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

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ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method with subsequently validateas per ICH guidelines for the determination ofLinagliptin(LIN) and Metformin hydrochloride (MET) using mobile phase [mixture of Phosphate buffer- pH-3.6 and acetonitrile in the ratio of 65:35] as the solvent. The proposed method involves the measurement of Retention time at selected analytical wavelength. 235.0 nmwas selected as the analytical wavelength.The retention time of LIN and MET was found to be 5.055 and 2.838 respectively. The linearity of the proposed method was investigated in the range of 1-5 µg/ml (r= 0.9998) for LIN and 10-50µg/ml (r= 0.9999) for MET respectively. The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

Keywords: RP – HPLC, Linagliptin, Metformin Hydrocholiride, Validation.

INTRODUCTION:

Linagliptin is an orally administered anti-diabetic drug in the DPP-4 inhibitor class. Linagliptin inhibits dipeptidyl peptidase 4 (DPP-4), which normally degrades the incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1). The inhibition of DPP-4 increases the amount of active plasma incretins which helps with glycemic control. Only one liquid chromatography method has been reported for the estimation of Linagliptin indudivally. No other methods are available for individual drug and in any combinations. Metformin is a biguanide antihyperglycemic agent used for treating non-insulin-dependent diabetes mellitus [NIDDM]. It improves glycemic control by decreasing hepatic glucose production, decreasing glucose absorption and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Metformin may induce weight loss and is the drug of choice for obese NIDDM patients. There are very few reports on analytical methods for estimation of Metformin individually and in other combinations, which includes bioanalytical methods using rat plasma, RP-HPLC, Spectrophotometric methods. The combination of Linagliptin and Metformin was significantly superior with proper diet and high blood sugar. On literature survey, it was found that no method have been reported for simultaneous estimation of Linagliptin and Metformin in combined dosage form and no method is available in pharmacopeia's. In the view of the need for a suitable method for routine analysis in combined formulations, attempts are being made to develop a simple

precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in formulation. As chromatographic methods of analysis is a pre-requisit for the marketing of most of formulations.

EXPERIMENTAL:

Chemicals and reagents:

The working standards of LINand MET were gifted from Swapnroop drug & pharmaceuticals (Aurangabad, Maharashtra) and Aarti drugs ltd (Goa)respectively. The tablet formulation of LIN and MET (Label claim: Linagliptin 25 mg and Metformin hydrochloride250 mg), Ondero Met tablets were purchased from the local market. Distilled water is obtained from local market for analytical work and rinsing purpose.

Instrument used:

A Shimadzu class HPLC unit accomplished with SPD-20AD UV-Visible detector;Enable C18 (250*4.6*5) Column (Shimadzu); LC-20 AD Pump; Quantitative HPLC was performed on a isocratic mode with 20 µl injection of sample loop (manual). The output signal was monitored and integrated using software class LAB SOLUTIONS (Shimadzu).

Preparation of Mobile phase:

The HPLC grade acetonitrile was filtered through 0.4 µm membrane filter paper. Buffer (0.585 gm. of anhydrous disodium hydrogen phosphate and 0.843gm. citric acid monohydrate in 650 ml distilled water) was filtered through 0.4 µm membrane filter paper. Mobile phase was prepared by mixing 650 ml of buffer with 350 ml of acetonitrile and sonicated for 15 min.

Preparation of standard stock solution:

50 mg each of standard LIN and MET was weighed accurately and transferred to two separate 50 ml volumetric flasks. Both the drugs were dissolved in 50 ml of mobile phase with sonication for 15 min and then volume was made up to the mark with mobile phase (solution–A). Further the stock solutions were diluted to get 50 µg/ml final concentration of standard stock solution of each drug (solution–B). These stock solutions were filtered through 0.4 µ membrane filter paper.

Preparation of calibration curves:

Appropriate dilutions were prepared separately and 20 µl of each was injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditionsas described below. Peak areaswere recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Chromatographic condition:

The mobile phase containing both Buffer and acetonitrile in the ratio of 65:35 was selected as the optimum composition of mobile phase, because it was found that this solvent system resolved both the components ideally. The flow rate was set to 1.0 ml/min and UV detection was carried out at 235.0 nm. The mobile phase and samples were degassed by sonication for 15 min and filtered through 0.4 µm membrane filter paper. All determinations were performed at constant column temperature (25^0C).

Selection of analytical concentration range:

Appropriate aliquots were pipetted out from the standard stock solution (solution B- 50 µg/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 1-5 µg/ml and 10-50 µg/ml of LIN and MET respectively. Triplicate dilutions of each of the above mentioned concentrations was prepared separately and from these triplicate solutions, 20 µl of each concentration of the drug were injected into the HPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Analysis of tablet formulation:

Twenty tablets of LIN and METin combination were weighed and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to 25 mg ofLIN and 250 mg of MET was weighed and transferred to 100 ml volumetric flask and dissolved in sufficient quantity of mobile phase. The contents were

sonicated for 5 minutes and the final volume was made up to the mark with mobile phase.
The above prepared solution was filtered through 0.4 µmembrane filter paper and was used as standard stock solution. Appropriate aliquot was pipetted out from the standard stock solution and was further diluted with the mobile phase to obtain a mixture containing 3µg/ml of LIN and 30 µg/ml of MET. A replicate mixtures containing 3µg/ml of MET and 30 µg/ml of RAM were prepared as above from the standard stock solution. A 20 µl volume of each sample mixture was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described above. The area of each peak was determined at 235.0 nm and the amount of drug present in the sample mixture was determined.

Method validation:
The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies and reproducibility as per the ICH guidelines.

RESULT AND DICUSSION:

The present manuscript deals with simultaneous estimation of LIN and MET in combined tablet dosage form by RP- HPLC method using mobile phase as the solvent. The developed method is based upon estimation of both the drugs by determining the area under curve of the chromatogram at selected analytical wavelength.
The linearity of the proposed method was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 1-5µg/ml for LIN (r = 0.9998) and 10-50 µg/ml MET (r = 0.9999) respectively as shown in the Table 9.
Recovery studies were also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 80%, 100% and 120% of the labeled amount of both the drugs (25mg LIN and 250 mg MET) in tablet formulation as shown in Table 1. Three replicate samples of each concentration levels were prepared and the percentage recoveryateach level (n = 3), and mean % recovery (n = 3) were determined and summarized in Table 1 and 2. Intra-day precision was estimated by assaying samples of the tablet formulation containing 1µg/ml of LIN and 10 µg/ml of MET, three times and the results were averaged for statistical evaluation. The statistical validation data for intra-day precision is summarized in Table 3 & 4. Inter-day precision was evaluated by analyzing a set of quality control samples of the tablet formulation containing 1µg/ml of LIN and 10 µg/ml of MET, three levels analyzed on three consecutive days. The statistical validation data for inter-day precision is summarized in Table 5. Both intra-day and inter-day variation showed less % RSD value indicating high grade of precision of the method as shown in table 6. The Robustness was evaluated by analysing the samples by varying few parameters like wavelength and flow rate. The statistical validation data is summarized in table 7 and 8. The validation results obtained confirm the suitability of the proposed RP-HPLC method for simple, accurate and precise analysis of LIN and MET in pharmaceutical preparations. The proposed method does not need prior separation of LIN and MET before analysis. In addition it is suitable for application without interference of excipients and can be applied directly to the commercial preparation without previous treatment.

Table 1: Recovery of LIN and MET in spiked standard drug solution.

Level of (%) Recovery	Amount present (mg)		Amount added (mg)		Amount found (mg)		Recovery* (%)	
	LIN	MET	LIN	MET	LIN	MET	LIN	MET
80%	25	250	20	200	44.95	449.90	99.88	99.98
	25	250	20	200	44.98	449.99	99.95	99.99
	25	250	20	200	44.95	449.87	99.80	99.97
100%	25	250	25	250	49.95	499.97	99.90	99.99
	25	250	25	250	49.99	499.97	99.98	99.99
	25	250	25	250	50.01	499.85	100.02	99.85
120%	25	250	30	300	55.05	549.85	100.09	99.97
	25	250	30	300	54.92	549.90	99.85	99.98
	25	250	30	300	54.99	549.99	99.98	99.99

Where n *= 3

Table 2: Recovery of LIN and MET in spiked standard drug solution

Level of (%) Recovery	Mean*		Standard deviation*		% Coefficient of Variation*		Standard Error*		%Recovery ± Standard Deviation	
	LIN	MET	LIN	MET	LIN	MET	LIN	MET	LIN	MET
80%	99.903	99.98	0.0404	0.01	0.0404	0.0100	0.023	0.005	99.94 ± 0.0738	99.97 ± 0.0297
100%	99.96	99.95	0.0611	0.0692	0.0611	0.0693	0.035	0.04		
120%	99.97	99.98	0.1201	0.01	0.1201	0.0100	0.069	0.005		

Where n *= 3

Table 3: Determination of intra-day precision of LIN and MET respectively

Sr. No	Amount present (µg)		Amount found (µg)		Label Claim* %	
	LIN	MET	LIN	MET	LIN	MET
1	1	10	0.98	9.99	99.97	99.90
2	1	10	0.93	10.01	99.91	100.1
3	1	10	0.80	9.94	99.71	99.40
4	1	10	1.05	9.88	100.05	98.82
5	1	10	0.99	9.99	99.98	99.90
6	1	10	0.98	9.96	99.97	99.60

Table 4 : Statistical validation data for determination of intra-day precision

Components	Mean*	Standard deviation*	% Coefficient of Variation*	Standard Error*
LIN	99.931	0.117374	0.1174	0.479
MET	99.62	0.463896	0.46566	0.1893

Where n *= 6

Table 5: Determination of inter-day precision of LIN and MET respectively

Sr. no	Amount present (µg)		Amount found (µg)		Label Claim* %	
	LIN	MET	LIN	MET	LIN	MET
DAY-1						
1	1	10	0.98	10.01	99.97	100.10
2	1	10	0.86	9.99	99.81	99.90
3	1	10	1.05	9.93	100.05	99.32
4	1	10	0.99	9.88	99.98	99.82
5	1	10	0.98	9.96	99.97	99.60
6	1	10	0.99	9.99	99.98	99.90

Sr. no	Amount present (µg)		Amount found (µg)		Label Claim* %	
	LIN	MET	LIN	MET	LIN	MET
DAY- 2						
1	1	10	0.96	9.88	99.95	98.82
2	1	10	0.98	9.96	99.97	99.60
3	1	10	0.99	9.99	99.98	99.90
4	1	10	0.99	9.93	99.98	99.32
5	1	10	0.98	9.93	99.97	99.32
6	1	10	1.05	9.99	100.05	99.90
DAY- 3						
1	1	10	1.05	9.32	100.05	99.32
2	1	10	0.98	9.96	99.97	99.60
3	1	10	0.96	9.88	99.95	98.82
4	1	10	0.98	9.32	99.97	99.32
5	1	10	0.99	10.01	99.98	100.1
6	1	10	0.99	9.99	99.98	99.90

Table 6: Statistical validation data for determination of inter-day precision

Components	Mean*	Standard deviation*	% Coefficient of Variation*	Standard Error*
LIN	99.975	0.013279	0.0132	0.00766
MET	99.586	0.101488	0.101909	0.05859

Where n*= 3

Table 7: Determination of Robustness of LIN and MET respectively.

Levels	Retention time		Tailing factor	
	LIN	MET	LIN	MET
Flow Rate				
-1	2.853	5.136	1.193	1.532
0	2.838	5.055	1.164	1.472
+1	2.822	5.023	1.135	1.432
Wavelength				
-2	2.828	5.067	1.133	1.483
0	2.838	5.055	1.164	1.472
+2	2.858	5.053	1.173	1.453

Table 8 : Statistical validation data of determination of Robustness for change in method parameters.

Parameters	Mean		Standard Deviation		(%) Coefficient of variance	
	LIN	MET	LIN	MET	LIN	MET
Flow Rate						
Retention time	2.837	5.071	0.0155	0.0582	0.0546	0.1147
Tailing factor	1.164	1.4786	0.029	0.0503	2.4914	3.403
Wavelength						
Retention time	2.8413	5.583	0.01527	0.00757	0.5374	0.1496
Tailing factor	2.8413	5.0583	0.01527	0.00757	0.5374	0.1496

Table 9: Summary of validation and System suitability parameters of LIN and MET

Parameters	LIN	MET
Linear range (µg/ml)	1-5	10-50
Slope	134614	17206
Intercept	85.905	5819.4
Regression coefficient (r^2)	0.9998	0.9999
Limit of Detection (µg/ml)	0.0723	0.0417
Limit of Quantification (µg/ml)	0.1219	0.1265
Retention time (min)	5.055	2.838
Tailing factor	1.164	1.472
Resolution factor	12.282	
Theoretical plate	9089	5553

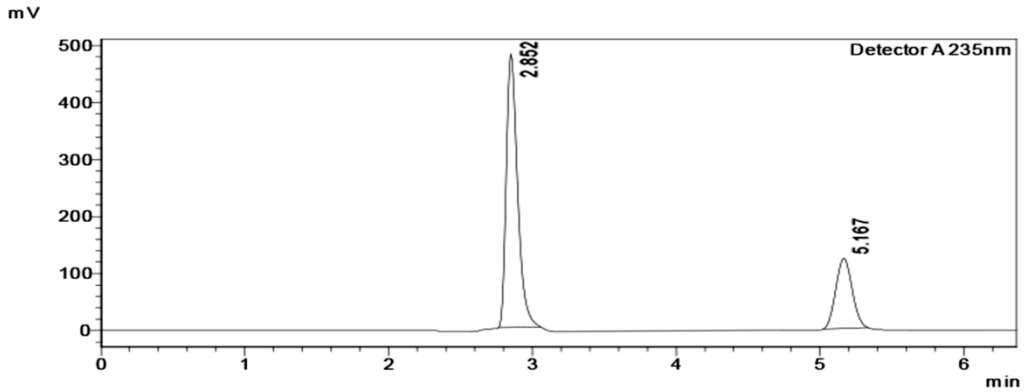

Fig.1 Chromatogram showing retention times of Linagliptin and Metformin respectively.

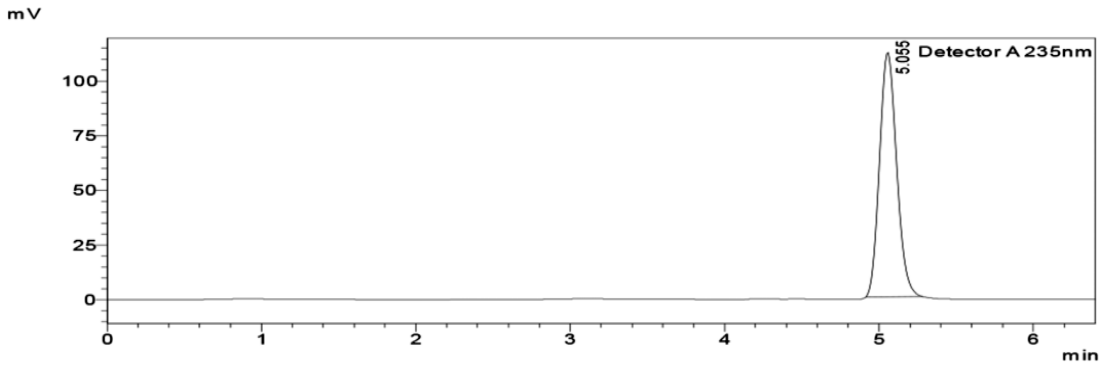

Fig.2 Chromatogram showing retention time of Linagliptin

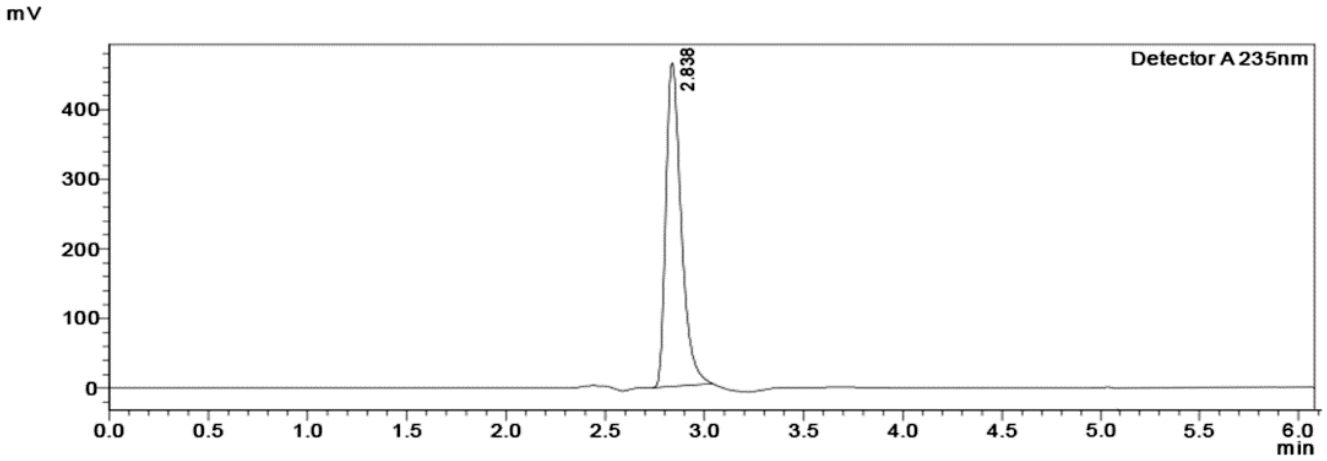

Fig.3 Chromatogram showing retention time of Metformin

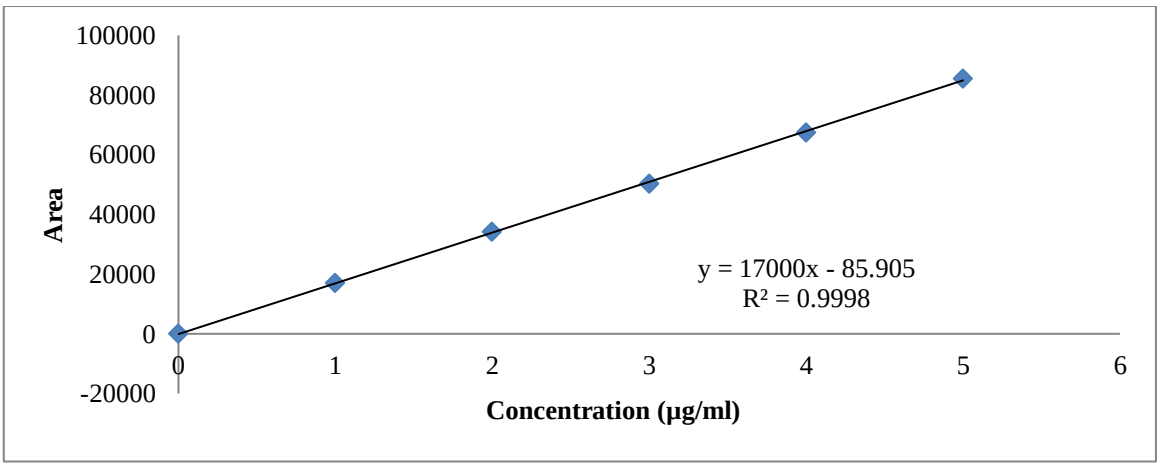

Fig. 4Calibration curve of Linagliptinat 235.0 nm in Acetonitrile and Buffer by RP-HPLC Method

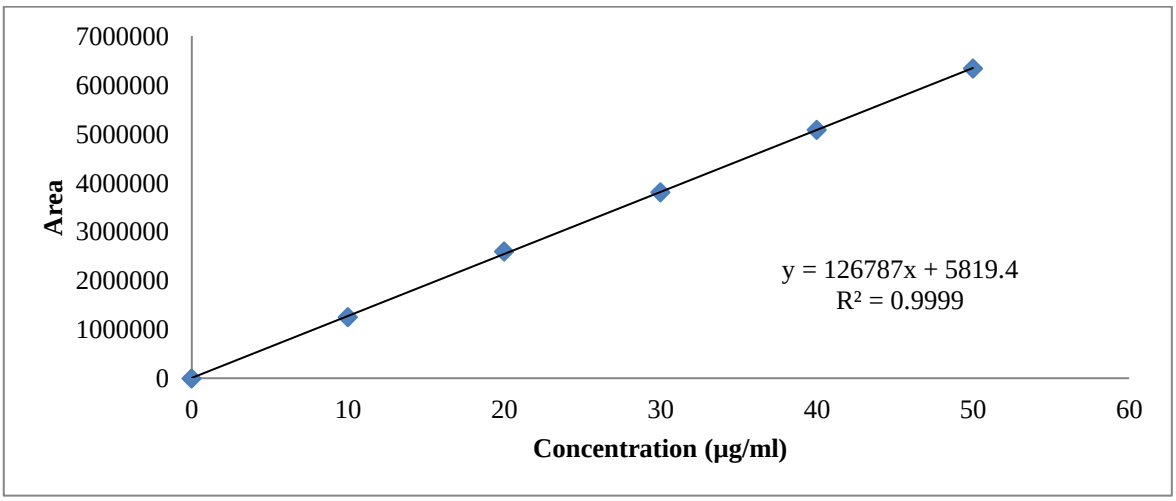

Fig. 5Calibration curve of Metformin at 235.0 nm in Acetonitrile and Buffer by RP-HPLC Method.

CONCLUSION:

The proposed HPLC method was found to be simple, sensitive, accurate and precise for determination of LIN and MET in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of LIN and MET hence the method was also economic for estimation of LIN and MET from synthetic mixture

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