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Analytical Method Development and Validation by RP-HPLC for the Simultaneous Estimation of Linagliptin and Metformin in Bulk and Combined Tablet Dosage Form

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A B S T R A C T

A new simple accurate and suitable reverse phase high performance liquid chromatographic method was developed for the determination of Metformin and Linagliptin in bulk and tablet dosage form. The separation was eluted on a Zodiac C18 column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of mixed phosphate buffer 6.5 and acetonitrile in a ratio of 50:50 v/v at a flow rate of 1.0ml/min. The detection was made at 248 nm. The retention times were 2.080.1min for Metformin and 5.730.1min for Linagliptin. Calibration curve was linear over the concentration range of 125-750µg/ml for Metformin and 0.625 - 3.750 µg/ml for Linagliptin. The propose method was validated as per the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Keywords: Method development and validation, Metformin Linagliptin, Tablets, RPHPLC

A R T I C L E I N F O

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1. Introduction

Pharmaceutical analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. Pharmaceutical analysis comprises those procedures necessary to determine the

"identity, strength, quality and purity" of such articles on the synthesis of new compounds, the analyst is an indispensable team mate of the synthesis.[1,2] Pharmaceutical analysis includes both qualitative and quantitative analysis of Drugs and Pharmaceutical substances starts from bulk drugs (starting materials) to form product. In the new current practice of medicine, there are so many Analytical procedures are used in the analysis of chemical constituents found in human body whose altered concentrations during diseases states serve as diagnostic aids and also used to analyze the medicinal agents and their metabolites found in biological system.

High Performance Liquid Chromatography

Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the coloured substances by filtration through column, followed by developments with pure solvents. High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids [3].

IUPAC name of Metformin carbamimidamid N, dimethyl methanimidamide. Linagliptin 8-[(3R)-3-amino piperidin-1-yl]-7-(but-2-yn-1-yl) 3-methyl-1[methylquinazoliyl)methyl] -2, 3, 6, 7-tetrahydro-1H-purine-2,6-dione. [4,5,6].

Structures:

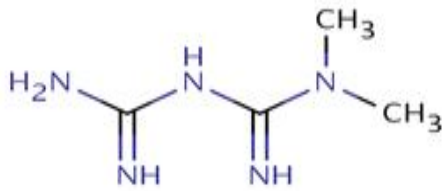

Figure 1: Structure of Metformin

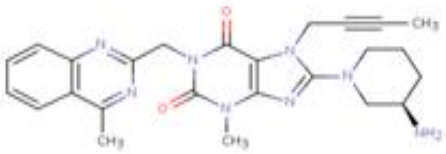

Figure 2: Structure of Linagliptin

2. Materials and Methods

Materials:

Metformin and Linagliptin, Combination Metformin and Linagliptin tablets distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument:

HPLC instrument used was of WATERS HPLC 2695 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer Systrnonics Instruments and matched quartz was used for measuring absorbance for Metformin and Linagliptin solutions.

Methods:

Standard Preparation:

Accurately Weighed and transferred 250mg of Metformin and 10mg Linagliptin of working Standards into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation:

For analysis of commercial formulation, 20 tablets of Metformin and Linagliptin 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 filterd it through a whatmann filter paper.

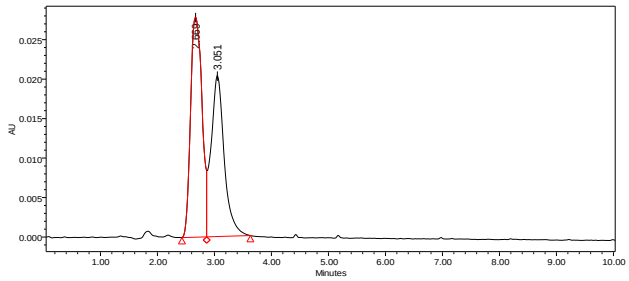

Figure 3: Trial chromatogram 1

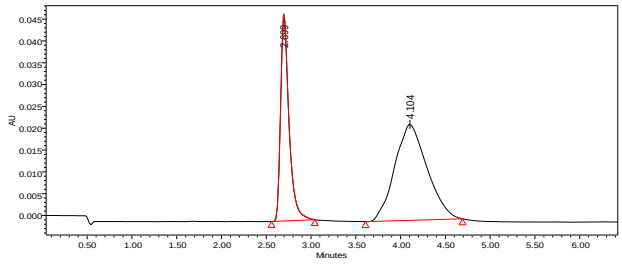

Figure 4: Trial chromatogram 2

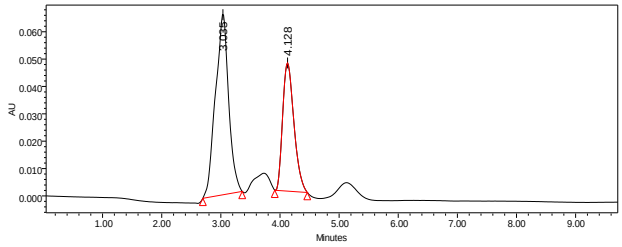

Figure 5: Trial chromatogram 3

Observation:

Metformin, Linagliptin peaks were sharp, but extra peak was observed. so further tail is carried out **Observation:** peak shapes are not good.

Observation: peak shapes are not good.

Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : Inertsil -ODS C18(250 x 4.6 mm, 5 µ)

Mobile phase : Methanol: Acetonitrile (80:20)
Flow rate : 1ml/min
Wavelength : 248nm
Temperature : Ambient
Injection Volume : 20µl

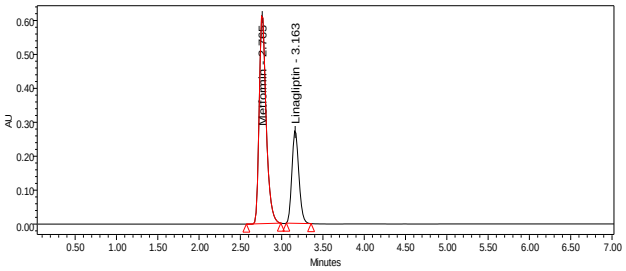

Figure 6: Optimized chromatogram of Metformin and Linagliptin

Observation: peak shape and retention time is good

3. Results and Discussion

1. System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Table 1: System suitability studies of Metformin and Linagliptin method

Property	Metformin	Linagliptin
Retention time (tR)	2.764 min	3.162 min
Theoretical plates (N)	3980± 63.48	7881 ± 63.48
Tailing factor (T)	1.75 ± 0.117	1.09± 0.117

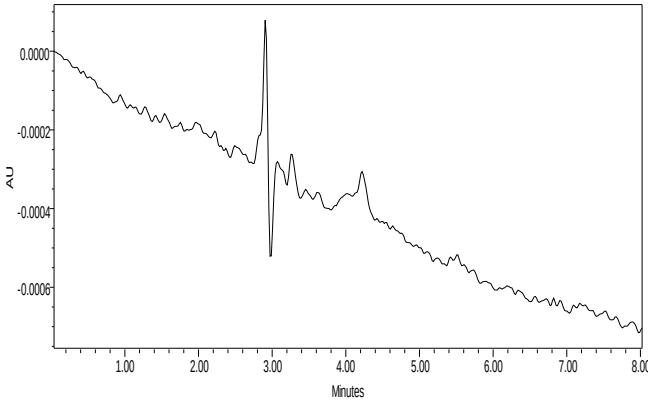

Figure 7: Chromatogram of blank

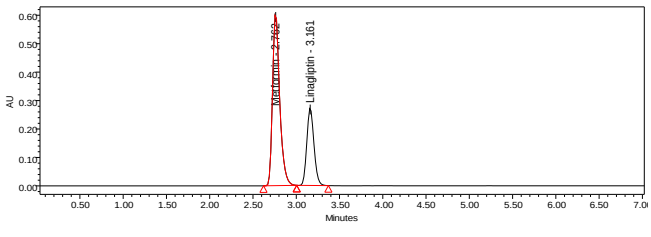

Figure 8: Typical chromatogram of Metformin and Linagliptin.

2. Linearity:
Six Linear concentrations of Metformin (20-80ppm) and Linagliptin (2.5-30ppm) are prepared and injected. Regression equation of the Metformin and Linagliptin are found to be, y = 10831x - 34273, and y = 213030x + 31232. And regression co-efficient was 0.999.

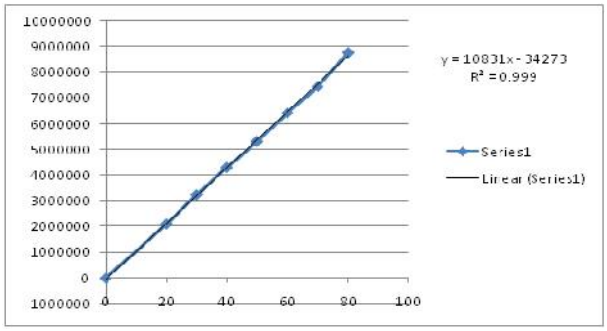

Figure 9: Calibration curve of Metformin

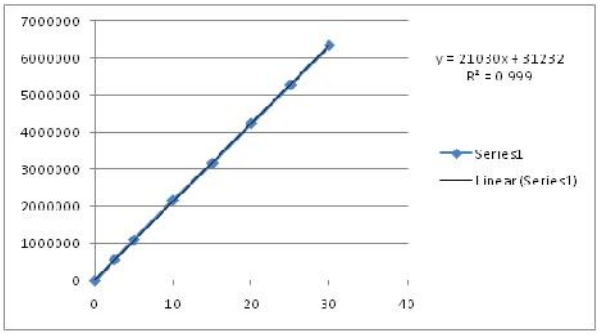

Figure 10: Calibration curve of Linagliptin

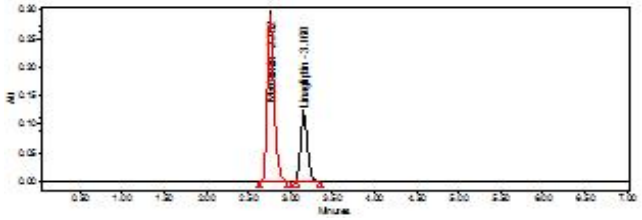

Figure 11: Linearity 20% Chromatogram of Metformin and Linagliptin.

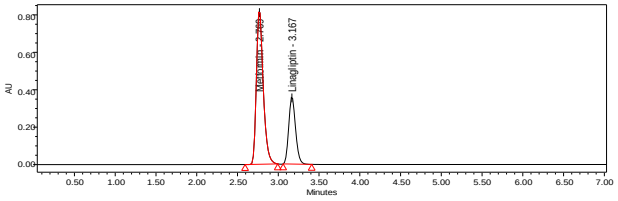

Figure 12: Linearity 60% Chromatogram of Metformin and Linagliptin.

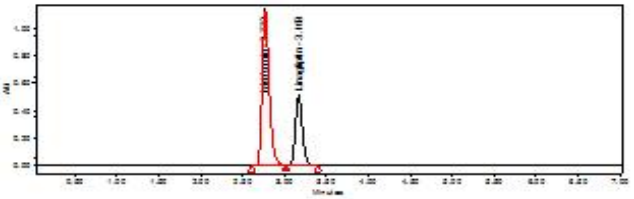

Figure 13: Linearity 80% Chromatogram of Metformin and Linagliptin.

3. Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RS for Metformin and Linagliptin were found to be 0.65% and 0.90% respectively.

Table 2: Repeatability results for Metformin and Linagliptin

S.NO	Metformin	Linagliptin
1	4196762	2245703
2	4237539	2291408
3	4219201	2278639
4	4278401	2239286
5	4235847	2267407
6	4219201	2278639
Mean	4231159	2266847
Std. Dev.	27435.85	20436.91
%RSD	0.648424	0.901557

*Average of six determinations

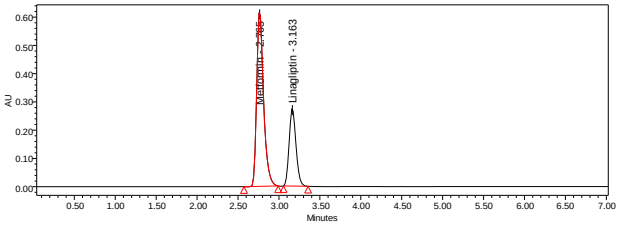

Figure 14: Repeatability Chromatogram of Metformin and Linagliptin

Inter day precision:

Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Metformin and Linagliptin were 0.38% and 0.72%.

Table 3: Inter day precision results for Metformin and Linagliptin.

S.NO	Metformin	Linagliptin
1	4219201	2278639
2	4237216	224732
3	4235847	2267407
4	4195611	2254490
5	4226557	2231236
6	4237216	2267407
Mean	4225275	2260652
Std. Dev.	16219.94	16338.36
%RSD	0.383879	0.722728

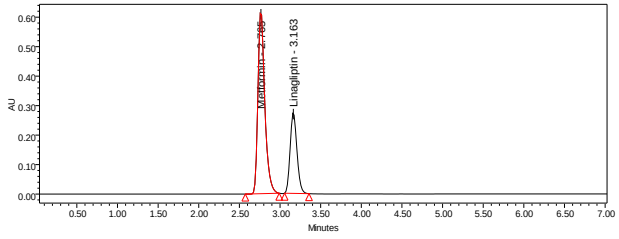

Figure 15: Inter Day precision Chromatogram of Metformin and Linagliptin

4. Accuracy:

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 6.5.

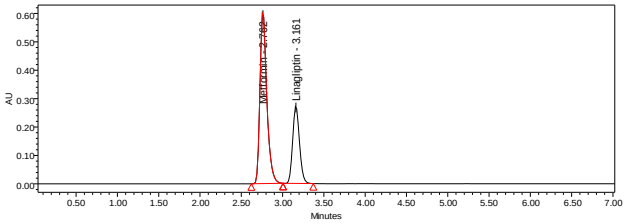

Figure 16: Accuracy 100% Chromatogram of Metformin and Linagliptin

5. LOD:

Limit of detection was calculated by STD deviation method Metformin and Linagliptin and LOD for Metformin and Linagliptin were found to be 0.491and 0.522respectively.

$$LOD = \frac{3.3\,\sigma}{S}$$

$$= \frac{3.3\times1621.94}{10893} = 0.491$$

6. LOQ:

Limit of Quantification was calculated by STD deviation method Metformin and Linagliptin and LOQ for Metformin and Linagliptin were found to be 1.48 and 1.58 respectively.

$$LOQ = \frac{10\,\sigma}{S}$$

$$= \frac{10\times1621.94}{10893} = 1.48$$

7. Robustness:

Small deliberate changes in method like Flow rate, mobile phase ratio and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Table 4: Robustness data of Metformin and Linagliptin

S.NO	Robustness condition	Metformin %RSD	Linagliptin %RSD
1	Flow minus	0.5	0.3
2	Flow Plus	0.1	0.7
3	Mobile phase minus	0.3	0.5
4	Mobile phase Plus	0.5	0.2
5	Temperature minus	0.4	0.5
6	Temperature Plus	0.1	0.2

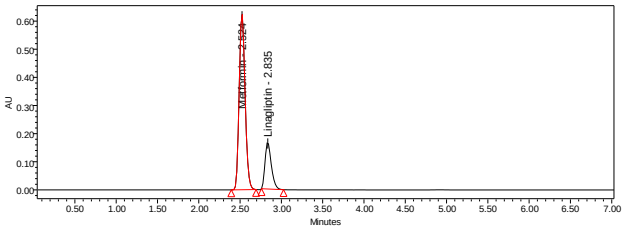

Figure 17: Flow plus Chromatogram of Metformin and Linagliptin

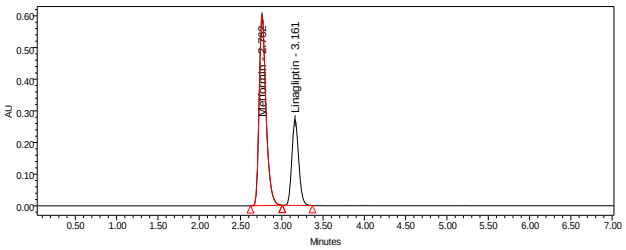

Figure 18: Mobile phase minus Chromatogram of Metformin and Linagliptin

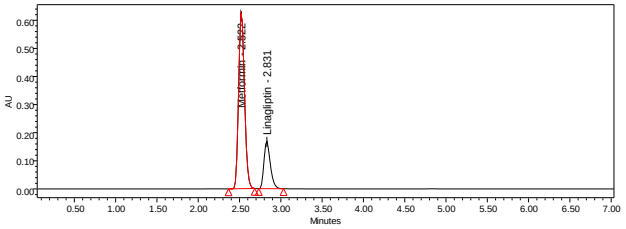

Figure 19: Temperature plus Chromatogram of Metformin and Linagliptin

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 99.24% and 99.82% for Metformin and Linagliptin respective.

Figure 5: Assay Chromatogram

S.NO	Metformin	Linagliptin
1	98.54	99.55
2	99.58	99.88
3	98.86	99.40
4	99.56	100.30
5	99.86	100.53
6	99.06	99.28
Mean	99.24333	99.82333
Std. Dev.	0.503812	0.505754
%RSD	0.507653	0.506649

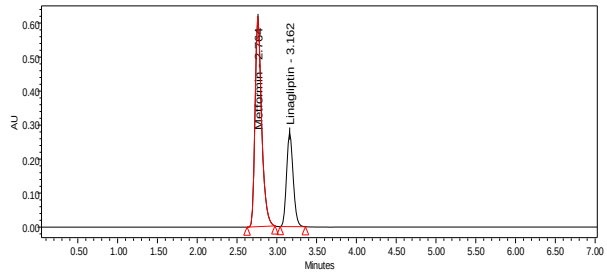

Figure 20: Assay of Metformin and Linagliptin

Table 6: Calibration data of Metformin and Linagliptin method

S.NO	Conc. Metformin (µg/ml)	Response	Conc. Linagliptin (µg/ml)	Response
1	0	0	0	0
2	10	2110652	2.5	560960
3	20	3250149	5	1102034
4	30	4307216	10	2164732
5	40	5320468	15	3171457
6	50	6427385	20	4138838
7	60	7452108	25	5276830

Table 7: Accuracy results of Metformin and Linagliptin

Sample	Concentration (%)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Metformin	50	49.83	100.75	0.92
	100	100.03	99.70	0.41
	150	150.07	100.21	0.31
Linagliptin	20	20.04	100.22	0.18
	40	39.78	100.02	0.091
	60	59.88	99.98	0.09

4. Conclusion

Finally with the above experimental data and results the developed RP-HPLC method is suitable for determination of Metformin and Linagliptin. The developed method is having the following advantages. Requires less runtime for recording chromatograms were less than 10min. percentage of recovery shows that the proposed method is free from interferences of excipients used in the formulation.

Therefore the proposed method is simple, precise, and accurate that can be effectively applied for routine analysis in quality control department of bulk drugs and tablet dosage forms.

5. Acknowledgement

I gives my immense pleasure to express my sincere thanks to my guide V.Pavan kumar, Ratnam Institute of Pharmacy, Nellore, A. P, India.

6. References

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