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**DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ESTIMATION OF ANAGLIPTIN IN TABLET DOSAGE FORM BY U.V. SPECTROPHOTOMETRIC METHOD**

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**Abstract**

A simple and cost effective spectrophotometric method is described for the determination of anagliptin in pharmaceutical formulations. Anagliptin shows absorption maximum at 246 nm and obeys Beer's law in the concentration range 3-15 µg/ml. Calibration curves show a linear relationship between the absorbance and concentration with coefficient of correlation 0.999. The validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The percentage recovery was found to be 98.18-99.98% ± SD. The optimized method showed good reproducibility and recovery with standard deviation < 1 % and percent relative standard deviation less than 2 %. The proposed method will be suitable for analysis of anagliptin in pharmaceutical formulations in quality control purpose.

**Keywords:** Anagliptin, Validation, U.V. Spectroscopy, Method development.

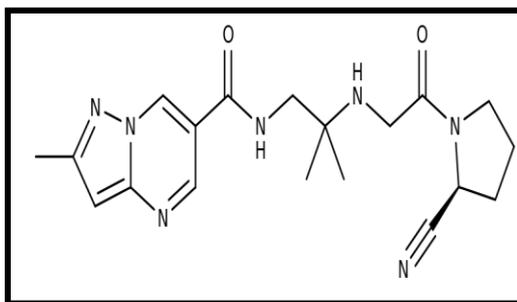
**Introduction**

Anagliptin is available in tablet dosage form. Chemically, Anagliptin N-[2-[[2-[(2S)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl]amino]-2-methylpropyl]-2-methylpyrazolo[1,5-a]pyrimidine -6-carboxamide.

Anagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor. It is used in Non-insulin dependent type-2 diabetes mellitus. It is available as white or pale yellow, crystalline powder or which is very soluble in water, freely soluble in methanol.

Anagliptin significantly inhibited the plasma DPP-4 activity and increased the plasma active GLP-1 levels in both the type diabetes. Anagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the GLP-1, gastrointestinal hormones released in response to a meal. By preventing GLP-1 inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the

pancreas. This drives blood glucose levels towards normal level.



**Figure 1: Structure of Anagliptin.**

## **Material and Methods**

### **Instrumentation**

Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

### **Chemicals and Reagents**

The bulk drug Anagliptin obtained from Intas Pharmaceuticals, Ahmedabad. Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

### **Selection of a Solvent**

Methanol was selected as solvent for studying spectral characteristic of drugs.

### **Preparation of Standard Solution**

#### **(A) Preparation of Standard Solution of Anagliptin**

##### **Preparation of Standard Stock Solution of Anagliptin (100µg/ml)**

Accurately weighed quantity of ANA 10 mg was transferred to 100 ml volumetric flask, dissolved in 10 ml of Methanol and diluted up to mark with Methanol to give a stock solution having strength of 100µg/ml.

##### **Preparation of Working Standard Solution of Anagliptin**

From the 100µg/ml stock solution 0.3 ml, 0.6ml, 0.9ml, 1.2ml, and 1.5ml of solution were taken and transferred to 10 ml volumetric flask and make up the volume up to 10 ml with methanol to produce concentration 3, 6, 9, 12 and 15 µg/ml respectively.

### **Selection of Analytical Wavelength**

The absorbance of solutions containing 3µg/ml was determined in UV range 200-800 nm using methanol as a blank. The  $\lambda_{max}$  was found to be 246 nm.

## **Preparation of Calibration Curve**

### **Calibration Curve for Anagliptin**

Various aliquots of different concentrations of standard ANA solution ranging from 3-15 µg/ml. The absorbance of the solutions was measured at 246 nm against Methanol as a blank. Calibration curve was plotted at wavelength and equation was formed using the absorbance.

### **Validation <sup>(1)</sup>**

#### **Linearity and Range**

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 3-15 µg/ml for ANA. (n = 5).

The calibration curve of absorbance against concentration was plotted and correlation coefficient and regression equations for ANA was calculated.

#### **Precision**

##### **(A) Repeatability**

Aliquots of 0.9 ml of working standard solution of ANA (100 µg/ml) were transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 9µg/ml solution of ANA. The absorbance of solution was measured six times and % RSD was calculated.

##### **(B) Intraday precision**

Aliquots of 0.6, 0.9, and 1.2 ml of working standard solution of ANA (100 µg/ml) were transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 6, 9 and 12µg/ml solution of ANA. Solution was analyzed 3 times on the same day and % RSD was calculated.

##### **(C) Interday Precision**

Aliquots of 0.6, 0.9, and 1.2 ml of working standard solution of ANA (100 µg/ml) were transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 6, 9 and 12µg/ml solution of ANA. Solution was analyzed 3 times on the 3 different days and % RSD was calculated.

#### **Limit of Detection (LOD)**

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

$$LOD = 3.3 *SD/Slope$$

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

### Limit of Quantification (LOQ)

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

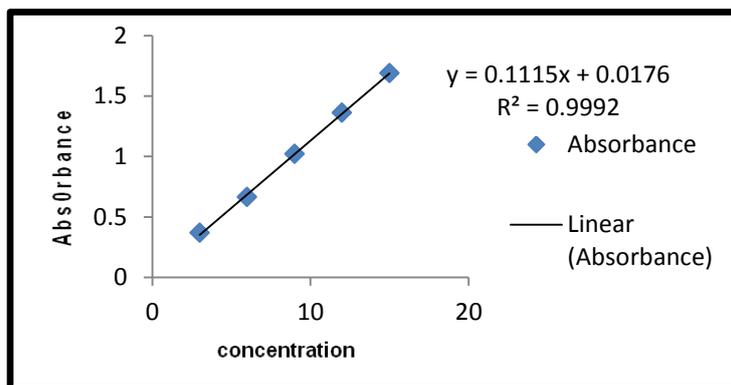
$$LOQ = 10 *SD/Slope$$

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

**Accuracy:** The accuracy of the method was determined by calculating recovery of ANA by the standard addition method. Aliquots of 0.48, 0.6, and 0.72 ml of working standard solution of ANA (100 µg/ml) were added at 80, 100 and 120 % level to pre-analyzed 0.6 ml sample solutions of ANA (100 µg/ml) transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 10.8, 12 and 13.2µg/ml solution of ANA. Absorbance of solution was measured at selected wavelength for ANA. The amount of ANA was calculated at each level and % recoveries were calculated by measuring the absorbance and fitting the values in equation. Accuracy was assessed using three concentrations and three replicates of each.

**Results and Discussion:** A reliable method was developed for estimation of Anagliptin by UV Spectrophotometer. Beer's law was obeyed in concentration range of 3-15 µg/ml for Anagliptin at 246 nm wavelength. The correlation coefficient of Anagliptin was found to be  $R^2 = 0.999$ . The mean % recoveries were found to be in the range of 98.18-99.98% for Anagliptin. The LOD and LOQ were 0.273µg/ml and 0.816µg/ml of Anagliptin respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analyte, which can be applied for the analysis of Anagliptin in tablet form.



**Figure 3: Calibration curve of Anagliptin (3-15 µg/ml) at 246nm.**

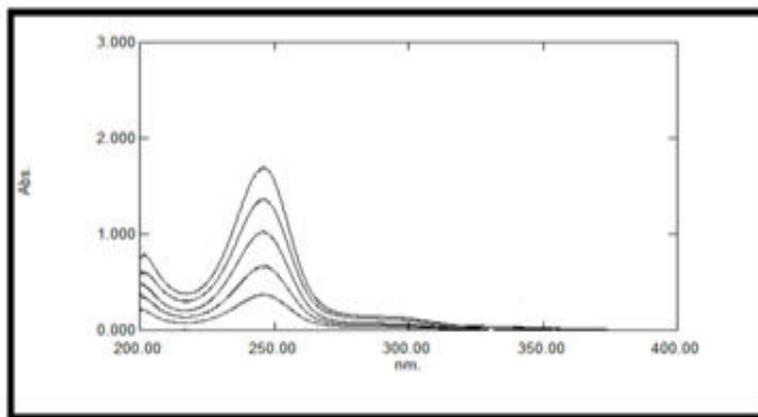


Figure 4: Overlay spectra of Anagliptin (3-15 µg/ml) at 246nm.

Table-1: Linearity data of Anagliptin.

AT 246 nm		
Con (µg/ml)	Mean absorbance ±SD(n=5)	%RSD
3	0.368±0.0018	0.508377
6	0.663±0.0027	0.407273
9	1.010±0.0100	0.990099
12	1.363±0.0022	0.167279
15	1.690±0.0068	0.406856

Table-2: Repeatability data of Anagliptin.

AT 246 nm		
Con (µg/ml)	Mean absorbance ±SD (n=6)	%RSD
9	1.0106±0.01078	1.060985

Table-3: Intraday Precision data of Anagliptin.

AT 246 nm		
Con (µg/ml)	Mean absorbance ±SD (n=3)	%RSD
6	0.663±0.001225	0.184728
9	1.0198±0.000837	0.082042
12	1.3626±0.00114	0.083676

**Table-4: Interday Precision data of Anagliptin**

AT 246 nm		
Con (µg/ml)	Mean absorbance ±SD	% RSD
6	0.66422±0.0025	0.376381
9	1.021±0.00103	0.100881
12	1.3698±0.002315	0.169003

**Table-5: Accuracy data of Anagliptin.**

Name of sample	Level	Amount taken (µg/mL)	Amount added (µg/mL)	Recovered Concentration (µg/mL)	% Recovery ± SD (n=3)
ANA	80	6	4.8	10.8	98.18
	100	6	6	12	98.33
	120	6	7.2	13.2	99.98

**Table 6: Assay Study Parameter.**

Anagliptin		
Concentration mg	Amount found mg	% Assay ± SD (n=3)
100	99.98	99.89333±0.090185

## Conclusion

The proposed Spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of ANA in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of ANA hence; the method is economic for estimation of ANA in tablet dosage form. Hence it can be conveniently adopted for routine quality control analysis of the drug.

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