



## Original Article

# Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin and Linagliptin in Combined Pharmaceutical Dosage Form

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### ABSTRACT

The objective of present work was to develop and validate a simple, accurate, precise HPLC method for the estimation of Metformin and Linagliptin. The chromatographic separation was achieved on a Hypersil BDSC18column (4.6x250 mm, 5µm particlesize). Different mobile phase systems in different proportions were tried. For HPLC method a mobile phase consisting of KH<sub>2</sub>PO<sub>4</sub> and acetonitrile(40:60) produced symmetric peak shape with good resolution for both the drugs. Next, the drugs were chromatographed under different flow rates from which a flow rate of 1.0 ml/min was selected. The retention times of Metformin and Linagliptin were found to be 2.464 min and 4.011 min, respectively. The proposed method was found to have excellent linearity in the concentration range of 100-600 and 0.5-3 µg/ml with correlation coefficient r<sup>2</sup>=0.999 and 0.999 for Metformin and Linagliptin respectively. The method was validated for linearity, precision, LOD, LOQ and robustness. The proposed method optimized and validated as per ICH guidelines.

**Keywords:** Metformin, Linagliptin, robustness.

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## 1. INTRODUCTION

Metformin and Linagliptin both are Antidiabetic Agents. Metformin is chemically C<sub>4</sub>H<sub>11</sub>N<sub>5</sub> 1-carbamimidamido-N, N dimethylmethanimidamide. Metformin is an oral hypoglycemic drug. it belongs to Biguanides. it is freely soluble in water. Sparingly

soluble in methanol. Linagliptin is chemically  $C_{25}H_{28}N_8O_2$ -(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-2,3,6,7-tetrahydro-1H-purine-2,6-dione. Linagliptin belongs to the class of Dipeptidyl peptidase 4-inhibitor, protease inhibitor. It is very soluble in Ethanol, Water.<sup>1-3</sup>

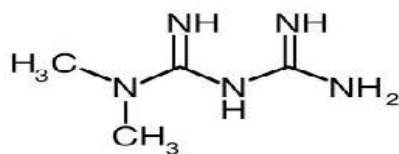


Fig 1: Metformin

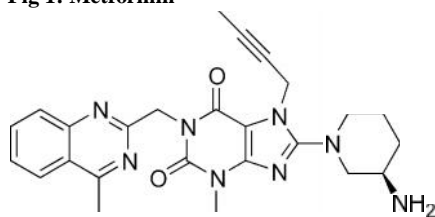


Fig 2: Linagliptin

Literature survey revealed that several methods were reported for Metformin and Linagliptin individually and in combinations Thamma Narendra Kumar et al 2011; Madhukar et al 2011; Kavitha.k et al 2013; Janardhan Swamy et al 2013; Shyamala.M et al; Pradeep G Sheelke et al; Dhirender Singh et al; Deepthi Jain et al; Lakshmi Raju Badugu et al; Himlal Paudel Chhetri et al; Sujatha.K et al. Therefore, the main objective of this study was to attempt to develop a simple and rapid analytical method for simultaneous estimation of Metformin and Linagliptin in a combined dosage form and validate the proposed assay.<sup>4-6</sup>

## 2. MATERIALS AND METHODS

**Apparatus:** The HPLC waters 2690/5 liquid chromatograph equipped with a PDA detector, the software installed was Empower, with 20 $\mu$ l loop, Hypersil-BDS C18 column (250mmx4.6mm, 5 $\mu$ l). The other instrument included are (SARTORIOUS) electronic balance and a sonicator (Fast clean).

### Chemicals and reagents

Active pharma ingredient of Metformin and Linagliptin was obtained as a gift sample from Arch pharma ltd, purified water HPLC grade was prepared by triple glass distillation and filtered through a 0.45 $\mu$  membrane filter.  $KH_2PO_4$  and Acetonitrile HPLC was run at a flow rate of 1.0ml/min, 20 $\mu$ l of the sample was injected in the chromatographic system. Mobile phase comprising of  $KH_2PO_4$  Acetonitrile at the ratio (40:60). The column temperature was ambient with a detection wavelength of 250nm.

### Preparation of standard solution

Stock solutions were prepared by dissolving 40mg of Metformin and 2mg of Linagliptin in mobile phase separately. Aliquots of standard solution of Metformin and Linagliptin were transferred into 10ml volumetric flasks and solutions were made up to the volume to yield concentrations of Metformin and Linagliptin.

### Pharmaceutical formulation

Formulation JENTADUETO-D2, manufactured by Boehringer Ingelheim Pharmaceuticals was purchased from the local pharmacy in Hyderabad.

### Preparation of sample solution

For analysis of commercial formulation, 20 tablets of Metformin 500mg and Linagliptin 2.5mg were weighed the average weight was calculated and powdered. A quantity equivalent to 500mg of Metformin and 2.5mg of Linagliptin was weighed and transferred to a 100ml volumetric flask which contain mobile phase and then shake it for 10mins and sonicate it for 20mins. The solution was allowed to stand at a room temperature for 20-30mins and filtered it through a whatmann filter paper. Then suitable aliquots of formulation solution were prepared and injected into HPLC to obtain concentration in linearity range.<sup>6-9</sup>

### Validation of analytical method:

**Accuracy:** Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. Recovery studies

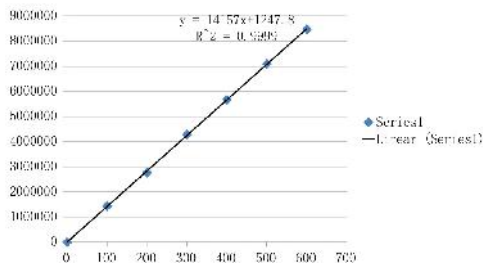
of the drug were carried out for determining accuracy parameter. Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out in 50% 100% and 150% levels.

**PRECISION:** The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The Precision expressed as standard deviation or relative standard deviation.

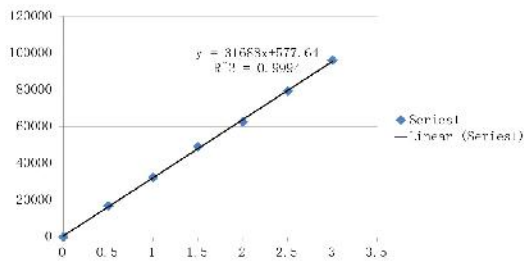
**a. System precision:** Standard solution prepared as per test method and injected five times.<sup>10</sup>

**b. Method precision:** Prepare five sample preparations individually using the single as per test method and injected each solution

**linearity:** The linearity of analytical method is the ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the given range. The linearity was performed by seven different concentrations, which were injected and calibration curve were plotted. The linearity of Metformin and linagliptin was found to be in the range of 100-600 and 0.5-3µg/ml respectively. The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for Metformin and Linagliptin is shown in figure.



**Fig 3: Plot of linearity and range study for Metformin**



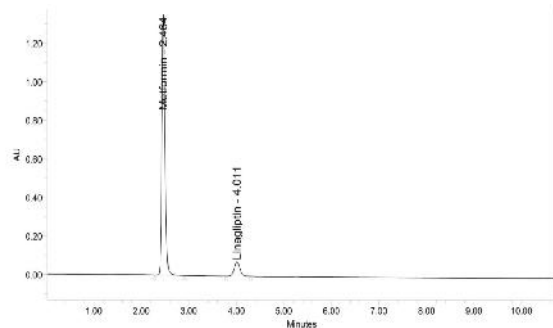
**Fig 4: Plot of linearity and range study for Linagliptin Ruggedness**

**a) System to System variability:** System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. A comparison of both the results obtained on two different HPLC systems, shows that the assay test method is rugged for System to system variables.<sup>11</sup>

**Robustness:** The robustness of an analytical procedure are a measure of its capacity to remain unaffected by small, but deliberate changes in the method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of combination of mobile phase and flow rate. % RSD of assay was calculated.

**Limit of detection (LOD) and Limit of quantification (LOQ)**

LOD of an analytical procedure is the lowest concentration of an analyte in a sample which can be detected but not necessarily quantitated as an exact value where as LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.



**Fig 5: Optimized chromatogram**

**Table 1: Results of validation parameters of RP-HPLC**

Sl. no	Validation Parameter	Metformin	Linagliptin	Acceptance Criteria
1.	Linearity (in $\mu\text{g}$ )	100-600	0.5-3	Correlation coefficient ( $R^2 = 0.999$ )
2.	Regression Line Equation	$y = 14157x + 1247.8$	$y = 31688x + 577.64$	
3.	$R^2$ Value	0.999	0.999	
4.	Precision			
	System Precision (%RSD)	0.72	0.66	
	Method Precision(%RSD)	0.68	0.43	RSD<2%
5.	LOD	0.29	0.06	
6.	LOQ	0.88	0.18	-
7.	Assay of marketed formulation	100.9%	100.8%	95-105%
8.	% Recovery	99.61-99.82	100.60-102.50	95-105%
9.	Ruggedness	0.4	0.5	RSD<2%

### 3. RESULTS AND DISCUSSION

- The slope, intercept and correlation coefficient values were found to be 14157, 1247.8 and 0.999 and 31688, 577.64 and 0.999 for Metformin and Linagliptin respectively.
- The LOD of Metformin and Linagliptin were found to be  $0.29\mu\text{g/ml}$  and  $0.06\mu\text{g/ml}$  respectively. The LOQ of Metformin and Linagliptin found to be  $0.88\mu\text{g/ml}$  and  $0.18\mu\text{g/ml}$  respectively.
- Precision of the developed method was studied. Low % RSD values indicate that the method is precise.

### 4. CONCLUSION

The proposed RP-HPLC method for the estimation of the Metformin and linagliptin in the pharmaceutical dosage form were simple, reliable and selective

providing satisfactory accuracy and precision with lower limits of detection and quantification. The recoveries achieved was good by RP-HPLC method. The methods can be recommended for routine and quality control analysis of these drugs in the pharmaceutical dosage forms. In this proposed method symmetrical peaks with good resolution were obtained.

**Table 2: Summary of analysis of Metformin and linagliptin by RP-HPLC method**

Drugs	Labeled amount, mg/tablet	Estimated Amount, mg/tablet	% Label claim	% *RSD
MET	500	500.08	100.9	0.715
		2.52		
LIN	2.5		100.8	0.652

### 5. ACKNOWLEDGEMENT

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