

Stability Indicating UV Spectrophotometric Method For Lina- gliptin and Metformin in Pharmaceutical Dosage Form

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ABSTRACT

Introduction: Linagliptin and metformin are newly introduced FDA drug combination for the treatment type 2 diabetic patients. This study reveals the development of three methods for the UV spectrophotometric simultaneous estimation of linagliptin and metformin in pharmaceutical dosage and forced degradation studies on four different stress conditions. **Methods:** Development of method was based upon simultaneous equation, absorbance ratio and absorbances correction method with a simple solvent system of distilled water. Absorption maxima was found to be 230.4nm, 294.4nm for metformin and linoglipitin respectively without mutual interference. Isobestic point for both drugs was chosen at 250.4 nm. Stability studies were carried out on acid, alkaline, peroxide and thermal stress conditions. **Conclusion:** Degradation studies reveals that method capability on different stress condition. This method obeyed Beer's law in the concentration range of 2-14 µg /ml for metformin and 10-40 µg /ml for linoglipitin. The results of the analysis has been validated statistically.

Method proven to be rapid, precise and cost effective for the routine analysis in pharmaceutical dosage form. Forced degradation outcomes can be used for the development of stable formulation and helps in the planning of proper storage requirement.

Key words: Forced degradation studies, ICH guidelines, Simultaneous, linagliptin, Metformin.

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INTRODUCTION

Linagliptin and metformin are regularly prescribed as a fixed drug combination for the treatment type 2 diabetes. Linagliptin assist pancreas to release more insulin and also indicates the liver to stop producing glucose when high glucose level present blood thereby it maintains glucose level of the diabetic patient. Metformin reduces the absorption of glucose from the stomach, bring down the release of stored glucose from the liver. Linagliptin belong to new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs and chemically¹ is 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]- 2, 3, 6, 7-tetrahydro-1 H-purine-2, 6-dione. Metformin² chemically N, N-diethyl imido dicarbonimidic diamide hydrochloride and belongs to the biguanide class of anti-diabetic drug.

Literature studies reveals that no stability indicating simultaneous RP-HPLC and UV method with degradation studies for titled drug combinations were not reported so far till date with simple solvent system like distilled water.³⁻⁵ Though LC published article are available, this work stands apart by being simple interms of solvent consumption. Development of method was based upon three different approaches like simultaneous equation, absorbance ratio and absorbances correction method. Moreover LOD and LOQ establishes rapid and sensitive of the developed technique. Robustness study was performed on two different parameters demonstrates method capacity to withstand the deliberate changes. Forced degradation study carried out by UV spectroscopy at four different stress state reveals that method is stable to specified period of time

MATERIAL AND METHOD

Reagents and chemicals

Linagliptin and metformin was obtained from Mylan laboratories laboratories limited, hyderabad, India respectively. Formulation was procured from the local market having strength of 2.5 and 500 mg of Linagliptin and metformin respectively.

Instrument

Systronics spectrophotometer 2202 UV/Visible double beam spectrophotometer with spectral band width of 2 nm, 0.5 nm of wavelength was used for all spectral measurements using a pair of 1cm matched quartz cell .

Selection of diluents

Diluent suitable for both Linagliptin and metformin was found to be distilled water. Metformin was freely soluble but linagliptin made solubilised in distilled water with vigorous shaking.

Parameter fixations

In the UV region of 400-200 nm, absorption maxima was found to be 230.4 nm and 294.4 nm was for metformin, Linoglipitin respectively. Isobestic point for both drugs was chosen at 250.4 nm.

Stock and working solution

A quantity of 50 mg each of Linoglipitin, metformin were weighed into a 50 ml separate standard flask and made up to the mark with diluent. From the stock, solution 5 ml was pipetted into 50ml volumetric flask and diluted with the diluents to a get concentration of 100µg/ml. Subsequent dilution was made with diluent to get a concentration of 2-14µg/ml, 10-40µg/ml for metformin and Linoglipitin respectively.

Standard and sample preparation

The developed procedure was extended to formulation of Linoglipitin and metformin. Tablets were crushed to a fine powder, powder equivalent to 50 mg was transferred to a 50 ml volumetric flask, dissolved in distilled water and then the solution was made up to the mark with the same and filtered through whatman filter paper no 42. Further dilution are done to get a concentration of 100µg/ml. Subsequent dilutions of this solution were made to a get final concentration of 10 µg/ml and 40µg/ml of metformin and Linoglipitin respectively for method one and two.⁶

Final dilution for method three⁷ was made to get concentration of 3 µg/ml and 40µg/ml of metformin and Linagliptin respectively.

Method 1: Vierordt's method of simultaneous determination

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad \text{..... (1)}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \quad \text{..... (2)}$$

Where, A_1 and A_2 are absorbance of mixture at 230.4 nm (λ_1) and 294.4 nm (λ_2) respectively, a_{x1} and a_{x2} are absorptivities of metformin at λ_1 and λ_2 respectively, a_{y1} and a_{y2} are absorptivities of linagliptin at λ_1 and λ_2 respectively, C_x and C_y are concentrations of metformin and linagliptin respectively

Method 2: Absorbance ratio method (Q-Absorbance method)

$$C_x = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{a_{x1}} \quad C_Y = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A_1}{a_{y1}}$$

$$Q_M = A_2/A_1 \quad Q_X = a_{x2}/a_{x1} \quad Q_Y = a_{y2}/a_{y1}$$

Where, C_x is the concentration of metformin and C_y concentration of linagliptin, A_1 & A_2 are the absorbance of the mixture at λ_1 nm (250.4 nm isobetic point) & λ_2 nm (294.4 nm) respectively; a_{x1} and a_{y1} are absorptivity of metformin and linagliptin respectively at λ_1 nm; a_{x2} and a_{y2} are absorptivity of metformin and linagliptin respectively at λ_2 nm.

Method 3: Absorbance correction method

$$A = abc \\ C_x = A_1/ab \\ C_x = A_1/a_{x1} * b \quad \text{..... (1)}$$

$$A_2 = A \text{ metformin} + A \text{ Linagliptin} \\ A_2 = (a_{y2} * c_y * b) + (a_{x2} * c_x * b) \\ A_2 = (a_{y2} * c_y * b) + (a_{x2} * c_x * b)$$

$$C_y = [A_2 - (a_{x2} * c_x)] / a_{y2} \quad \text{..... (2)}$$

where A_1 , A_2 are absorbance of mixture at 230.4 nm (λ_1) and 294.4 nm (λ_2), respectively, a_{x1} and a_{x2} are absorptivities of metformin at λ_1 and λ_2 , respectively, a_{y1} and a_{y2} are absorptivities of linagliptin at λ_1 and λ_2 , respectively, c_x and c_y are concentrations of metformin and linagliptin respectively.

Forced degradation study⁸⁻¹²

Forced degradation is a procedure that includes the degradation of drug molecule at different stress conditions that can be used to find out the stability of the drug substance.

Objectives of forced degradation study

To expose the degradation mechanisms at different stress conditions of the drug formulation

To check the intrinsic stability of a drug molecule in dosage form

To purpose stability indicating nature of a developed techniques

Acidic and Alkaline degradation

From the working solution, 1.4 ml of metformin and 4 ml of linagliptin was taken into separate 10 ml flasks and degradation studies were carried out. 3 ml of 0.1N HCl was added to flasks containing solution of metformin, linagliptin for acidic condition and same procedure repeated

with 3ml of 0.1N NaOH for alkaline condition. Then, the volumetric flasks were kept at 60-70°C in reflux condition 15 minutes in dark for acid and alkaline condition respectively. Finally, volume made up to 10 ml with diluents and absorbance was measured.

Oxidative and thermal degradation

From working solution, transfer 1.4 ml of metformin and 4 ml of linagliptin into separate flask and to each 1ml of 6 % w/v of hydrogen peroxide was added and the volume was made up to 10 ml the mark with diluent. Then, the volumetric flasks were kept in dark for 15 min and absorbance was measured.

Thermal study was carried out by taking 1.4 ml of metformin and 4 ml of linagliptin from working solution into separate flasks 10 ml flask. Then kept in hot air oven at 90°C for 15 min and vol was made up to 10 ml with diluents and absorbance was measured.

Method Validation¹²⁻¹⁴

Accuracy

The accuracy of the method was determined by preparing solutions at different concentrations such as 80%, 100% and 120% in which the amount of formulation was kept constant and the amount of pure drug was varied at three different levels. The solutions were prepared in triplicates and the accuracy was indicated by percentage of recovered amount of drug.

Precision

Precision studies were performed for intermediate/ruggedness and in-traday. Ruggedness were carried out by using different UV instrument. Aliquot portions of stock solution of both drug was further diluted to get concentration of 2,10µg/ml for metformin and for linagliptin 10,40µg/ml.

Linearity

Linearity was established in the range of 2-14 µg/ml for metformin and 10-40 µg/ml of linagliptin. The correlation coefficient of the linearity was found to be 0.999 at each wavelength for both drugs. Residual plot displayed excellent linearity.

Stability of analytical solution

The stability of analytical solution for the standard and sample preparation was determined by taking the absorbances at 4, 8, 12, 24 and 48 h.

Specificity study

Specificity study was conducted by comparing the spectrum of tablet solution and that of standard solution. It was noted that there was no interference of the excipients in the formulations. Forced degradation studies also confirms the specificity of the developed method.

Robustness study

It was conducted by the minor variations in the optimized parameters like pH of solvent system and the absorption maxima of the determination. It was observed that there were no much changes in the absorption value of the two titled drugs.

LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated under the optimized conditions. Limit of detection can be calculated using the following equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times F/S$$

F = Standard deviation of the response, S = Slope of the corresponding calibration curve.

LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under optimized conditions. Limit of quantification can be calculated using the following equation as per ICH guidelines.

$$\text{LOQ} = 10 \times F/S$$

F = Standard deviation of the response, S = Slope of the corresponding calibration curve.

RESULT

Forced degradation studies

Metformin degraded up to 19.72 % that was slightly high in contrast to linagliptin under acidic condition whereas as in alkali condition linagliptin degraded little more than metformin. Moreover Slight shift of lamda max was detected for linagliptin under alkaline forced degradation study. This may be due to break down of ionisable functional group present in the titled drug molecule. Linagliptin undergone high degradation under peroxide stress condition in comparison to metformin and may be due electron transfer mechanism to form reactive cations and anions. Thermal degradation explained on the basis of arrhenius equation and linagliptin was slightly sensitive to thermal conditions. Forced degradation studies proves that these methods are apt for its proposed use. Forced degradation results can be used for the development of stable formulation and helps in designing proper storage requirement (Table 1 & Figure 1,2).

Optimization and validation of method

Three methods were used for the estimation of metformin and linagliptin in pharmaceutical dosage form with a simple solvent system (Table 2 & Figure 3, 4). Validation studies were carried out on different parameters

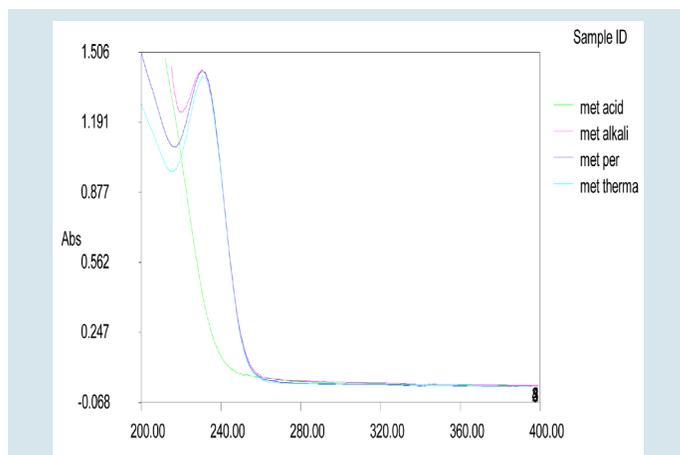


Figure 1: Forced degradation studies of Metformin.

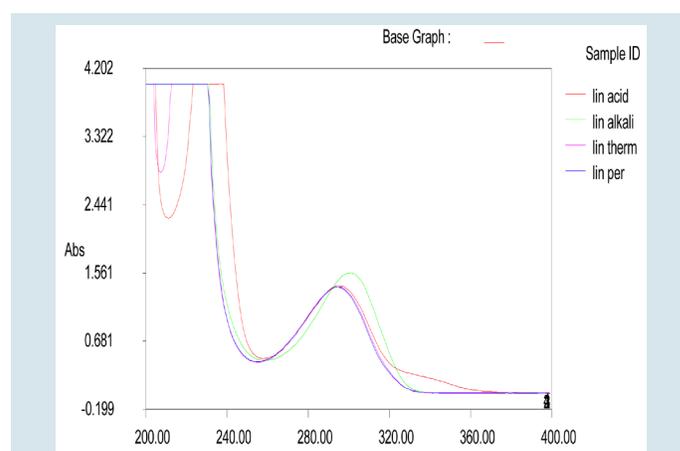


Figure 2: Forced degradation studies of Linagliptin.

Table 1: Forced degradation study

Stress condition	Meformin			linagliptin		
	Amount found (µg/ml)	%Drug degraded	% Drug obtained	Amount found (µg/ml)	%Drug degraded	% Drug obtained
Acid (0.1N) 15 min with reflux condition	11.23	19.72	80.27	33.82	15.42	84.57
Alkali (0.1N) 15 min with reflux condition	13.70	2.09	97.90	35.47	11.31	88.68
Hydrogen peroxide (6%w/v) 15 min Without reflux condition	13.46	3.83	96.16	33.39	16.51	83.48
Thermal 60 min 80-90°C	13.60	2.78	97.21	33.42	16.39	83.60

Table 2: Analysis of sample preparations

Methods	Drug	Label claim (mg)	Amount found (mg)	% label claim
Method 1	metformin	500	491.12	98.22
	linagliptin	2.5	2.46	98.60
Method 2	metformin	500	490.68	98.13
	linagliptin	2.5	2.49	99.79
Method 3	metformin	500	495.88	99.17
	linagliptin	2.5	2.46	98.59

Average mean of six determinations

as per ICH guideline such as recovery study, intraday and intermediate precision and range, LOD and LOQ, Specificity and robustness study. Linearity was established at seven different concentrations for both titled drugs with good correlation coefficient and residual plots. Residual are the vertical line between regression line and recorded data. Residual plot does not displayed obvious pattern and relative smaller in size. Overall perditied y value and observed y value are closer that indicate good linear relationship between x and y variables (Figure 5, 6).

Accuracy or recovery conducted at three different levels (Table 3). Intra

and Inter day precision were carried out using same optimized conditions and and RSD¹⁵⁻¹⁶ was less than 2 % (Table 4). LOD and LOQ indicate that method was highly sensitive and fast (Table 5). The method was found highly specific since there is no interference form the excipients of the formulations. Analytical solution was found stable up to 48 hrs on stable storage condition. The method has been highly robust since deliberate changes in the optimized parameters does not effect to a certain extent (Table 6).

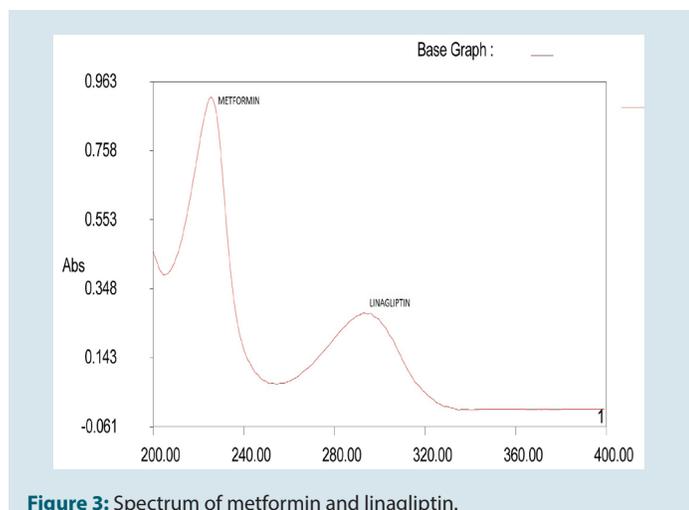


Figure 3: Spectrum of metformin and linagliptin.

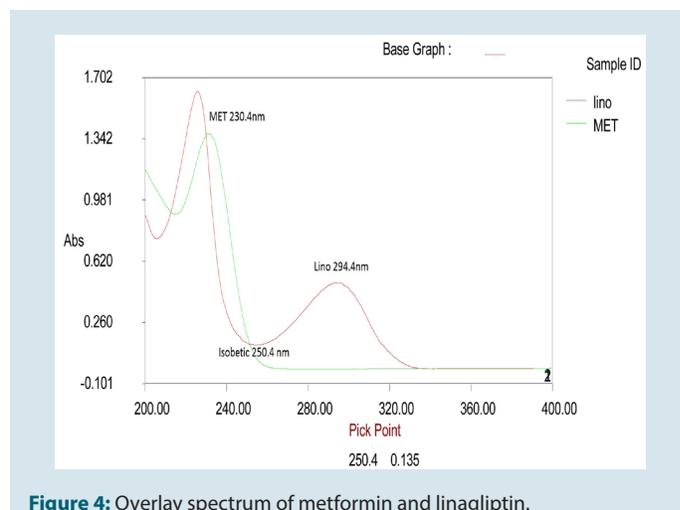


Figure 4: Overlay spectrum of metformin and linagliptin.

Table 3: Recovery Study							
Drug	Amount taken µg/ml	Amount added µg/ml	Recovery level in %	Total Amount of drug µg/ml±SD	Amount recovered	% Recovery*	
metformin	2	1.6	80	3.61±0.08	1.60	100.08	
		2	100	3.98±0.07	1.98	99.32	
		2.4	120	4.43±0.06	2.38	99.42	
linagliptin	10	8	80	17.73±0.27	7.73	96.72	
		10	100	19.66±0.47	9.66	96.66	
		12	120	12.09±0.29	12.0	100.75	

Average mean of three determinations in each level

Table 4 : Precision study									
Precision	metformin				linagliptin				
	2µg/ml (n=6)		10µg/ml(n=6)		10µg/ml (n=6)		40µg/ml(n=6)		
	mean	%RSD	mean	%RSD	Mean	%RSD	mean	%RSD	
Intermidate/ Ruggdness	0.245	1.02	0.971	1.10	0.255	1.02	1.21	0.11	
Intraday	0.238	0.63	0.969	0.91	0.252	0.75	1.32	0.09	
Method	0.232	0.68	0.958	0.89	0.242	0.71	1.29	0.08	

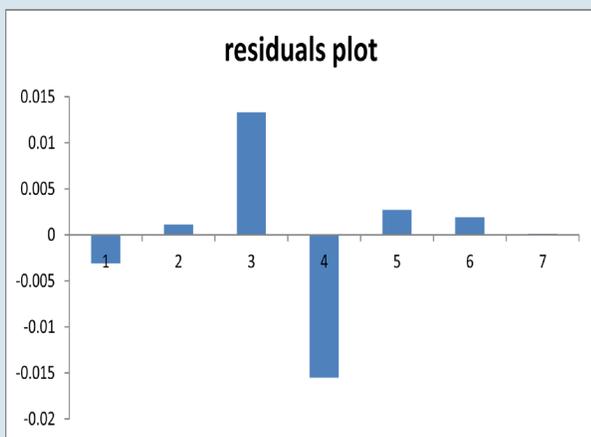


Figure 5: Metformin residual plot.

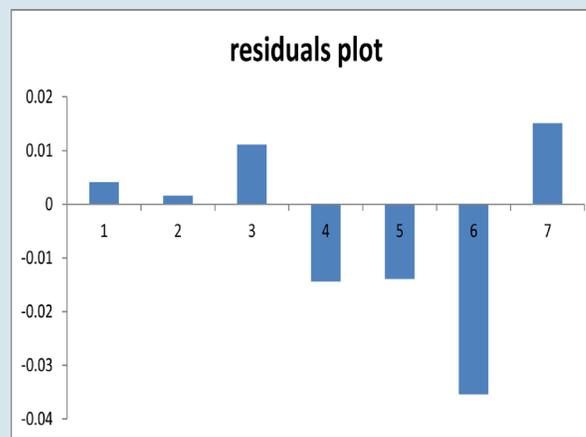


Figure 6: Inagliptin residual plot.

Table 5: LOD and LOQ

Drug µg/ml	LOD	LOQ
Metformin	0.34	1.71
linagliptin	1.03	5.18

Table 6: Robustness study

Parameters	Modifications	Absorbance		% RSD	
		metformin	linagliptin	metformin	linagliptin
Mobile phase	pH 6.5	0.407	0.419		
	Optimized	0.422	0.435	1.83	1.92
	pH 7.5	0.417	0.424		
Absorption maxima	+2 nm	0.418	0.432		
	Optimized nm	0.422	0.435	0.49	0.58
	- 2nm	0.421	0.43		

CONCLUSION

The developed spectroscopic methods are found to be rapid, cost effective, accurate and precise and can be used for routine analysis of metformin and linagliptin. The developed methods were validated as per ICH guidelines. The % RSD for the validation parameters was found to be less than 2%. Hence proposed method may be used for routine analysis of these drugs in pharmaceutical dosage forms. Stability indicating nature of the method well established for a specified period which indirectly point out the method competence under different stress condition and thereby useful for the designing of storage requirement of the formulation. This work can be extended to characterize unknown degradants with integrated approach.

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CONFLICT OF INTEREST

None

ABBREVIATIONS USED

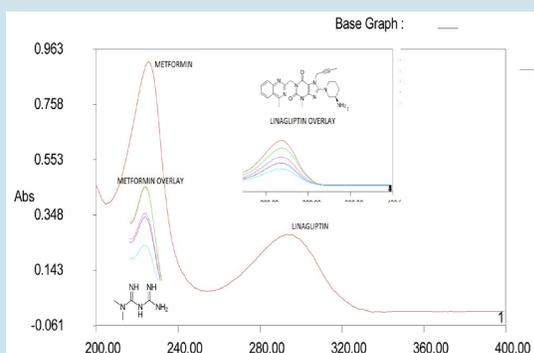
UV spectroscopy: Ultra violet spectroscopy; ICH-International Conference on Harmonisation; DPP: dipeptidyl peptidase; LOD: Limit of detection; LOQ: Limit of quantification.

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PICTORIAL ABSTRACT



SUMMARY

- This contains overlay spectrum of metform and linagliptin along with binary combination mix of two titled drugs.
- This spectroscopic analytical technique are found to be rapid, cost effective, accurate and precise and can be used for routine analysis of metformin and linagliptin.

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