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## Original Research Paper

### A SIMPLE AND SENSITIVE STABILITY-INDICATING HPTLC ASSAY METHOD FOR THE DETERMINATION OF LINAGLIPTIN

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#### ABSTRACT

A simple, sensitive, accurate and stability-indicating economical densitometric method has been developed and validated for the quantitative determination of linagliptin in bulk and pharmaceutical dosage forms. Separation of the drug was carried out using ethyl acetate: IPA : Ammonia 7:3:0.4 (v/v/v) as mobile phase on precoated silica gel 60 F<sub>254</sub> plates. The retention factor ( $R_f$ ) for linagliptin was  $0.61 \pm 0.05$ . The detection of band was carried out at 225 nm. The method is validated with respect to linearity, accuracy, precision, robustness and forced degradation studies, which further proves the stability-indicating supremacy of the method. During forced degradation studies, linagliptin is observed to be labile to acid, base hydrolysis and oxidative stress and stable in photolytic and thermal stress. The degradation products are well separated from the linagliptin peak, thus proving the stability-indicating superiority of the method. The calibration curve was linear in the concentration range 100 to 700 ng per band with correlation coefficient ( $r^2 = 0.997$ ) and is found to be sensitive for linagliptin, with a detection limit of 47 ng per band and a quantification limit of 142 ng per band. The proposed method is simple, rapid and economical when compared to liquid chromatographic method for the determination of linagliptin in bulk and pharmaceutical dosage form.

**Keywords:** Linagliptin, Densitometric method, Stability-indicating, Validation.

#### INTRODUCTION

Linagliptin is a highly potent, selective CD26 (DPP-4) inhibitor with IC<sub>50</sub> of 1 nM. Linagliptin is described chemically as 1H-Purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyln-1-yl)-3,7-dihydro-3-methyl-1-[(4-methyl-2quinazolin-yl) methyl]. 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, dipeptidyl peptidase-4 (DPP-4). 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. In summary, Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP. Linagliptin was approved by the FDA in May 2011. Results in 2010 from a Phase III clinical trial of Linagliptin showed that the drug can effectively reduce blood sugar.<sup>1,4</sup> Literature survey reveals that Linagliptin can be estimated by RP-HPLC<sup>5</sup> and also in combination with other drugs.<sup>6,7</sup> U.V. Spectrophotometric method reported for

the estimation of linagliptin in bulk and pharmaceutical dosage forms.<sup>8</sup>

To date there have been no published reports for quantitation of Linagliptin by HPTLC in bulk and in tablet dosage form. The proposed method is validated as per ICH guidelines.<sup>9</sup> Present work describes a simple, accurate, precise and stability-indicating method for the estimation of linagliptin in bulk and tablet formulations.

## MATERIAL AND METHODS

### Materials

Pure drug Linagliptin, Tradjenta® tablets (Linagliptin-5 mg), a pro drug was provided by our APL Research Centre-II. (A Division of Aurobindo Pharma Ltd). All the reagents and chemicals used were of analytical grade from Merck Chemicals, India.

Standard stock solution (1 mg mL<sup>-1</sup>) of Linagliptin was prepared by dissolving 50 mg of drug in 50 mL methanol. This solution (5 mL) was further diluted to 50 mL to furnish stock solution of 100 ng µL<sup>-1</sup>

### Chromatography

The samples were spotted in the form of bands of width 6mm with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel 60 F<sub>254</sub> aluminum HPTLC plates (20 cm x 10 cm) with 250 µm thickness (E. Merck, Darmstadt, Germany) using a CAMAG Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min, prior to chromatography. Linear ascending development was carried out in a 20 cm x 10 cm twin-trough glass chamber (CAMAG) using the mobile phase ethyl acetate: IPA: Ammonia 7:3:0.4 (v/v/v). The chamber saturation time was 15 min. The development distance was 9 cm and the development time 20 min. The plates were dried in a current of air with the help of a hair dryer. Densitometric scanning was performed with a CAMAG TLC Scanner 3 at 225 nm operated by Wincats software version 4.06. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 200 and 400

nm. The slit dimension were 5 mm x 0.45 mm and the scanning speed of 20 mm s<sup>-1</sup>.

### Method Validation

The proposed method was validated as per ICH guidelines

#### *Specificity*

The specificity of the method was ascertained by analyzing standard drug and samples of equivalent concentration (500 ng per band). The band for linagliptin in the sample was confirmed by comparing the R<sub>f</sub> values and spectra of the band from the sample with those from the standard. The peak purity of linagliptin was assessed by comparing the spectra at three different levels, i.e., peak start, peak apex and peak end.

#### *Precision*

The repeatability of sample application and of measurement of peak area was determined by performing six replicate measurements of the same band using 500 ng per band of linagliptin. The intra-day and inter-day variation in the analysis of linagliptin was measured.

#### *Limits of Detection and Quantification*

To estimate the limits of detection (LOD) and quantification (LOQ), blank methanol was applied to a plate six times by the method described above. The signal-to-noise ratio (S/N) was determined. LOD and LOQ was regarded as the amounts for which S/N was 3:1 and 10:1 respectively.

#### *Linearity*

For preparation of the calibration plot aliquots of (1-7 µL) of the fresh standard stock solution (100 ng µL<sup>-1</sup>) of linagliptin were applied by over spotting on an TLC plate and the plate was developed and scanned as described above. Each standard was analyzed in five replicates and peak areas were recorded. Calibration plot for linagliptin was constructed separately by plotting peak area against respective concentration of linagliptin.

#### *Accuracy*

To check accuracy recovery was studied by addition of standard drug solution at three

different levels of 50, 100, 150% to pre-analyzed sample solution. Densitograms were obtained and the peak areas was noted. The concentrations of drug and thus mean percentage recovery was calculated from respective calibration curves.

#### *Robustness*

The robustness of the method was studied, by small but deliberate variation of chamber saturation period ( $\pm 10\%$ ), mobile phase composition ( $\pm 2\%$ ), and time from development to scanning (0, 30, 60, 90 min). One factor at a time was changed to study the effect. The robustness of the method was checked at concentration of 500 ng per band to study the effect on the peak area of the drug.

#### *Solution Stability*

Sample solutions for the estimation of the assay were prepared and stored in tightly capped volumetric flasks, protected from light on a laboratory bench and evaluated at different time intervals up to 24 h. They were then applied to a TLC plate and analyzed by the method for the presence of any band other than that of the drug peak.

#### *Analysis of Tablet Formulation*

Tradjenta® tablets nominally containing 5 mg Linagliptin per tablet were weighed accurately and finely powdered. A quantity of powder equivalent to (25mg of linagliptin) was weighed and transferred to a 25 mL volumetric flask containing approximately 15 mL methanol and sonicated to dissolve, and the volume was made up to the mark with the methanol. The solution was filtered using Whatman no.41 paper. From the filtrate 5 mL was further diluted to 50 mL to furnish sample stock solution of linagliptin (100 ng  $\mu\text{L}^{-1}$ ). From sample stock solution 5  $\mu\text{L}$  was applied to an HPTLC plate to furnish final amount of 500 ng per band for linagliptin. After chromatographic development peak areas of the bands were measured at 225 nm and amount of drug present per tablet was estimated from the respective calibration plots. The procedure was repeated six times for analysis of homogenous sample.

#### *Accelerated Degradation of Linagliptin*

Stock solution of 50 mg linagliptin in 50 mL methanol was used for forced degradation study

#### *Acid and Base Degradation*

HCl (1 M, 5 mL) and NaOH (1M 5 mL) were added separately to 5 mL stock solution of linagliptin. The mixtures were heated under reflux at 85°C on a water bath for 3 h. The forced degradation was performed in the dark to exclude the possible degradative effect of light. The resulting solution (1  $\mu\text{L}$ , 500 ng per band) were applied to a TLC plate and the chromatograms were run as described above.

#### *Hydrogen Peroxide-Induced Degradation*

Hydrogen peroxide (10.0% v/v, 5 mL) was added to 5 mL stock solution of linagliptin and the mixture was heated under reflux at 85°C on a water bath for 1 h. The resulting solution (1  $\mu\text{L}$ , 500 ng per band) was applied to TLC plate and the chromatogram was run as described above.

#### *Photochemical Degradation*

Photolytic studies was also carried out by exposure of drug to UV light up to 200 watt hours/ $\text{m}^2$  and subsequently cool fluorescent light to achieve an illumination of 1.2 million Lux hours. A solution of 50 mg of linagliptin in 100 mL methanol was prepared from the photochemical degraded samples and 1  $\mu\text{L}$  (500 ng per band) were applied to TLC plate and the chromatogram was run as described above.

#### *Thermal Degradation*

Linagliptin drug powder was placed in oven at 105 °C for 2 h to study the thermal degradation. A solution of 50 mg linagliptin in 100 mL methanol was prepared from the thermal degraded sample and 1  $\mu\text{L}$  (500 ng per band) was applied to a TLC plate and the chromatogram was run as described above.

## **RESULTS AND DISCUSSION**

#### *Method Development and Optimization*

The TLC procedure was optimized with the objective of developing a stability indicating assay. Initially prepared mobile phase ethyl acetate : ethanol 7:3 (v/v) no movement observed.

Introduce isopropanol (IPA) in presence of ethanol and tried in different ratios, slight moment was observed. Ammonia also introduced as a trail part in different concentration with the above composition then observed good results. Finally the mobile phase ethyl acetate : IPA : Ammonia 7:3:0.4 (v/v/v) was selected as optimum for obtaining well defined peak . The retention factor for linagliptin was  $0.61 \pm 0.05$ . The wavelength 225 nm was selected for detection because drug showed considerable absorbance at this wavelength.

### Method Validation

#### Linearity

The standard calibration plots were linear over a range 100-700 ng per band with correlation coefficient of 0.997. The validation data is summarized in (Table 1). Which gives Beer's law limit, linear regression equation, correlation coefficient, LOD and LOQ for drug.

#### Accuracy

Recovery study results ranged from 99.63 to 100.17% with RSD values ranging from 0.256 to 0.882%. Results of recovery studies are reported in (Table 2).

#### Precision

Intra-day variation, as RSD, was 0.512% and inter-day variation, as RSD, was 0.671% summarised in (Table 3). These low levels of the RSD show the repeatability and precision of the method was good.

#### Robustness

The robustness of the method was checked at concentration of 500 ng per band to study the effect on the peak area of the drug. Results of Robustness studies are reported in (Table 4).

### Assay of Tablet formulation

The spectra acquired for linagliptin extracted from the tablet was also compared with those

acquired for linagliptin standard, good correlation between the corresponding spectra indicated that the method is specific for the drug and no other tablet components interfere with the drug. The assay [%] was  $99.623 \pm 0.203$  in tablet formulation tested.

### Stability-Indicating Property

The chromatograms obtained from samples degraded by treatment with acid, base, hydrogen peroxide contained well-separated spots of the pure drug and some additional peaks at different  $R_F$  values. It is apparent from that the spots of the degradation products were well resolved from those of the drug. The peak of linagliptin was not significantly shifted in the presence of the degradation peaks, which indicated the stability-indicating nature of the method. Photochemical and thermal degradation shows no additional peaks other than drug peak.

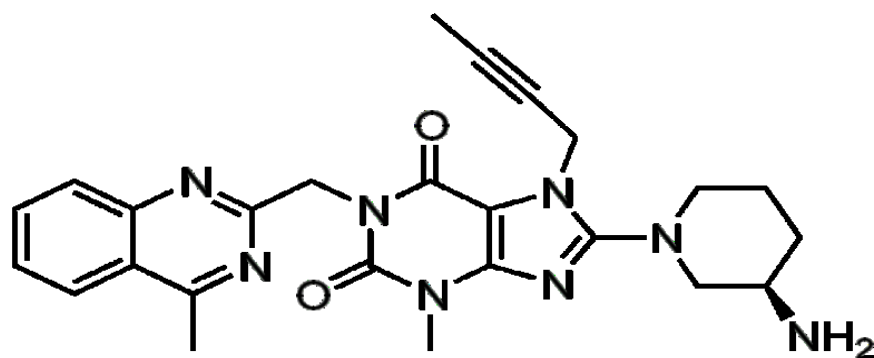
### CONCLUSION

The developed HPTLC technique is accurate, precise, specific, robust and stability indicating one, Statistical analysis proves that the method is reproducible and selective for the analysis of lingalipitin as bulk drug and pharmaceutical formulations. It is proposed for routine analysis of this drug in presence of degradation products in stability study.

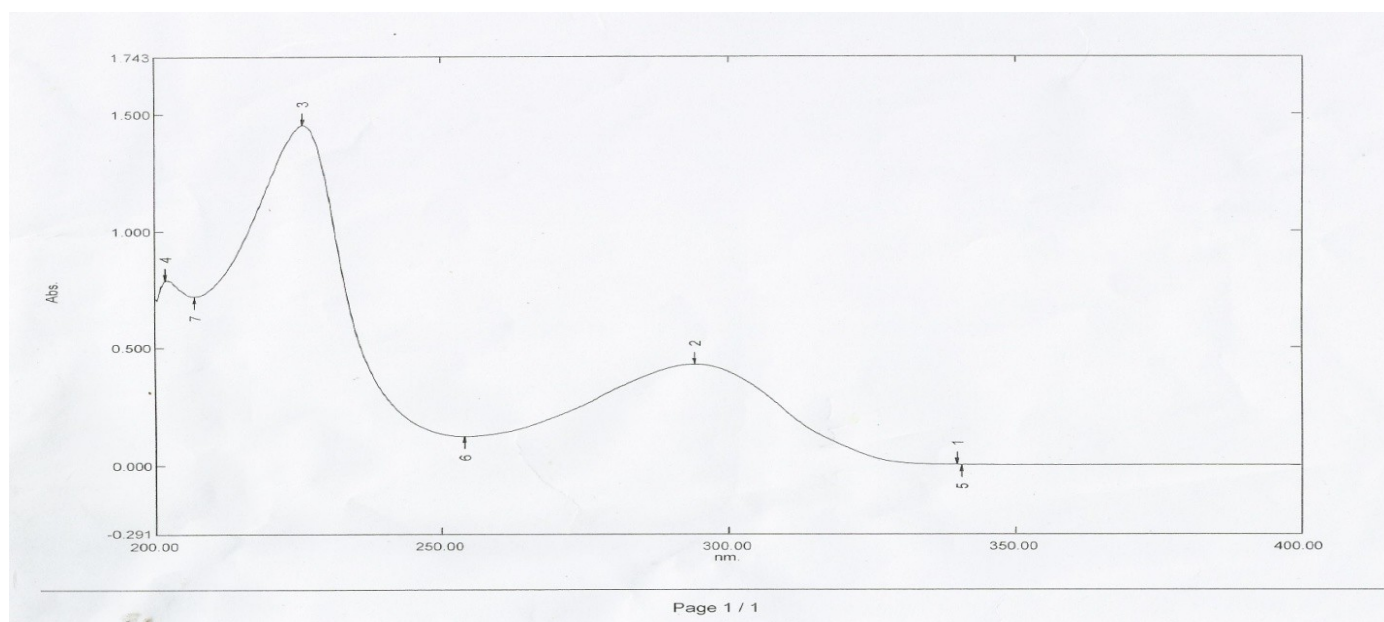
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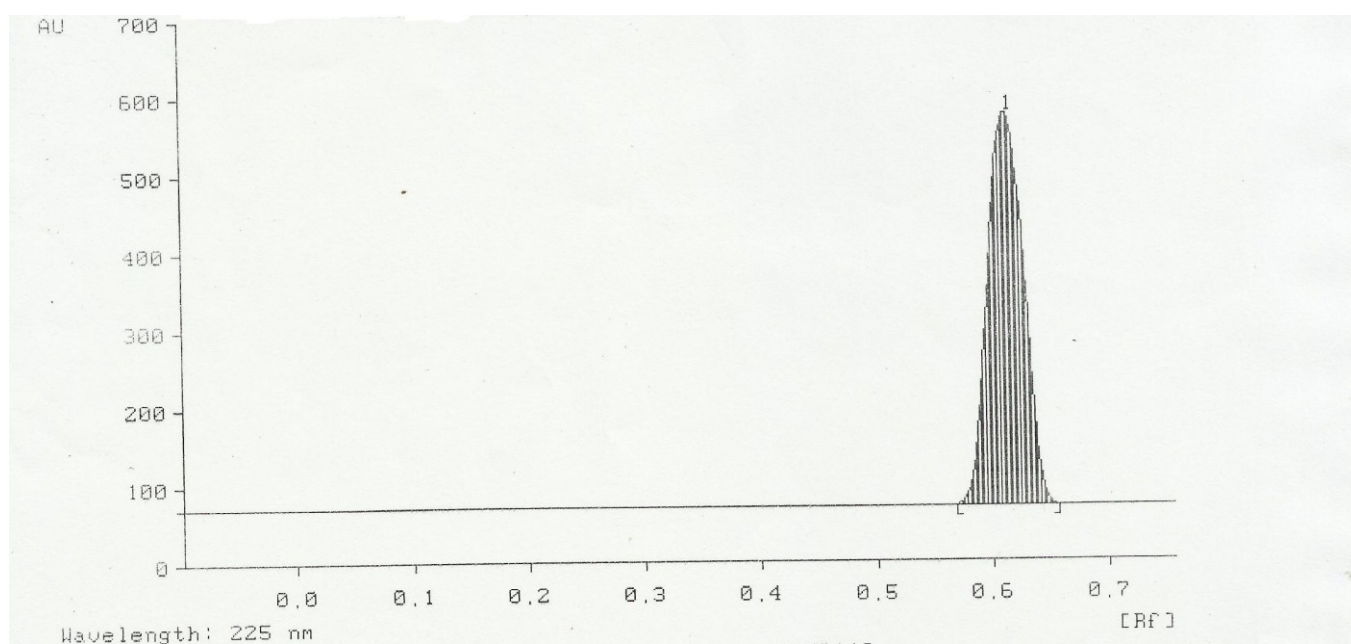




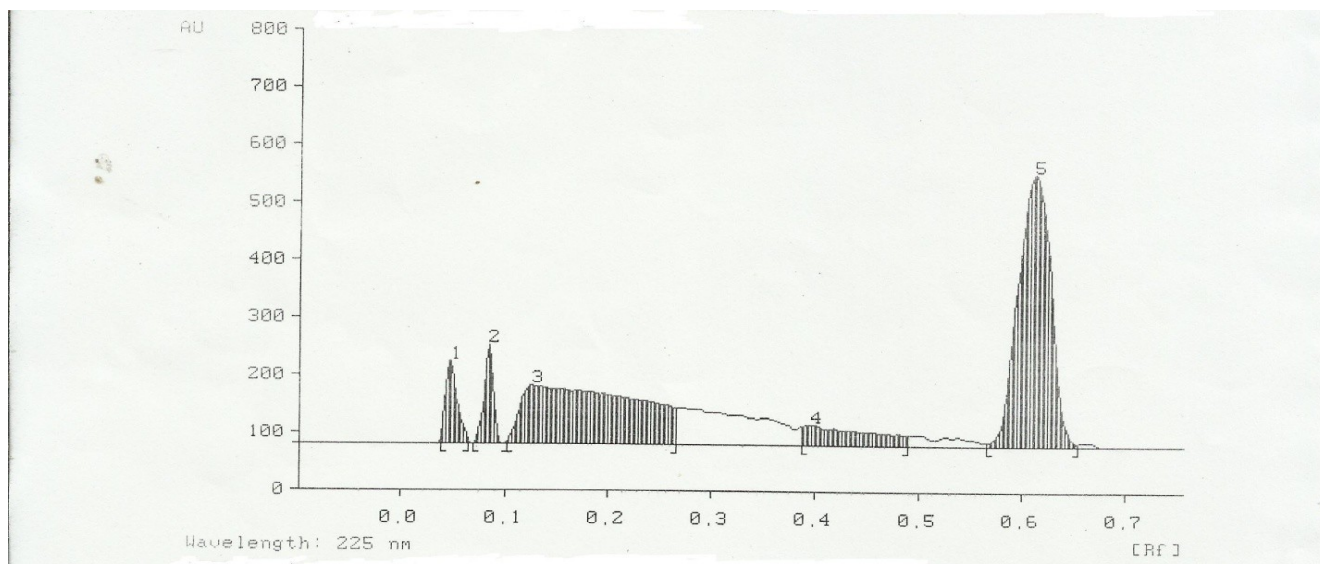
**Figure 1:** Chemical structure of Linagliptin



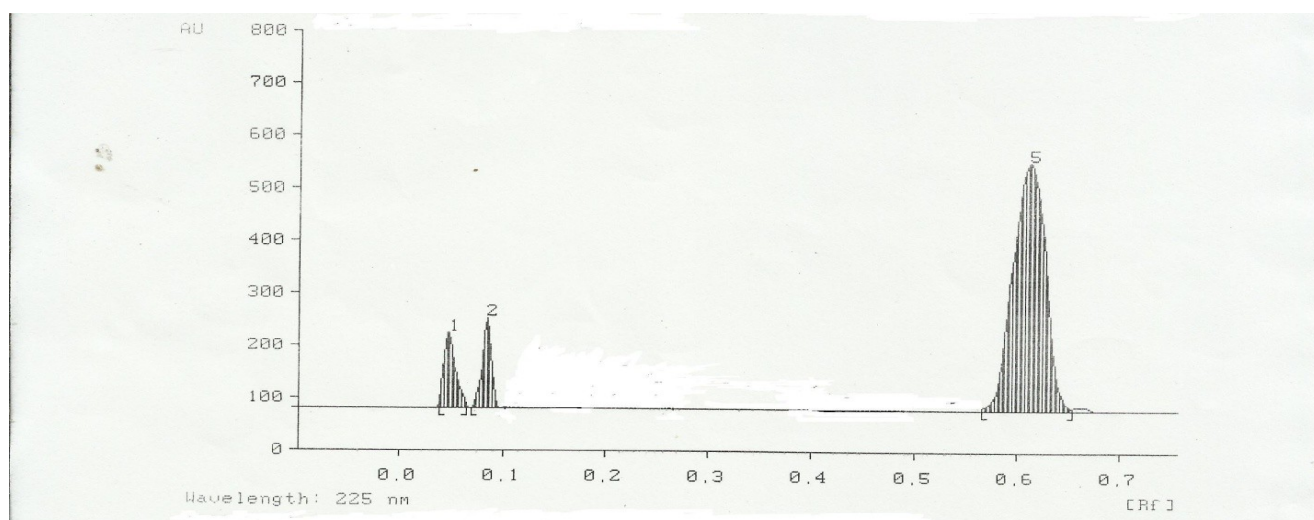
**Figure 2:** Spectra of linagliptin measured from 200 to 400 nm



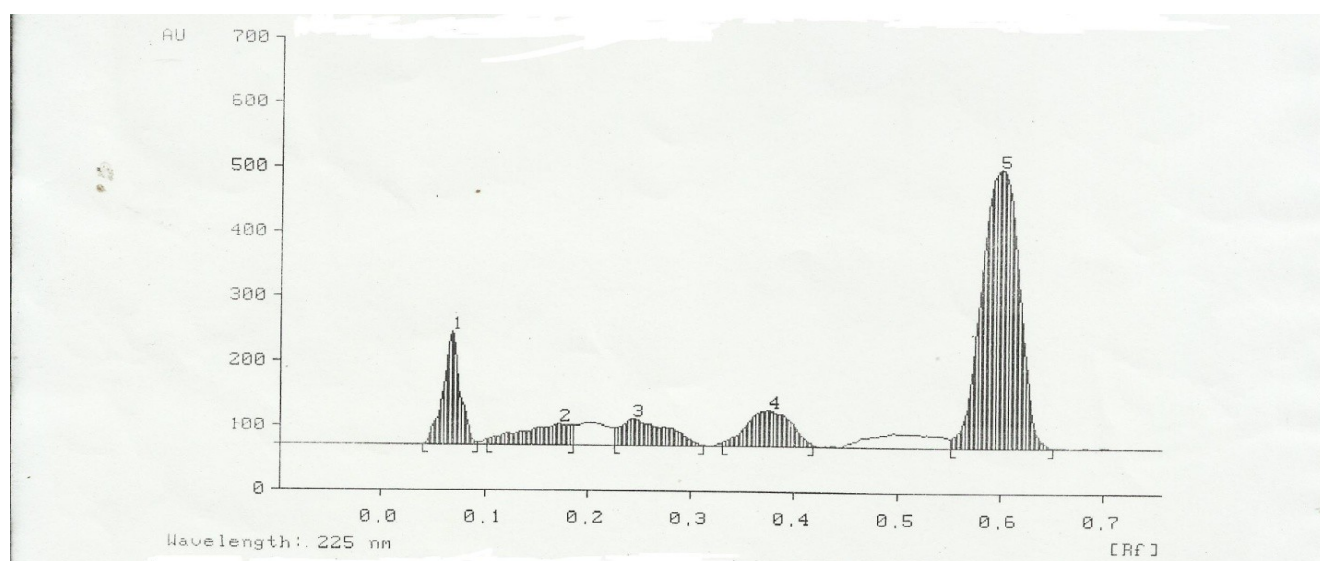
**Figure 3:** Standard chromatogram of linagliptin ( 500 ng per band, R<sub>f</sub> 0.61 ± 0.05 )



**Figure 4:** Acid-Induced degradation chromatogram of Linagliptin



**Figure 5:** Base-Induced degradation chromatogram of Linagliptin



**Figure 6:** Hydrogen peroxide-Induced degradation Chromatogram of Linagliptin

**Table 1:** Summary of validation data for the proposed method

Linagliptin	
Detection wavelength [nm]	225
Beer's law limit [ng per band]	100-700
Correlation coefficient (r)	0.997
Linear regression equation <sup>a)</sup> (y = mx + c)	
Intercept (c)	-74.961
Slope(m)	5.4064
Detection limit [ng per band]	47
Quantitation limit [ng per band]	142

<sup>a)</sup>Where y is peak area and x is concentration (ng per band) n = 5

**Table 2:** Results from study of recovery of linagliptin (n = 3)

Drug	Amount taken [ng/band]	Amount added [ng/band]	Amount found [ng/band]	Mean recovery[%]	RSD[%] <sup>n</sup>
AM	200	100	99.68	99.63	0.568
	200	200	200.00	99.95	0.882
	200	300	300.66	100.17	0.256

**Table 3:** Intra-day and inter-day precision (n = 6)

Amount (ng per band Linagliptin)	Mean area	SD(±)	RSD[%] <sup>n</sup>
Intra-day precision 500	2508.80	12.79	0.512
Inter-day precision 500	2515.97	16.77	0.671

**Table 4:** Results from testing of robustness, as RSD [%] of peak area

Condition varied	Linagliptin
Time from chromatography to scanning [min]	0.355
Chamber saturation period [min]	0.425
Mobile phase composition [%]	0.652

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