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Analytical Method Development And Validation For Simultaneous Estimation Of Metformin And Linagliptin

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Abstract- The aim of this paper was to develop a simple and validated RP- HPLC method for simultaneous estimation of Metformin HCL (MET) and Linagliptin (LINA) in tablet dosage form. Chromatographic separation was achieved on a BDS hypersil C18, 250mm × 4.6mm, 5µ (particle size), and thermo scientific. The mobile phase comprised of Methanol: Water: OPA (50:50:0.05 v/v/v) (Phosphate buffer pH 3.0 was adjusted with H3PO4)at flow rate of 0.9 ml/min and all eluents were detected at 234 nm. The retention times were 2.8000 ± 0.10 and 7.6147 ± 0.10 min for Metformin and Linagliptin respectively. The method was validated according to ICH guidelines. It was found to be accurate and reproducible, linear, and precise. Calibration curves at seven levels for Metformin and Linagliptin were linear in the range of 50-250 µg/mL and 50-250 µg/mL, with r2= 0.999, respectively. There was no interference from excipient in the analysis of Metformin and Linagliptin. Hence, the proposed method can be used for analysis of routine quality control samples of Metformin and Linagliptin tablets.
Index Terms: HPLC; Metformin; Linagliptin; Development; Validation.

1. INTRODUCTION:

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

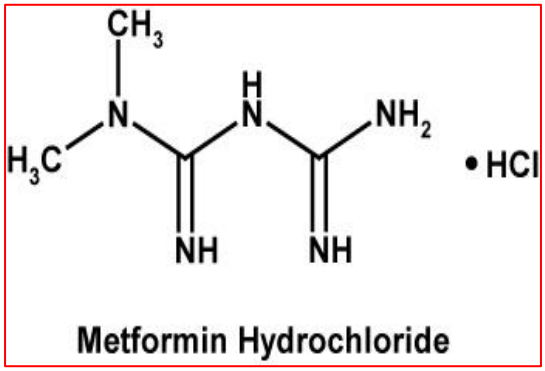

Fig.1 Structure of MET

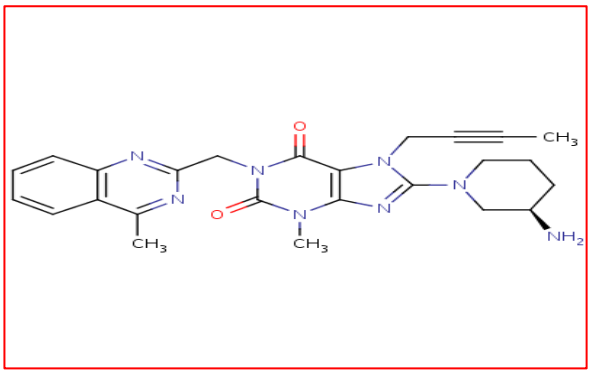

Fig.2 Structure of TENE

The detailed survey of literature revealed that several Spectrophotometric methods, HPLC methods, Stability indicating methods and Plasma extraction methods 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, 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 MET and LINA from formulated dosage form. The developed method is validated as per ICH and all relevant guidelines for broad linearity range than other available methods and with better retention times.

2. MATERIALS AND METHODS:

Chemicals: Pure Standard of MET and LINA were obtained from McCoy Pharma Pvt. Ltd. (Tarapur, India.). ONDERO MET® tablets were purchased from the local medical store. HPLC grade Methanol, OPA and Potassium dihydrogen ortho phosphate were

obtained from Merck, Rankem. High purity deionised water was obtained from a Millipore, Milli-Q purification system. All solvents and reagents were of analytical grade.

Instrumentation: Younglin (S.K) Gradient System HPLC equipped with Shimadzu-1800 UV detector (UV 730 D) was used throughout the analysis. The data was acquired using Lab- Solutions Autochro - 3000 software. The analytical column BDS hypersil C18, 250mm × 4.6mm, 5µ (particle size), thermo scientific was used as a stationary phase. AX200 Electronic balance was used for weighing the contents. The instrumental settings were a flow of 1.0mL/min the injection Rheodyne injector volume was (20 µl Capacity). Column oven temperature was ambient.

Optimization of chromatographic conditions: The chromatographic conditions were optimized by different means (Using different column, different buffer and different mode of HPLC run).

Chromatographic conditions: The chromatographic elution was carried out in isocratic mode using a mobile phase consisting of Methanol: Water: OPA (50:50:0.05 v/v/v) (Phosphate buffer pH 3.0 was adjusted with H_3PO_4) and the column was maintained at ambient temperature. The analysis was performed at a flow rate of 0.9 mL/min with a run time of 10 min. The eluents was monitored at wavelength of 234 nm. The 25µl volume of sample was injected by auto sampler.

Preparation of mobile phase: Methanol: Water: OPA (50:50:0.05 v/v/v**):** water and methanol taken in beaker adjusted PH at 0.05 using o-phosphoric acid. Sonicate for 30 minute and filter through 0.20 µ size membrane filter. Diluents: Mobile Phase.

Preparation of standard solutions

Accurately weighed quantity of MET and LINA 500 and 2.5 mg respectively, was transferred into 100 mL volumetric flask, was added 200 and 50 mL respectively, of diluent then sonicated for 10 minutes. Final volume of solution was made up to mark with diluent to get stock solution containing 5 and 0.025 mg/mL respectively of MET and LINA in 100 mL volumetric flask, the resultant stock solution of MET and LINA having strength of 5000 and 25 µg/mL respectively.

3. RESULT AND DISCUSSION:

Optimization of chromatographic conditions

The wavelength of maximum absorption for both the drugs MET and LINA were observed 206nm (λ1) and 234nm (λ2) respectively. The isoabsorptive point was obtained at 234nm. The overlain spectrum of MET and LINA was shown in [**Fig-2]**

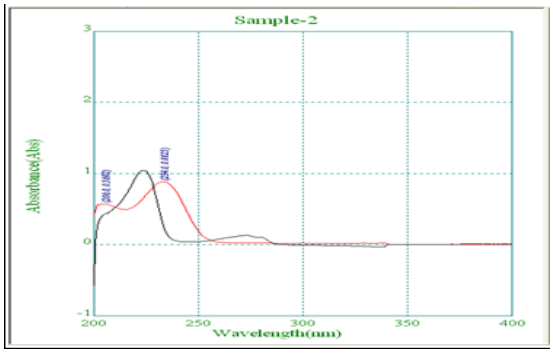

Fig.3 The overlain spectrum of MET and LINA

The column selection has been done by backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution between Metformin and Teneligliptin peak. Satisfactory results were found with Thermo scientific BDS Hypersil C18, 250 mm × 4.60 mm, 5µ was selected.

Optimized chromatographic conditions for estimation of MET and LINA are finalized as shown in **Table 1.**

Table 1: Optimized chromatographic conditions for estimation of MET and LINA:

Column	Thermoscientific, BDS hypersil C18, 4.6 x 250 mm, 5µ
Flow rate	0.9 mL/min.
Mobile Phase	Methanol : Water : OPA (50:50:0.05 v/v/v)(pH 3.0 by o-phosphoric acid)
Detection	234 nm
Injection Volume	20 µl
Runtime	10 Minute
Diluent	Mobile Phase

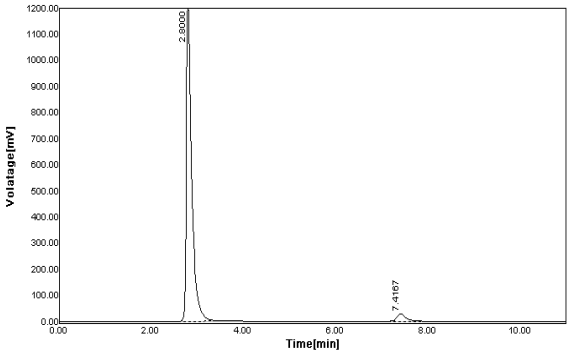

Fig-3: Chromatogram of Sample MET and Sample LINA by using optimized method**.**

Retention time was obtained With mobile phase containing Water, Methanol and 0.05% of Ortho phosphoric acid (50:50:0.05) buffer pH 3 adjusted with 0.9M/min-flow rate in low pressure gradient

mode. So finally low pressure gradient elution was selected for the development of method.

System suitability test:
Standard solution of MET and LINA were injected into the chromatographic system and recorded the chromatograms for observing system suitability test such parameter avg. peak area of standard, no. of theoretical plates, retention time, asymmetry,% RSD, Resolution were observed and it was noted that all parameters were in accepted criteria shown in **table 10**.

Table 10: Observed system suitability parameter

Sr. No	Parameters	MET	LINA
1.	Avg. peak area of standard	9942.51	384.37
2.	No. of theoretical plates	3194.0	6497.6
3.	Retention time (min)	2.800	7.416
4.	Asymmetry	1.000	1.291
5.	% RSD	0.96%	0.3%
6.	Resolution	-	13.85

Linearity & Range
The calibration curves were prepared by plotting the peak areas of the drug to which were linear in the concentration range of 50-300 µg mL-1, 1-6 µg mL-1 for MET and LINA (Internal standard) respectively **(Fig-4 and 5).** The correlation coefficient (R2) was found to be 0.999 and 0.9962 which are greater than 0.995, ensure that a good correlation existed between the peak area ratio of sample with Internal standard and Analytes.

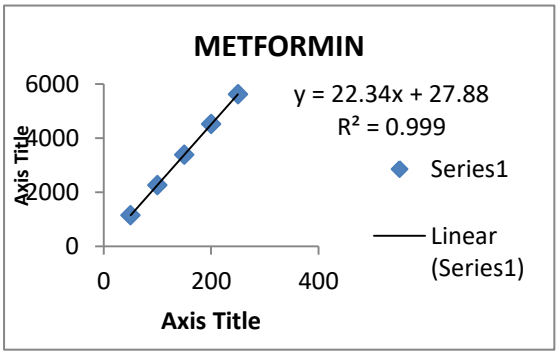

Fig.6 Calibration Curve of MET

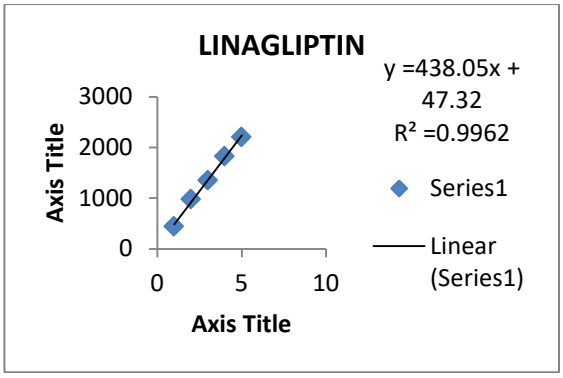

Fig.6 Calibration Curve of LINA

Table 3: Calibration data for standard MET

Sr. No.	% Conc.	Conc. (µg/mL)	Peak Area mean*	SD	%RSD
1	50	200	10260.9	0.78	0.01
2	100	400	17378.25	229.6	1.32
3	150	600	24817.5	69.08	0.28
4	200	800	31514.5	66.37	0.21
5	250	1000	371518.7	543.7	1.45
Correlatn Coefficient			0.999		
Regression Equation			Y=35.32x+3302		

Table 4: Calibration data for standard LINA

Sr. No.	% Conc.	Conc. (µg/mL)	Peak Area mean*	SD	%RSD
1	50	1	439.08	0.57	0.13
2	100	2	982.235	2.55	0.26
3	150	3	1350.87	14.06	1.04
4	200	4	1827.79	5.32	0.29
5	250	5	2206.94	4.03	0.18
Correlation Coefficient			0.996		
Regression Equation			y=438.0x+47.32		

Intraday precision:
Combined standard solutions containing mixture of 400 ppm, 600 ppm and 800 ppm of MET and 2 ppm, 3 ppm and 4 ppm of LINA were analysed 3 times on the same day. The % R.S.D for the MET and LINA was calculated and shown in **Table 6**.

MET				TENE			
Conc Ppm	**Peak Area Mean**	**SD**	**% RSD**	**Conc Ppm**	**Peak Area Mean**	**SD**	**% RSD**
400	17402	133.5	0.77	02	943.5	16.6	1.78
600	24837.8	584.5	2.39	03	1391.8	4.1	0.30
800	31902.9	450.5	1.43	04	1730.2	15.8	0.91

Table 5: Intraday precision for MET and TENE

Assay of Marketed Formulation:
Twenty tablets were weighed. The powder from twenty tablets were collected and weighed. The Powder equivalent to 500 mg of MET and 2.5 mg of LINA was transferred to a 100 mL volumetric flask and dissolved in mobile phase. The solution was ultra sonicated for 30 min and filtered through 0.20 micron membrane filter to obtain concentration about 5000 µg/mL MET and 25 µg/ml LINA respectively. And a solution of 500 µg/mL of MET and 2.5 µg/mL of LINA solution was prepared by diluting 1 mL of sample stock solution with diluents in 10 mL volumetric flask up to the mark. Then, this solution was forwarded for chromatographic study.

4. CONCLUSION:

A novel RP- HPLC method has been developed for the simultaneous estimation of Metformin and Linagliptin in marketed formulations. The method gave good resolution for both the drugs with a short analysis time below 6 minutes. The developed method was validated. It was found to be simple, precise and accurate. The good % recovery in tablet forms suggests that the excipients present in the dosage forms have no interference in the determination. The % RSD was also less than 2 % showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of Metformin and Linagliptin in combined dosage form. It can be also used in the quality control in bulk manufacturing.

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