97	99.53	99.5866± 0.19139	0.19
	4	6	10	5.96	99.43		
	4	6	10	5.98	99.80		

**Table 7: Accuracy Data of Linagliptin**

% Level	Target Conc. (µg/ml)	Std. Spiked conc. (µg/ml)	Total conc. (µg/ml)	Standard conc. (µg/ml)	% Recovery	Mean %Recovery ± SD	% RSD
0%	10	0	10	-	-	-	-
50%	10	5	15	4.94	98.88	99.2533± 0.33842	0.34
	10	5	15	4.96	99.34		
	10	5	15	4.97	99.54		
100%	10	10	20	9.85	98.57	98.9033± 0.29023	0.29
	10	10	20	9.90	99.04		
	10	10	20	9.91	99.10		
150%	10	15	25	14.87	99.13	99.2400± 0.10148	0.10
	10	15	25	14.89	99.26		
	10	15	25	14.90	99.33		

4. **Precision:** Low value of %RSD clearly ensures precision of the measuring device.

**Table 8: Repeatability of GLIM and LINA**

Name of Drug	Conc. (µg/ml)	Mean Abs. ± SD (n=3)	%RSD
Glimepiride	4	0.3726±0.00115	0.30
	6	0.5634±0.00148	0.26
	8	0.7427± 0.00145	0.19
Linagliptin	10	0.4326± 0.00165	0.38
	15	0.6232± 0.00173	0.27
	20	0.8154± 0.00261	0.32

**Table 9: Intraday Precision of GLIM and LINA**

Name of Drug	Conc. (µg/ml)	Mean Abs.± SD (n=3)	%RSD
GLIM	4	0.3732± 0.00160	0.42
	6	0.5627± 0.00220	0.39
	8	0.7433± 0.00204	0.27
LINA	10	0.4331± 0.00226	0.52
	15	0.6258± 0.00368	0.58
	20	0.8151± 0.00352	0.43

**Table 10: Interday Precision of GLIM and LINA**

Name of Drug	Conc. (µg/ml)	Mean Abs.± SD (n=3)	%RSD
GLIM	4	0.3729± 0.00197	0.52
	6	0.5647± 0.00255	0.45
	8	0.7438± 0.00238	0.32
LINA	10	0.4341± 0.00330	0.76
	15	0.6253± 0.00454	0.72
	20	0.8162± 0.00530	0.65

**5. Detection Limit (DL) and Quantitation Limit (QL):**

**Table 11: DL and QL of GLIM and LINA**

Name of Drug	DL(µg/ml) (n=3)	QL(µg/ml) (n=3)
GLIM	0.13	0.39
LINA	0.22	0.67

**6. Robustness: %: %RSD was found to be, for Glimepiride 0.42-0.48 % and for Linagliptin 0.24-0.30 %.**



**Table 12: Robustness of GLIM**

Sr. No.	Standard Absorbance	Glimepiride (244 nm)	
		+2 nm	-2 nm
1)	0.5622	0.5619	0.5625
2)	0.5664	0.5662	0.5667
3)	0.5617	0.5613	0.5621
<b>Mean</b>	0.5634	0.5631	0.5638
<b>SD</b>	0.0026	0.0027	0.0024
<b>%RSD</b>	0.46	0.48	0.42

**Table 13: Robustness of LINA**

Sr. No.	Standard Abs.	Linagliptin (296 nm)	
		+2 nm	-2 nm
1)	0.6217	0.6204	0.6227
2)	0.6238	0.6236	0.6239
3)	0.6203	0.6202	0.6208
<b>Mean</b>	0.6219	0.6214	0.6224
<b>SD</b>	0.0017	0.0019	0.0015
<b>%RSD</b>	0.27	0.30	0.24

7. **Assay:** The percentage purity of Glimepiride and Linagliptin was found to be  $98.666 \pm 0.76376\%$  and  $99.333 \pm 0.50332\%$  respectively.

**Table 14: Analysis of Synthetic Mixture**

Total Amount (mg)		Amount Found (mg)		%Purity (%)	
GLIM	LINA	GLIM	LINA	GLIM $\pm$ SD (n=3)	LINA $\pm$ SD (n=3)
2	5	1.97	4.99	98.666 $\pm$ 0.7637	99.333 $\pm$ 0.5033
2	5	1.96	4.97		
2	5	1.99	4.94		

#### IV. CONCLUSION

The UV Spectroscopic method is Developed and Validated for the Simultaneous Estimation of Glimepiride and Linagliptin in their synthetic mixture. Each validation parameters are performed in accordance with ICH Q2 (R2) guidelines and produces result within predetermined range, indicating that this method is

appropriate for routine quantitative analysis. The method is specific as there are no interference of impurities and excipients. The proposed method is found to be simple, accurate and precise.

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