



Research Article

SIMULTANEOUS ESTIMATION OF LINAGLIPTIN AND METFORMIN HCL IN HUMAN PLASMA BY RP-HPLC METHOD

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ABSTRACT

A new and simple method was developed for quantitative determination of Linagliptin (LINA) and Metformin (MET) spiked with plasma using an Onyx C₁₈ Monolithic column connected with an Onyx C₁₈ guard cartridge and PDA detection at 220 nm. The optimized mobile phase acetonitrile (ACN), methanol (MeOH), formic acid (HCOOH) was pumped at 0.892 mL/min. The method was linear between 0.02-0.1 µg/mL, statistically validated for its linearity, precision, and accuracy. The precision was found to be less than 1% in the assay method. It was found that the additives in the commercial tablet did not interfere with the method. The currently developed method can routinely use for the estimation of LINA and MET related compounds from the tablet dosage form spiked with plasma.

Keywords: Linagliptin, Metformin, Plasma, RP-HPLC.

INTRODUCTION

Linagliptin (LINA) (**Fig.1**), chemically (S)-3-amino-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,3]triazolo[4,3-a]pyrazin-7(8H)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one. LINA is a white to off-white powder. The solubility of drug substance is soluble in water and N, N-diethyl formamide, slightly soluble in methanol, very slightly soluble in ethanol, acetone, and acetonitrile, insoluble in isopropanol and isopropyl acetate. LINA is a potent oral hypoglycemic drug of the dipeptidyl peptidase4(DPP4) inhibitor.

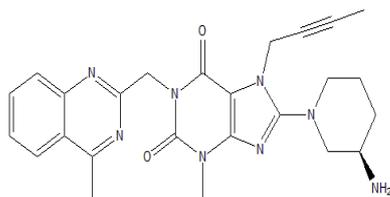
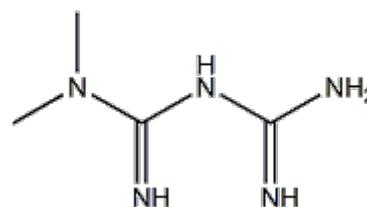


Fig 1: Linagliptin

Metformin HCL (MET) (**Fig.2**), chemically N, N-diethyl imidodicarbonimidic diamide hydrochloride as shown in Fig 2. Metformin is a white powder. MET is freely soluble in water, slightly soluble in ethanol (95%), practically insoluble in acetone, ether, and chloroform. Literature review reveals that some analytical procedures have been accounted for estimation of LINA and MET individually as stability indicating and in biological fluids or in combination with different drugs in pharmaceutical dosage forms.

As of late HPLC [1-2], UV-Spectroscopy [3-4] and UP-LC [5] and Quality by design technique [6-7] have been accounted for the simultaneous determination of LINA and MET in pharmaceutical dosage forms and biological fluids which are either monotonous or costly techniques.



HCl

Fig 2: Metformin HCL

MATERIALS AND METHODS

Chromatographic measurements were made on an RP-HPLC Shimadzu (Tokyo, Japan) model which consisted of an LC-20AD solvent delivery module, SPD-M20A prominence diode array detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20µl loop. The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1-11SP1) installed on it. The mobile phase was degassed using Branson sonicator (Branson Ultrasonic Corporation, USA). Absorbance spectra were recorded using an UV-double beam spectrophotometer (Systronics 2202 Model UV-1601PC, Japan) employing a quartz cell of 1 cm of path length.

Chemicals and reagents

Working standards of LINA and MET were purchased from Biotech Solutions, New Delhi. ACN, MeOH of HPLC grade and HCOOH was of analytical- reagent grade supplied by M/S SD Fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, and Bangalore, India. The pharmaceuticals TRAJENTA DUO (Batch No:

659913A, MFG: APR 2016, EXP: APR 2019) were purchased from Medicine Chamber, Park Town, Chennai, India. Plasma sample has been collected from the Blood bank of RMMC&H, Annamalai University, Chidambaram, as gift sample for this research work.

Chromatographic condition

Chromatographic separations were carried out on an Onyx C₁₈ Monolithic column (100mm× i.d., 5µm) connected with an Onyx C₁₈ guard cartridge (4mm×3mm i.d., 5µm). The mobile phase consisted of ACN, MeOH and 0.01% HCOOH (pH 3 ±0.5) (30:13.59:56.41). The method was optimized by using Box-Behenken Design (BBD) from Design of Expert software. A wavelength of 220 nm was selected for detection. The injection volume of the sample was 20µl. The HPLC system was used in an air-conditioned laboratory atmosphere (20±2°C).

Preparation of Standard Solutions

Stock standard solutions of LINA and MET (1mg/ml) were prepared in the mobile phase. The readied stock arrangements were put away at 4°C ± 0.05, subsequently shielded from light. Working standard solutions were freshly arranged by diluting the stock solutions with mobile phase during analysis day. Calibration curves revealing peak area ratios of LINA and MET were built up in the range of 0.02-1µg/ml.

Selection of detection wavelength

LINA and MET showed significant absorbance at 220 nm using PDA detector.

Preparation of Sample Solution

Serial dilutions of analyte were prepared in the mobile phase and 1ml of each dilution was spiked into 100µL of plasma in a polypropylene centrifuge tubes. Then all the tubes were centrifuged for 20 min at 3000 rpm. Supernatant was collected in another Eppendorf tube and 20µL supernatant was injected into the analytical column.

Assay validation

The optimized RP-HPLC method was validated as per the guidelines of the [ICH] Q2 [R1] for various parameter [8].

Linearity and range

For linearity a concentration ranges from 0.02 to 1 µg/mL of LINA and MET was prepared. A calibration graph was plotted by taking peak area versus concentration. The correlation coefficient, intercept, slope and linear regression analysis was done [9].

Sensitivity

With the formula $3.3 \sigma/S$ and $10 \sigma/S$, limit of detection (LOD) and limit of quantitation (LOQ) was calculated respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot [10].

Specificity

The specificity was calculated by comparing test results obtained from the analysis of sample solution containing excipients with the results obtained from standard drug [11].

Precision studies

Precision was calculated by taking 0.02, 0.04, 0.06 µg/mL concentration of LINA and MET sample were analyzed six times on a similar day to find out any differences in the results [12].

Accuracy studies

Accuracy is the closeness in the agreement between the accepted true value and the actual results obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the mixture of the sample to be analyzed. For accuracy studies, three different concentration of solution such as 0.08 µg/mL, 0.1 µg/mL, and 0.12 µg/mL were used. After injecting each concentration mean % recovery was calculated [13].

RESULT AND DISCUSSION

Chromatographic development

Chromatographic analysis was developed using an Onyx C₁₈ Monolithic column (100mm× i.d., 5µm) connected with an Onyx C₁₈ guard cartridge (4mm×3mm i.d., 5µm). The mobile phase consists of ACN, MeOH and 0.01% HCOOH (pH 3 ±0.5) (30:20:50) and that was supplied at a flow rate of 1 mL/min. The method was optimized by using Design of Expert software. A wavelength of 220 nm was selected for detection. Fig. 3 shows the optimized chromatogram of LINA and MET.

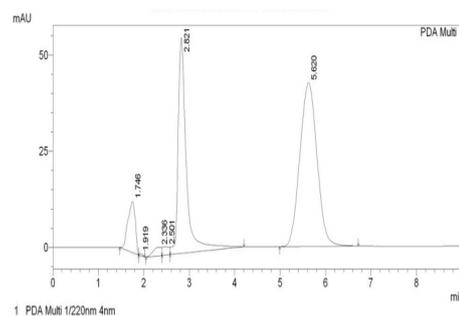


Fig. 3: Optimized chromatogram of LINA and MET

Validation of the method

Linearity

The analyte response was linear ($r^2=0.998$) over the concentration range of 0.02–0.1 µg/mL of LINA and MET. The results were shown in Table 1. Fig. 4 and Fig. 5 shows the calibration curve. The selected concentration gives acceptable accuracy and precision over a wide concentration range. The results demonstrate that an excellent correlation coefficient between the absorbance and concentration of LINA and MET.

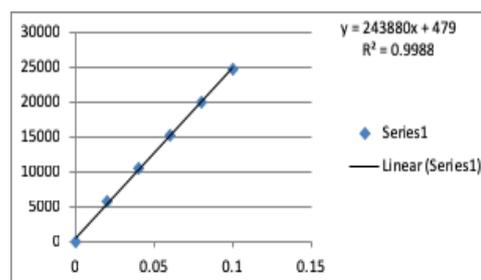


Fig. 4: Linearity graph of LINA

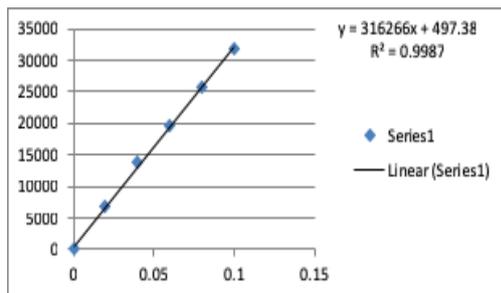


Fig. 5: Linearity graph of MET

Table 1: Linear regression analysis of LINA and MET

Parameters	LINA	MET
Linearity range (µg/mL)	0.02-0.1	0.02-0.1
Correlation Coefficient (r ²)	0.998	0.998
Slope	24388	31626
Intercept	479	497.3

Sensitivity

The LOD was found to be 0.0018 and 0.0039 µg for LINA and MET respectively. The LOQ for LINA and MET were found to be 0.0056 and 0.0118 µg respectively representing good sensitivity of the method.

Table 3: Results of recovery studies of LINA and MET

Amount taken		Amount added		% recovery		%RSD	
LINA	MET	LINA	MET	LINA	MET	LINA	MET
0.04	0.04	0.032	0.032	99.8	99.7	0.003	0.005
0.04	0.04	0.04	0.04	100	99	0.007	0.003
0.04	0.04	0.048	0.048	99.6	99.1	0.005	0.004

ANALYSIS OF A MARKETED PREPARATION WITH SPIKED PLASMA

The results obtained for the amount of LINA and MET in tablet powder spiked with human plasma against the label claims were in good agreement it indicates that there is no interference from the excipients presents in the tablet. The percent assay was found to be 99.9% and 99.4%, for LINA and MET respectively.

CONCLUSION

The plan to conduct this research work is to create and approve a method utilizing a simple, rapid, sensitive, precise, and accurate RP-HPLC for the routine determination of LINA and MET in bulk, plasma, and pharmaceutical preparations. The proposed method is appropriate for pharmaceutical investigation in different analytical laboratories. The retention time and run time were short, hence, requires less mobile phase for this technique, making it more economical and rapid. Consequently the technique can be utilized for the analysis of a large number of samples.

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Specificity

The chromatograms obtained from standard and sample solutions are not interfering which indicates method is highly selective.

Precision

In the estimation of LINA and MET (Table 2) showed that the % RSD was <2% during the analysis. These low values of RSD show that the precision of the method is good.

Table 2: Precision studies of LINA and MET

Drug	Actual Concentration	Precision Data	% RSD
LINA	0.04 µg	99.99	0.0333
MET	0.04 µg	99.99	0.0254

Accuracy

The study of accuracy reveals influences of additives that are usually present in the dosage forms on the quantitative parameters. The recovery study data presented in Table 3 indicates that the accuracy of the quantification of LINA and MET were more than 99%, which indicate that the proposed simultaneous RP-HPLC method is reliable for the estimation of marketed formulation used in the study.

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