

*Research Article*

**Stability-Indicating HPTLC Method for Quantitative estimation of Linagliptin and Metformin Hydrochloride.**

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**ABSTRACT**

A new simple, accurate, precise and selective stability- indicating high performance thin layer chromatographic (HPTLC) method was developed and validated for simultaneous estimation of Linagliptin and Metformin Hydrochloride in bulk and in tablet dosage form. The chromatographic development was carried out on precoated silica gel 60 F<sub>254</sub> aluminium plates using mixture of 1% ammonium acetate in MeOH: CHCl<sub>3</sub>: strong NH<sub>3</sub> solution (9: 3 :0.1v/v) as mobile phase and densitometry scanning of band at 225 nm using Camag TLC Scanner-3 with win CAT 1.4.3 version software. The R<sub>f</sub> value of Linagliptin and Metformin Hydrochloride were found to be 0.70± 0.02 and 0.50 ± 0.02, respectively. The method was validated with respect to linearity, accuracy, precision and robustness. The calibration curve was found to be linear over a range of 500 - 2000 ng/ band for Linagliptin and 250 – 1250 ng/ band for Metformin Hydrochloride. The drugs were subjected to stress conditions of hydrolysis under different pH conditions, oxidation, photolysis and thermal degradation.

**KEYWORDS**

Linagliptin, Metformin Hydrochloride, HPTLC, Stability.

## **1. INTRODUCTION**

Linagliptin is an inhibitor of DPP-4, an enzyme that degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP). Both GLP-1 and GIP increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose levels. GLP-1 also reduces glucagon secretion from pancreatic alpha cells, resulting in a reduction in hepatic glucose output. Thus, Linagliptin stimulates the release of insulin in a glucose-dependent manner and decreases the levels of glucagon in the circulation. <sup>[1]</sup> Metformin Hydrochloride is indicated in patients with type 2 diabetes to control hyperglycemia that cannot be controlled by diet management, exercise, or weight reduction, or when insulin therapy is not required or feasible. Metformin Hydrochloride is used in the treatment of type 2 diabetes (non-insulin-dependent diabetes); as a lipid-lowering agent; and in the treatment of obesity in patients with non-insulin-dependent diabetes mellitus. <sup>[2]</sup> Literature survey revealed that various analytical methods like spectrophotometric <sup>[3][4]</sup>, Simple HPLC <sup>[5-9]</sup>, SIM RPHPLC <sup>[10-13]</sup>, SIM HPTLC <sup>[14-15]</sup>, Bioanalytical HPLC <sup>[16]</sup> have been reported for the determination of Linagliptin and Metformin Hydrochloride but no stability indicating HPTLC method has yet been reported for estimation of Linagliptin and Metformin Hydrochloride in combination. Development of SIM is based on systematic exposure of API to various stress conditions. Systematic optimization trials are required to arrive at combination of “concentration of stress reagent and duration of exposure”, to obtain degradation preferably in the 10-30% range. Typical degradative conditions involve hydrolysis under different pH conditions, photolysis and thermal.

Achieving 100% degradation could possibly cause secondary degradation. Secondary degradation products are the degradation products, which are not likely to be formed under normal storage conditions.

## **2. MATERIALS AND METHODS**

### *2.1. Reagents and Chemicals*

Authentic sample of Linagliptin and Metformin Hydrochloride were obtained from Wolkhardt limited (Aurangabad) and Ajanta pharma limited (Mumbai), respectively. Methanol (AR grade), Chloroform (AR grade) were purchased from S. D. fine chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30% v/v) sodium hydroxide (NaOH) and Ammonium acetate were purchased from Loba Chemie Pvt. Ltd. Mumbai.

### *2.2. Preparation of Standard Stock Solution*

Standard stock solution of Linagliptin and Metformin Hydrochloride were prepared separately by dissolving 10 mg of drug in 10 ml of methanol(in case of Linagliptin) and in 10ml water(in case of Metformin Hydrochloride) to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100µg/ml of Linagliptin and 50µg/ml Metformin Hydrochloride separately using MeOH.

### *2.3. Selection of Detection Wavelength*

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that both the drugs showed considerable absorbance at 225 nm.

### *2.4. Chromatographic State and Instrumentation*

Chromatographic separation of drug was performed on Aluminum plates precoated with silica gel 60 F<sub>254</sub>, (10 cm × 10 cm with 250 µm layer thickness). Samples were applied on the plate as a band with 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of 1% ammonium acetate in methanol:CHCl<sub>3</sub>:NH<sub>3</sub>(9:3:0.1). 20 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 ml of organic solvent was used per run, migration distance was 90 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by win CATS software (Version 1.4.3, Camag), slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

### *2.5. Preparation of Assay Solution*

By geometric mixing with tablet excipients, a blend was prepared such that 1500mg blend is equivalent to 1000mg of Metformin Hydrochloride and 10mg of Linagliptin. Appropriate quantity of Blend was weighed and dispersed in 10ml MeOH. This solution was filtered and diluted appropriately to obtain final two solutions, one with concentration of 100 µg/ml of Linagliptin and another one with 50µg/ml of Metformin Hydrochloride.

### *2.6. Stress Degradation Studies of Bulk Drug*

Stress testing studies were carried out to provide evidence on how the quality of drug varies under the influence of variety of environmental conditions like hydrolysis under different pH conditions, oxidation, heat etc.

#### *2.6.1. Alkali Catalyzed Hydrolysis*

1 ml working standard solution of Linagliptin (1000 µg/ml) was mixed with 1 ml of 2 N NaOH (methanolic) and volume was made up to 10ml. Solution was kept for 3hrs and applied on TLC plate. Metformin Hydrochloride was treated in similar manner to Linagliptin and further diluted to get 50µg/ml as final concentration and was applied.

#### *2.6.2. Acid Catalyzed Hydrolysis*

1 ml working standard solution of Linagliptin (1000 µg/ml) was mixed with 1 ml of 0.1 N HCl (methanolic) and volume was made up to 10ml. Solution was kept overnight and applied on TLC plate. Metformin Hydrochloride was treated in similar manner to Linagliptin and further diluted to get 50µg/ml as final concentration and was applied on TLC plate

### *2.6.3. Neutral Hydrolysis*

1 ml working standard solution of Linagliptin (1000 µg/ml) was mixed with 1 ml water, volume was made up to 10ml with MeOH. Solution was kept for overnight and applied on TLC plate. Metformin Hydrochloride was treated in similar manner to Linagliptin and further diluted to get 50µg/ml as final concentration and was applied.

### *2.6.4. Oxidation Degradation*

1 Ml working standard solution of Linagliptin (1000 µg/ml) was mixed with 1ml 6% v/v H<sub>2</sub>O<sub>2</sub>, volume was made up to 10ml with MeOH. Solution was kept for 3hrs and applied on TLC plate. Metformin Hydrochloride was treated in similar manner to Linagliptin and further diluted to get 50µg/ml as final concentration and was applied.

### *2.6.5. Degradation under Dry Heat*

Dry heat study was performed by keeping both drugs in oven at 60<sup>0</sup> C. A sample was withdrawn after 24hrs, weighed and dissolved in methanol to get solution of 100µg/ml of Linagliptin and 50µg/ml of Metformin Hydrochloride and then applied on TLC plate.

### *2.6.6. Photo-Degradation*

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hrs/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux hrs. Sample was weighed, dissolved and diluted get 100 µg/ml as final concentration and was applied on TLC plate. Metformin Hydrochloride was treated in similar manner to Linagliptin and further diluted to get 50µg/ml as final concentration and was applied.

## **3. RESULTS AND DISCUSSION**

### *3.1. Optimization of Chromatographic Conditions*

The primary target in developing this stability indicating HPTLC method is to achieve the resolution between Linagliptin and Metformin Hydrochloride and its degradation products. Since peak purity values as per Win CATS software were within limits, various trials by varying the proportion of mobile phase components and pH were carried out. Optimized mobile phase is 1% ammonium acetate in methanol: CHCl<sub>3</sub>:NH<sub>3</sub> (9:3:0.1) that resulted in R<sub>f</sub> of 0.70 ± 0.02 and 0.50 ± 0.02 for Linagliptin and Metformin Hydrochloride respectively (Figure 2). Forced degradation study showed the method is highly specific and no degradation products were retained at R<sub>f</sub> of drugs.

### *3.2. Result of Forced Degradation Studies*

Degradation was observed for Linagliptin and Metformin Hydrochloride samples during stress conditions like hydrolysis under different pH conditions, oxidation, dry heat and light. Results of the stress degradation studies are presented in Table 1. No peak was observed for product of any reagent induced degradation

### 3.3. Method Validation

#### 3.3.1. Linearity

The calibration curve was obtained in the range of 250 -1250ng/band for Linagliptin and 500-2500 ng/band for Metformin Hydrochloride by applying different volumes on TLC of stock solution 50µg/ml and 100 µg/ml resp. Each standard in five replicates was analyzed and peak areas were recorded. Standard calibration graph was plotted of peak area Vs concentration applied. The equation of the calibration curve found for Linagliptin was  $y = 5.8922x + 3068.3$  and for Metformin Hydrochloride,  $y = 10.625x + 1348.6$ . The coefficient of correlation ( $r^2$ ) was found to be 0.993 for Linagliptin and 0.998 for Metformin Hydrochloride shown in Figure 3

#### 3.3.2. Precision

The precision of the system was demonstrated by intra-day and inter-day studies. In the intraday study 6 replicates of 1 standard concentrations (250ng/band for Linagliptin and 500 ng/band for Metformin Hydrochloride) were analyzed in a day and percentage RSD was calculated. For the inter day study, 3 replicates of 3 consecutive concentrations were analyzed and percentage RSD was calculated. For intraday system precision %RSD was found to be 0.13% for Linagliptin and 0.20 % for Metformin Hydrochloride. For inter-day precision % RSD found to be 0.05% for Linagliptin and 0.53 % for Metformin Hydrochloride

#### 3.3.3. Accuracy

To check accuracy of the method, recovery studies were carried out by spiking standard drugs to excipient blend at three different levels. Spiked blend was extracted using MeOH. These solutions were applied on TLC plates. The drug concentrations of Linagliptin and Metformin Hydrochloride were calculated by using linearity equations of Linagliptin and Metformin Hydrochloride, respectively. % Recovery was within 97% - 100% for both the drugs.

#### 3.3.4. Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity is spectral purity of particular peak that it is not co eluted with other impurities and calculated at peak start, peak middle and peak end point. The peak purity values were found to be more than 0.995, indicating the no interference of any other peak of degradation product, impurity or matrix at  $R_f$  value of drugs.

#### 3.3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated as  $3.3\sigma/S$  and  $10\sigma/S$ , respectively; where  $\sigma$  is the standard deviation of the response and S is the slope of the calibration plot. The LOD of Linagliptin and Metformin Hydrochloride were found 7.42ng/band and 9.67ng/band, respectively. The LOQ of Linagliptin and Metformin Hydrochloride were found to be 13.3202ng/band and 29.31ng/band, respectively.

#### 3.3.6. Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, chamber saturation time, Time from application to development and

Time from development to scanning are altered and the effects on the area were noted. The results obtained are shown in Table 2.

#### **4. DISCUSSION**

There is one paper available in literature for Stability indicating HPTLC method. In work of A. Rajasekaran et al mobile phase is quaternary and in degradation study hydrolysis under acidic condition i.e. 0.1N HCl for 3hrs at 80<sup>0</sup>C gave recovery 57.41%, in case of basic hydrolysis i.e. 2N NaOH for 3 hrs at gave 19.73% recovery while in oxidative hydrolysis i.e. 3% H<sub>2</sub>O<sub>2</sub> for 3 hrs at 80<sup>0</sup>C gave 46.64% recovery while in current work mobile phase is tertiary, and from acid hydrolysis (0.1N HCl for overnight) recovery was 82.27%, basic hydrolysis i.e. 2N NaOH for 3 hrs gave 77.4% recovery oxidative hydrolysis i.e. 3% H<sub>2</sub>O<sub>2</sub> for 4 hrs gave 71.01% recovery. Since this result do not match our observations, further study may be taken up for confirmation by using API from different sources.

#### **5. CONCLUSION**

The developed method is stability indicating and can be used for assessing the stability of Linagliptin and Metformin in bulk form. The developed method is specific, selective, accurate and precise.

#### **6. ACKNOWLEDGEMENT**

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**Table 1:** Summary of stress degradation of Linagliptin and Metformin Hydrochloride.

stress condition	Metformin Hydrochloride			Linagliptin		
	% Recovered	Peak purity r(s,m)	r(m,e)	% Recovered	Peak purity r(s,m)	r(m,e)
Acid (0.1N overnight)	82.27	0.999	0.999	71.96	0.999	0.999
Base (2N NaOH 3Hrs)	77.4	0.999	0.999	68.13	0.998	0.999
Neutral(Overnight)	91.71	0.998	0.998	79.65	0.999	0.998
Oxidative (3% H <sub>2</sub> O <sub>2</sub> 4hrs)	71.01	0.998	0.999	92.65	0.998	0.999
Thermal(60°C Overnight)	88.91	0.998	0.997	73.98	0.997	0.999
Photo Stability (UV, 200 Watt Hrs/Square Meter)	UV :86.24	0.999	0.999	UV: 89.66	0.999	0.999
(Florescence Million Lux. Hrs)	1.2 Flu : 82.09	0.999	0.999	Flu: 78.13	0.999	0.999

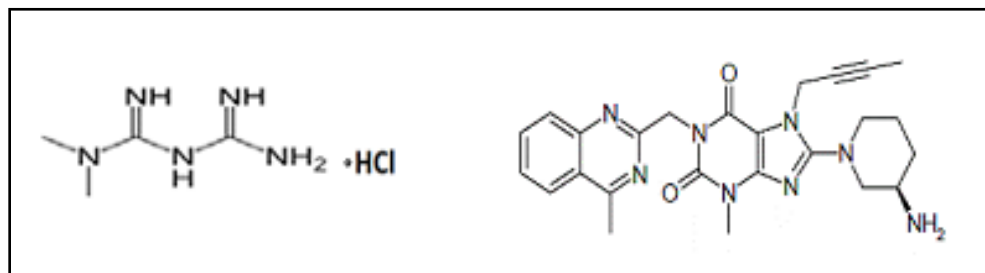
**Table 2:** Robustness of Linagliptin and Metformin Hydrochloride.

Sr. No.	Parameters	Robust condition	% RSD	
			Metformin Hydrochloride	Linagliptin
1.	Saturation time (15min)	13min	0.08	0.17
		17min	0.31	0.13
2.	Mobile composition (1% ammonium hydroxide in methanol:CHCl <sub>3</sub> :NH <sub>3</sub> (9:3:0.1)	1% ammonium acetate in methanol:CHCl <sub>3</sub> :NH <sub>3</sub> (9:2.5:0.1)	1.05	0.37
		1% ammonium acetate in methanol:CHCl <sub>3</sub> :NH <sub>3</sub> (9:3.5:0.1)	0.11	0.036
3.	Time from spotting to development(immediate)	After 30min	1.38	0.098
		After 2hrs	0.18	0.27
4.	Time from development to scanning(immediate)	After 2hrs	0.03	0.012
		After 24hrs	0.35	0.19

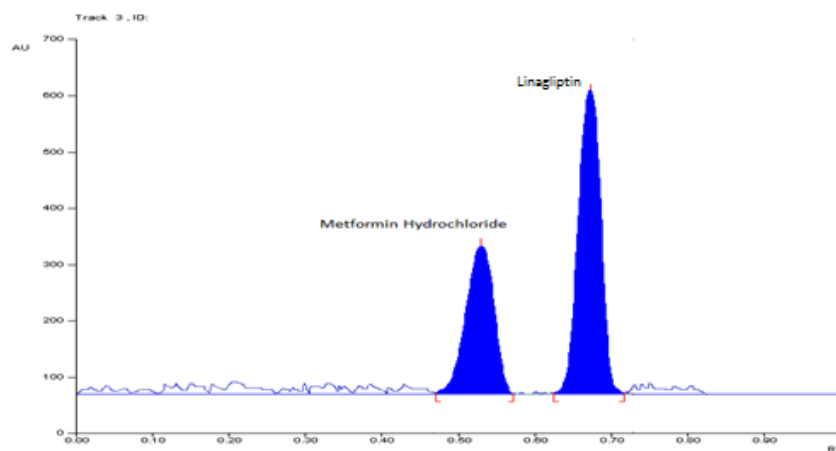


**Table 3:** Summary of validation study of Linagliptin and Metformin Hydrochloride.

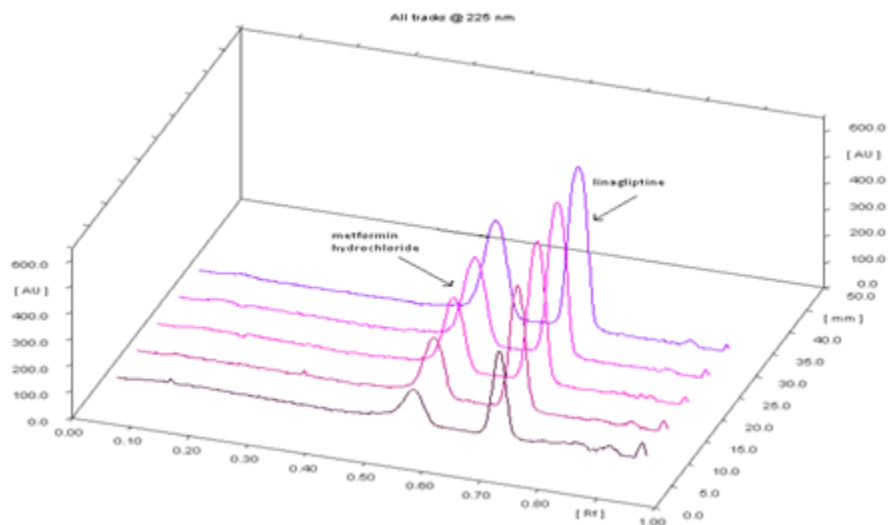
Validation Parameter	Linagliptin	Metformin Hydrochloride
Linearity Equation	$Y=5.892x + 3068$	$Y=10.62x + 1348$
( $r^2$ )	$r^2 = 0.993$	$r^2 = 0.998$
Range	500 –2500 ng/band	250 – 1250 ng/band
Precision (% RSD)		
Intra day	0.13%	0.20%
Inter day	0.05%	0.53%
Accuracy	Within limits	Within limits
(% recovery)	( $100 \pm 2$ %)	( $100 \pm 2$ %)
Limit of Detection	7.42 ng/ band	9.6747ng/band
Limit of Quantitation	13.3202ng/band	29.317ng/band
Specificity	Specific	Specific
Robustness	Robust	Robust



**Fig. 1(A):** Linagliptin **Fig. 1(B):** Metformin Hydrochloride



**Fig. 2:** Representative Standard densitogram of Linagliptin (500 ng/band,  $R_f 0.70 \pm 0.02$ ) and of Metformin Hydrochloride (250 ng/band,  $R_f 0.50 \pm 0.02$ )



**Fig. 3:** Linearity study of Linagliptin at 225nm in 500-2500 ng/band concentration and of Metformin Hydrochloride at 225nm in 250-1250 ng/band concentration.