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RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND LINAGLIPTIN IN TABLET DOSAGE FORM

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ABSTRACT

An economical RP-HPLC method using a PDA detector at 225 nm wavelength for simultaneous estimation of Metformin and Linagliptin in pharmaceutical dosage forms has been developed. The method was validated as per ICH guidelines over a range of 250-2500 µg/mL and 1.25-12.5 µg/mL for Metformin and Linagliptin respectively. Analytical column Water's X-Bridge C18, 150 × 4.6 mm, five µ was used at a temperature of 30°C ± 0.5°C. Acetonitrile and 0.02M phosphate buffer (pH 5.0) in the ratio of 35:65% v/v composition were used as mobile phase at a flow rate of 1.0 mL/min. Retention times of 1.6 and 4.6 min were obtained for Metformin and Linagliptin respectively. The percentage recoveries of Metformin and Linagliptin are 100.12% and 99.42% respectively. The goodness of fit was close to 1 for all the three components. The relative standard deviations are always less than 2%.

Keywords: Linagliptin, Metformin, RP-HPLC, Simultaneous analysis, Tablets

INTRODUCTION

Metformin[1] , chemically N, N-Dimethylimidodicarbonimidic diamide is an oral antidiabetic drug in the biguanide class[Fig.-1A]. It is the first-line drug of choice for the treatment of type-II diabetes[2]. Metformin suppresses glucose production by the liver. It helps in reducing LDL cholesterol and triglyceride levels.

Linagliptin[3], chemically, 8- [(3R)- 3 -aminopiperidin-1-yl] -7- (but-2-yn-1-yl) -3-methyl-1- [(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1H-purine-2,6-dione is an DPP-4 inhibitor developed by Boehringer Ingelheim for treatment of type-II diabetes[Fig.-1B]. Linagliptin is an inhibitor of DPP-4. It stimulates the release of insulin in a glucose- dependent manner and decreases the levels of glucagon in the circulation.[4]

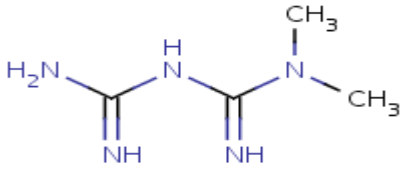

Fig.-1A: Metformin

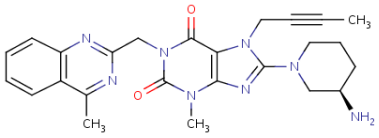

Fig.-1B: Linagliptin

The detailed survey of literature revealed that several Spectrophotometric methods[5-9], HPLC methods[10-12], Stability indicating methods[13,14] and Plasma extraction methods[15,16] were reported for the determination these drugs individually or in combination with other drugs in pharmaceutical dosage forms. A few

HPLC methods are available with the combination of above-cited drugs[17-21], with lower linearity range and or having longer retention times. The author made an attempt to develop and validate a cost-effective RP-HPLC assay method for estimation of Metformin and Linagliptin from formulated dosage form. The developed method is validated as per ICH and all relevant guidelines [22-27] for broad linearity range than other available methods and with better retention times.

EXPERIMENTAL

Reagents and Chemicals

Working standards of Metformin and Linagliptin obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples. HPLC grade, Water and Acetonitrile and methanol procured from Merck Chemical Division, Mumbai. JENTADUETO tablets containing 500mg of Metformin and 2.5 mg of Linagliptin were purchased from the pharmacy

Instrumentation and Chromatographic Conditions

2996 series of Waters Photodiode array detector attached to 2995 series of Waters HPLC, which is having Hamilton syringe and autosampler opted for chromatography. A degasser to remove the dissolved air and column oven to maintain the desired temperature is also available in the system. Mobile phase with a composition of Acetonitrile: 0.02 M phosphate buffer (pH5.0): 35:65 v/v with 1.0 mL flow rate and Waters Xbridge C18, 4.6*150mm, 5µ as a stationary phase with an injection volume of 10 µL were selected as chromatographic conditions. Detector wavelength was fixed at 225 nm.

Orking Standard Stock Solution Preparation

50 mg of Metformin and 10 mg of Linagliptin working standards were accurately weighed, and these samples were transferred to 10mL and 100mL volumetric flasks respectively, containing diluent. The mixture was sonicated for 5 minutes to aid dissolution and finally made up to the volume with the same diluent. The below table indicates the dilutions and the concentration of the stock solution.

PREPARATION OF CALIBRATION CURVE STANDARDS

Calibration curve spiking solutions were prepared from respective stock solutions in the range of 250-2500µg/mL for Metformin and 1.25-12.5µg/mL for Linagliptin respectively as shown in Table-1.

Table-1: Calibration curve standards Preparation

Concentration of standard stock solution (µg/mL) Metformin	Vol. taken (mL)	Final volume	Conc. of Metformin (µg/mL)	Concentration of standard stock solution (µg/mL) Linagliptin	Vol. taken (mL)	Final volume	Conc. Of Linagliptin (µg/mL)
5000	0.5	10	250	100	0.125	10	1.25
	1		500		0.25		2.5
	1.5		750		0.375		3.75
	2		1000		0.5		5
	3		1500		0.75		7.5
	5		2500		1.25		12.5

Tablet Solution (Sample) Preparation

Twenty tablets of JENTADUETO containing 500 mg of Metformin 2.5 mg of Linagliptin were weighed and ground into fine powder. Powder equivalent to the weight of five tablets was accurately weighed and transferred to a 500ml volumetric flask containing a few mL of diluent. After thorough mixing and sonication, this solution is filtered using 0.45-micron filter paper. 2.0 ml of this solution was transferred to 10 ml volumetric flask, and the solution was made up to the volume with diluent.

RESULTS AND DISCUSSIONS

Method development

After the selection of the drug combination, both the drugs were dissolved in suitable diluent to get a clear solution. Based on the literature reverse phase chromatography was identified as an appropriate chromatography separation method. The mobile phase was optimized by modifying different combinations of buffers and organic solvents. The pKa values of both the drugs were considered for optimization of pH of the buffer. The resolution and the peak shape of both the drugs found significant with the mobile phase composition of Acetonitrile: 0.02Mphosphate buffer (pH5.0): 35:65 v/v at a flow rate of 1mL/min and analyzed at 225 nm. The retention time observed (1.6 for MET and 4.6 for LIN) allows a rapid determination of these drugs. A typical chromatogram is shown below in Figure-2.

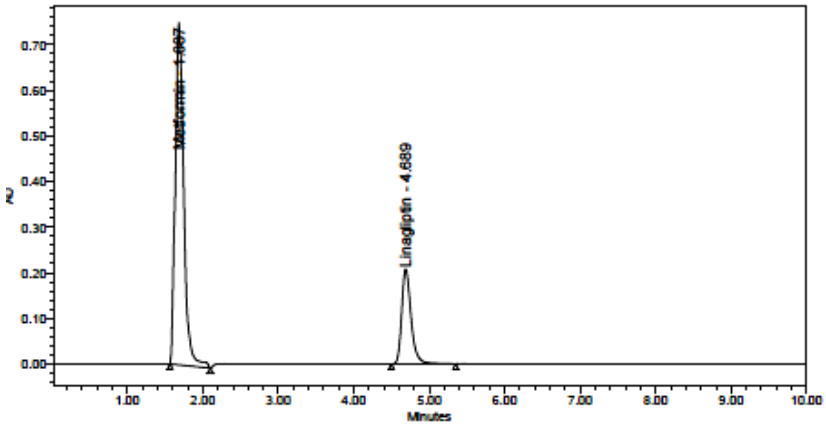

Fig.- 2: Typical chromatogram of Metformin and Linagliptin

Method Validation

System Suitability Test

System suitability test was performed by injecting six replicate injections 100% target solution of Metformin (MET) and Linagliptin (LIN). The parameters such as a number of theoretical plates, area and peak tailing were determined and were observed that all the parameters were within the limits. Results were shown in Table-2.

Table-2: System Suitability data of Metformin and Linagliptin

Parameters	MET	LIN
USP Tailing	1.31	1.32
Theoretical plates	2890	7408

System suitability chromatogram of Metformin and Linagliptin

Specificity

Specificity experiment was performed by injecting samples of mobile phase, placebo, the sample solution, unspiked and spiked sample. The results showed no interference at the retention time of Metformin and Linagliptin. The representative chromatogram of Placebo was shown in Figure-3.

Linearity

Standard solutions of Metformin (250-2500µg/mL) and Linagliptin (1.25-12.5µg/mL) and respectively were prepared and injected under the chromatographic conditions described above. Calibration curves were drawn the concentration of drug versus corresponding peak areas obtained at 210nm. The results showed a significant correlation between detector response and concentration level of each drug within the concentration range.

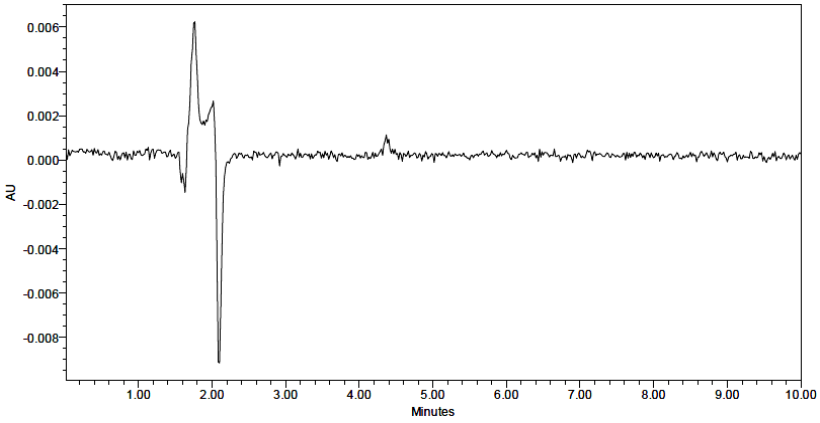

Fig.-3: Placebo Chromatogram

All the three drugs showed a linear response and the equation Y= (mx+c) was used to represent the linearity as follows-

Y (MET) = 5451.x +20295 and Y (LIN) = 23386.x+13347

The results are given in Table 6.3.-6.4 and the resulted chromatograms are shown in Figures-4 and 5.

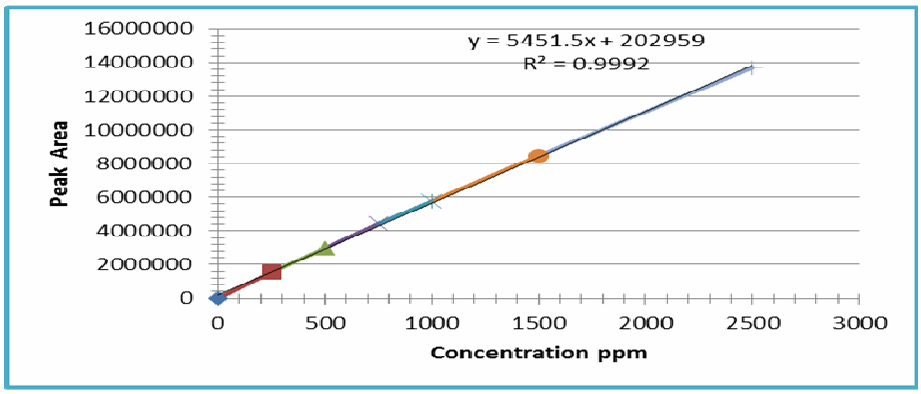

Fig.-4: Calibration curve of Metformin

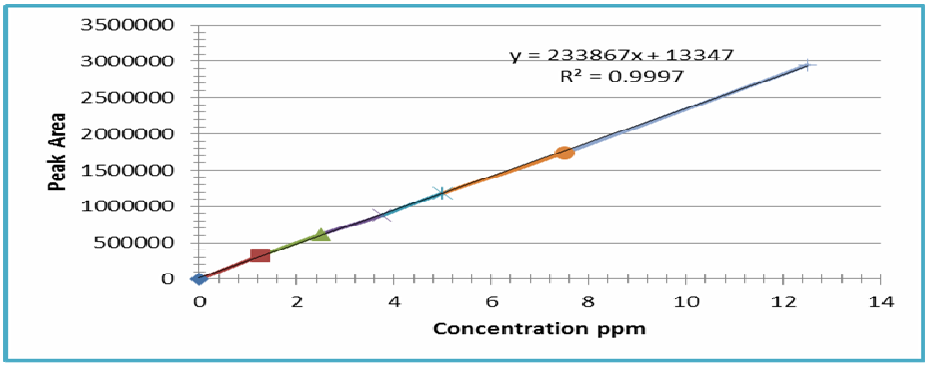

Fig.-5: calibration curve of Linagliptin

Accuracy

Known amounts of reference solution for all the three drugs Metformin and Linagliptin equivalent to 50%, 100% and 150% of the label claim were added to the tablet solutions of Metformin and Linagliptin. These results are summarized in Table- 3.

Table-3: Accuracy data of Metformin and Linagliptin

Level	Area		%Recovery	
	MET	LIN	MET	LIN
50%	8213264	178438	100.60	99.39
	8240351	178978	101.60	101.78
	8198159	178123	100.05	100.84
100%	10916308	237183	99.89	101.39
	10945081	235382	100.42	100.52
	10986734	236397	101.18	100.66
150%	13613858	294140	99.58	99.85
	13686560	294042	100.47	99.86
	13614056	295856	99.59	99.94
		AVG	100.38	100.47
		STD	0.69	0.79
		%RSD	0.69	0.78

Repeatability

The precision of the developed method was assessed for intraday (Precision) and interday (by varying the analyst and HPLC column called as intermediate precision). The % RSD for Metformin and Linagliptin were calculated, which is found to be within the acceptable limits (RSD < 2) and presented in Table-4.

Table-4: Precision data for MET and LIN

Validation Parameter	Intra-Day		Inter-Day	
	MET	LIN	MET	LIN
%Mean	99.99	100.00	99.83	99.99
SD	0.61	0.13	0.72	0.22
%RSD	0.61	0.13	0.72	0.22

Robustness

Robustness is performed by making slight variations in the Flow rate, column temperature and concentration of the mobile phase. The changes and the results were tabulated in Table-5.

Table-5: Robustness data of Metformin and Linagliptin

Column Temperature	Changed value	Retention time		Tailing factor		% Assay	
		MET	LIN	MET	LIN	MET	LIN
	25	1.643	3.706	1.76	1.33	98.70	98.30
	35	1.66	3.75	1.31	1.34	99.10	99.50
Flow Rate	0.9	1.76	4.135	1.55	1.36	101.50	101.20
	1.1	1.55	3.65	1.59	1.35	100.80	100.90
Mobile phase	60:40:00	1.64	3.06	1.35	1.36	100.20	100.50
	75:25:00	1.68	4.69	1.49	1.35	99.30	99.10
Average						99.93	99.91
STDEV						1.09	1.13
%RSD						1.09	1.13

LOD and LOQ

In the present chapter, LOD and LOQ of MET and LIN were determined by linearity curve method. LOD and LOQ were determined by using the equations-

LOD = 3.3 σ/S and LOQ= 10 σ/S

Where, "σ "is the standard deviation of the response, and "s" is the slope of the linearity curve. The LOD values were 2.66µg/mL and 8.05µg/mL for MET and LIN respectively. The LOQ values were 0.05µg/ml and 0.16µg/ml for MET and LIN respectively.

Stability of Sample Solution

The stability studies were carried out in mobile phase after 24hrs at ambient temperature using the mentioned chromatographic conditions. From these studies, it was revealed that Metformin and Linagliptin were stable in mobile phase for at least for 24hrs indicating the reliability of analysis in the proposed procedure. Results are shown in Table-6.

Table-6: Stability data of MET and LIN.

	Percentage of Assay	
Drug	%Assay at 0 hr	%Assay at 24hr
MET	101.58	99.13
LIN	99.43	99.92

Analysis of Formulations

The proposed method was applied for the estimation of Metformin and Linagliptin in tablet dosage form and the results are reported in Table-7.

Table-7: Assay of the marketed formulation

Sample No.	%Assay	
	MET	LIN
1	100.44	99.59
2	101.34	99.38
3	101.01	99.64
4	100.41	99.46
5	101.91	99.38
6	101.58	99.15
AVG	101.12	99.43
SD	0.61	0.17
%RSD	0.60	0.18

CONCLUSION

A new RP-HPLC method was developed and validated for simultaneous estimation of Metformin and Linagliptin in tablet dosage form. The calibration curve was found to be linear in the concentration range of 250-2500µg/mL and 1.25-12.5µg/mL for Metformin and Linagliptin respectively. A linear equation was established to provide the best fit for the concentration vs. detector response. The "r^2" value is equal to 0.99 during the validation. The obtained % RSD value of < 2, confirms that proposed method is effectively precise. Further, the separation of the analytes was completed in 6 minutes only, making the proposed RP-HPLC method conveniently adopted for the routine quality control analysis of other combination formulations containing these drugs.

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