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Analytical Method development and Method validation for the simultaneous estimation of Metformin HCL and Linagliptin in Bulk and tablet Dosage Form by RP-HPLC Method

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ABSTRACT

A rapid, highly sensitive, economical and accurate RP-HPLC method was developed for simultaneous estimation of Metformin HCL and Linagliptin in Bulk and Pharmaceutical Dosage form. The separation was achieved by Hypersil C18 column (250 × 4.6 mm, 5 µ particle size) with mobile phase consisting of phosphate buffer (pH 5.6, diluted with orthophosphoric acid), methanol and acetonitrile in the ratio of 40:5:55 v/v, using flow rate 1.0 mL/min and eluents monitored at 233nm .The developed method was validated as per ICH guidelines for specificity, linearity, precision, accuracy, robustness, limit of detection and limit of quantification. The retention times of Linagliptin and Metformin were 5.4 and 6.6 min respectively. The linearity was found to be in the range of 125-750 µg/mL and 0.625-3.75 µg/mL for Metformin and Linagliptin respectively, had regression coefficients (R^2) 0.999. The proposed method was successfully applied for simultaneous estimation of both drugs in Pharmaceutical formulation.

Keywords: RP-HPLC, Metformin, Linagliptin, Validation

INTRODUCTION

Linagliptin is described chemically as 1H-Purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1- yl)-3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl) methyl]-The empirical formula is $C_{25}H_{28}N_8O_2$. The structural formula is shown in fig (2). Linagliptin is a white to yellowish or only slightly hygroscopic solid substance. It is very slightly soluble in water (0.9 mg mL-1). Linagliptin is soluble in methanol (ca. 60 mg mL-1), sparingly soluble in ethanol (ca. 10 mg mL-1), very slightly soluble in isopropanol (<1 mg mL-1), and very slightly soluble in acetone (ca. 1 mg mL-1) [1-3]. Linagliptin is an oral drug that reduces blood sugar (glucose) levels in patients with type 2 diabetes. Linagliptin is a member of a class of drugs that inhibit the enzyme, di peptidyl peptidase-4 (DPP-4). Following a meal, incretion hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP) are released from the intestine, and their levels increase in the blood. GLP-1 and GIP reduce blood glucose by increasing the production and release of insulin from the pancreas. GLP-1 also reduces blood glucose by reducing the secretion by the pancreas of the hormone, glucagon, a hormone that increases the production of glucose by the liver and raises the blood level of glucose. The net effect of increased release of GLP-1 and GIP is to reduce blood glucose levels. Linagliptin inhibits the enzyme, DPP-4, that destroys GLP-1 and GIP and thereby increases the levels and activity of both hormones. As a result, levels of GLP-1 and GIP in the blood remain higher, and blood glucose levels fall. Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP [4-8].

Metformin hydrochloride (MH) chemically, 3-(diaminomethylidene)-1,1-dimethylguanidine hydrochloride[6]is an anti diabetic agent[7]. It is the drug of choice for the treatment of type II diabetes, particularly in overweight and obese people and individuals with normal kidney function. It works by lowering blood sugar and helping the body use insulin more efficiently. It is available in 500 mg, 850

mg and 1000 mg tablets (immediate release) and in 500 mg and 750 mg (slow release) for oral administration. BoehringerIngelheim Pharmaceuticals, Inc. LP in combination with MH in a single dosage form as Jentadueto™ .[9] In combination these are available in 2.5/500 mg, 2.5/850 mg and 2.5/1000 mg of Linagliptin and MH, respectively.

EXPERIMENTAL

Materials: Analytical pure samples of MH (Aurbindo Pharmaceuticals Ltd., Hyderabad, India) and LINA (Aurbindo Pharmaceuticals Ltd., Hyderabad, India) were used in the study. The pharmaceutical dosage form used in this study was Jentadueto™ (BoehringerIngelheim Pharmaceuticals, Inc. LP) labeled to contain 500mg of MET and 2.5mg of LIN. Acetonitrile, water, methanol used are of HPLC grade.

Instrumentation: Waters 2695 Series Alliance HPLC system consisting of an inbuilt auto sampler, a column oven, a quaternary pump and a photo-diode array detector (PDA) was employed throughout the analysis. The data was acquired through the Empower-2 software. The column used was ODS symmetry Hypersil C18 (250 × 4.6mm, 5µm). Meltronics sonicator was used for enhancing the dissolution of the compounds. Wensar electronic analytical balance was used for weighing the sample. Digisun pH meter was used for adjusting the pH of buffer solution.

Optimized Chromatographic Conditions: The chromatographic elution was carried out in the isocratic mode using a mobile phase consisting of phosphate buffer (pH 5.6, adjusted with orthophosphoric acid), methanol and acetonitrile in the ratio of 40:5:55. The analysis was performed at ambient temperature using a flow rate 1.0 mL/min with a run time of 10 min. The eluent was monitored using PDA at a wavelength of 233nm. The mobile phase was filtered through 0.45µm membrane filter paper prior to use.

Preparation of Standard Stock Solution: Accurately weighed and transferred 100mg of Metformin and 0.5mg of Linagliptin working Standards into separate each 10 ml clean dry volumetric flasks, 7ml of iluents was added , sonicated for 5 minutes and made up to the final volume with iluents.

Preparation of Sample Stock Solution: The formulation tablets of Linagliptin and Metformin (Jentaduetog) were crushed to give finely powdered material. Powder equivalent to 500 mg of drug was taken in 250 ml of volumetric flask containing 150 ml of iluents and was shaken to dissolve the drug and then filtered through Ultipor N66 Nylon 6,6 membrane sample filter paper. 1 ml of this solution was then diluted to 25 ml with mobile phase to obtain a concentration of 250µg/ ml for Metformin hydrochloride and 1.25µg/ ml Linagliptin solution was filtered through a 0.45 mm nylon filter before analysis.

Method Validation

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [10].

Specificity: Specificity of an analytical method is the ability to measure specifically the analyte of interest without interferences from blank and placebo.
Check for interference from blank: Diluent was used as blank. Standard and sample were prepared as per test procedure. Check for the interferences of blank and peaks with the analyte peak and calculate % interference with analyte peak against the standard peak area. (Fig. 3& 4)

Chromatogram and Precision: When the drugs METFORMIN HCL and LINAGLIPTIN were introduced together in one sample into the HPLC system using the chromatographic conditions mentioned earlier the peaks show an excellent resolution(Fig.3).This led us for further study of the system and method precisions. The intraday and interday precisions were below 2.0% indicating a high system precision (Table 1).

Linearity: By appropriate aliquots of the standard METFORMIN and LINAGLIPTIN solution with the mobile phase, five working solutions ranging between 125-750µg/mL and 0.625-3.75µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The graph of peak area obtained of the chromatograms was plotted against the concentration versus response of METFORMIN HCL and LINAGLIPTIN to obtain the calibration curve. (Fig. 5 & 6) The linearity data of METFORMIN HCL and LINAGLIPTIN are shown in Table 4.

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by method to the assay value. Accuracy must be established across the specified range of the analytical procedure. Accuracy was determined over the range of 50%, 100% and

150% of the sample concentration. The accuracy was then calculated as the percentage of analyte recovered by the assay. Mean recoveries (Mean ± S.D) for METFORMIN HCL and LINAGLIPTIN from the combination formulation are shown in Table 2 & 3 indicating good accuracy of the method.

Robustness: Variations in the flow rate, mobile phase and temperature have been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by such variations. Analytical concentration at level 100% was analyzed by preparations at each level (with duplicate readings) against a standard solution. The results show that the %RSD is less than 2.0%

Limit of detection and the limit of quantification: Limit of detection (LOD) value was found to be 1.68 and 4.08 for MET and LIN respectively. The limit of quantification (LOQ) was found to be 12.36 and 4.99 for MET and LIN respectively. The values were calculated based on the ICH guidelines. .(Table 7 & 8)

System suitability test: The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analysing each solute for their peak areas, theoretical plates (N), resolution ® and tailing factors (T).The system suitability of this method was good.(Table 6)

RESULTS AND DISCUSSION

In the simultaneous estimation of METFORMIN HCL and LINAGLIPTIN in injection dose the method was developed and validated according to ICH guidelines. RP-HPLC method has shown adequate separation for METFORMIN HCL and LINAGLIPTIN. Separation was achieved on Hypersil C18 (250 × 4.6mm, 5µm) column by using phosphate buffer (pH 5.6, diluted with orthophosphoric acid), methanol and acetonitrile in the ratio of 40:5:55 v/v, at a flow rate 1.0 mL/min and eluents monitored at 233nm. In the present study the specificity of the method was determined by assessing interference from the placebo & diluents. There were no other co-eluting, interfering peaks from excipients, impurities found and the method was specific for estimation of METFORMIN HCL and LINAGLIPTIN. The method was validated in terms of linearity, precision, accuracy, specificity, Robustness, limit of detection and limit of quantification. The linearity of the proposed method was investigated in the range of 125-750µg/ml and 0.625-3.75µg/ml for METFORMIN HCL and LINAGLIPTIN respectively. Accuracy was determined by recovery study and it was found to be 99.80% for METFORMIN HCL and 100.47% for LINAGLIPTIN. The percentage RSD value for the three assay values was 1.11 for METFORMIN HCL and 1.049 for LINAGLIPTIN. The precision of the method was assessed in accordance with ICH guidelines. The low %RSD (<2) values indicate that this method was precise. The robustness was determined by analysing the same sample under a variety of conditions. The factors consider being variations in the pH of the mobile phase, temperature and flow rate. There were no significant changes in the chromatography pattern when these modifications were made in the experimental conditions, showing that the method is robust. The system suitability tests were also carried out. The proposed method was applied to the analysis of marketed formulations.

CONCLUSION

The proposed RP-HPLC method is rapid, simple, specific, accurate, economical and precise for the simultaneous estimation of METFORMIN HCL and LINAGLIPTIN in Bulk and Pharmaceutical dosage form. There were no analytical methods reported so far for this estimation. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The proposed method involves direct quantification of both the components. Hence the developed RP-HPLC method can be conveniently adopted for the routine quality control analysis in the combination formulation.

Fig 1: Chemical structure of Metformin Hydrochloride

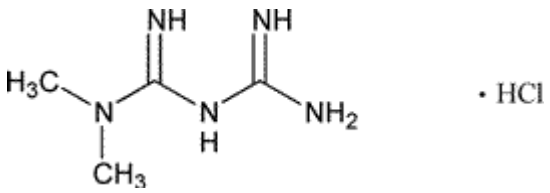

Fig 2: Chemical structure of Linagliptin

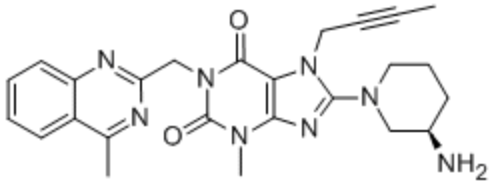

Figure 3: Chromatogram showing no interference with the placebo at the retention times of main peaks.

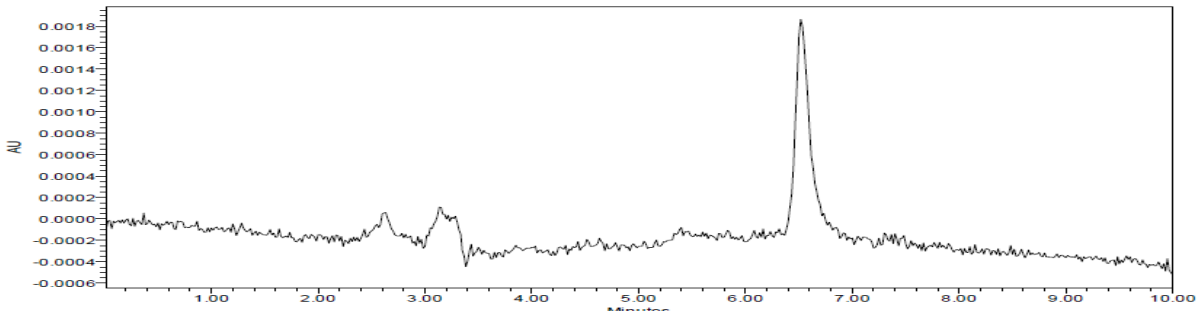

Figure 4: A typical chromatogram of Metformin Hydrochloride and Linagliptin

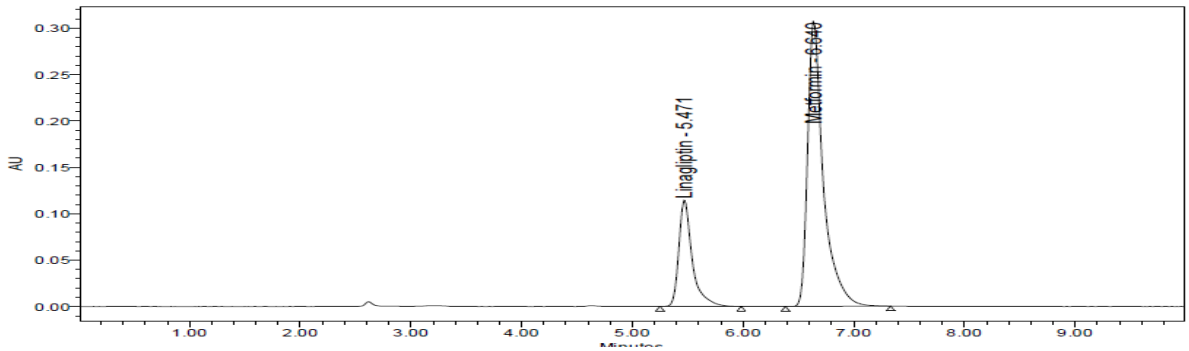

Figure 5: Linearity Graph for Metformin Hydrochloride

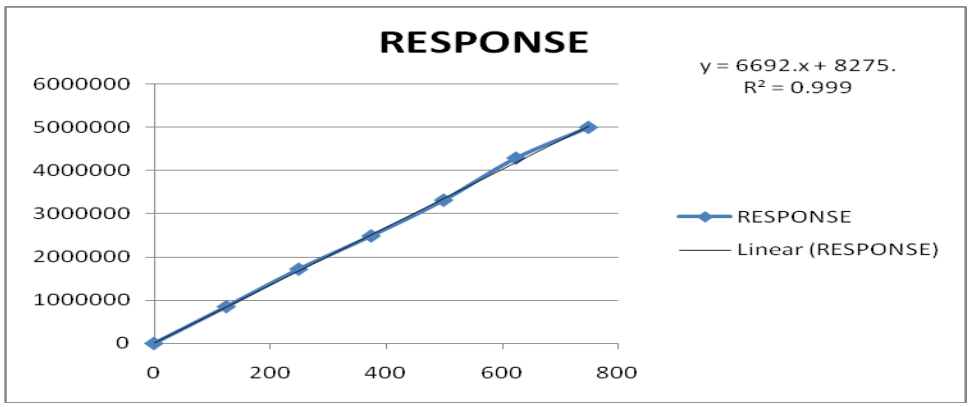

Figure 6: Linearity Graph for Linagliptin

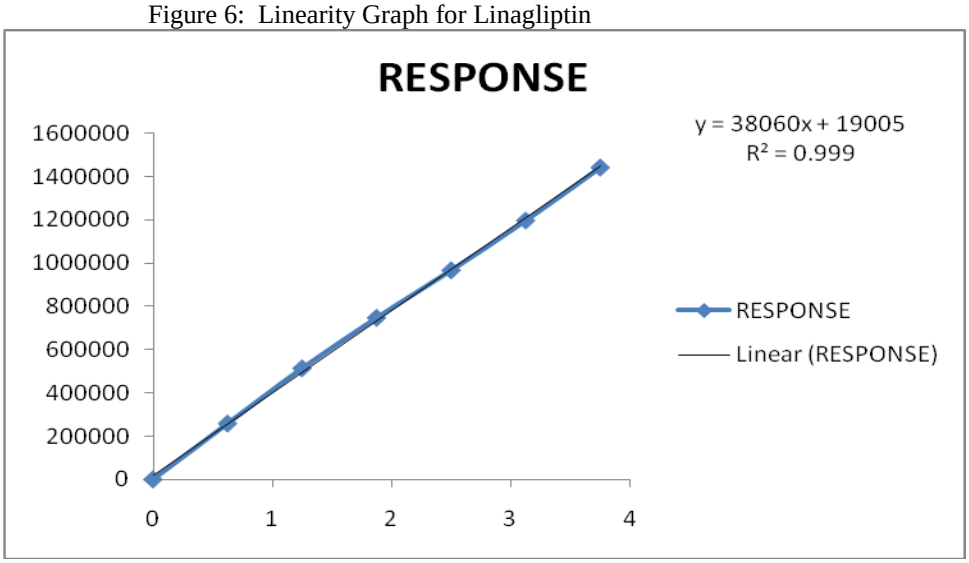

Table 1: Method Precision

Sample number	% Assay Metformin	% Assay Linagliptin
1	98.33	98.266
2	99.15	99.016
3	98.67	98.913
4	97.22	98.438
5	99.34	99.005
6	100.25	98.239
AVG	98.83	98.646
SD	1.026	0.371
% RSD	1.03	0.376

Table 2: Recovery Study for Metformin Hydrochloride

Level	Mean % Recovery	% RSD
50	100.00	0.19
100	99.4	1.33
150	101.05	0.53

Table 3: Recovery Study for Linagliptin

Level	Mean % Recovery	% RSD
50	100.3	0.11
100	100.01	0.91
150	99.11	0.03

Table 4: Linearity data of Metformin Hydrochloride and Linagliptin.

	MET		LIN	
S.No.	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	125	849307	0.625	258382
2	250	1715044	1.25	514246
3	375	2483388	1.875	747701
4	500	3309122	2.5	967616
5	625	4280262	3.125	1197640
6	750	4989762	3.75	1442910

Table 5: System Suitability data of Linagliptin

S.no	Areas	Theortical Plates	Tailing Factor
1	965560	11561	1.53
2	974797	11677	1.51
3	971672	11624	1.53
4	984651	11874	1.52
5	975598	11608	1.54
6	980042	11711	1.55
AVG	975387		
SD	6607.1		
% RSD	0.7		

Table 6: System Suitability data of Metformin Hydrochloride

S.no	Areas	Theoretical Plates	Tailing Factor
1	3054245	12765	1.66
2	3082594	13000	1.66
3	3073530	12839	1.66
4	3108153	12816	1.67
5	3095627	12792	1.66
6	3081726	13106	1.66
AVG	3082646		
SD	18491.5		
% RSD	0.6		

Table 7: LOD and LOQ of Metformin Hydrochloride.

Parameter	Measured Value (µg/ml)
Limit of Detection	4.08
Limit of quantification	12.3

Table 8: LOD and LOQ of Linagliptin.

Parameter	Measured Value (µg/ml)
Limit of Detection	1.64
Limit of quantification	4.99

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