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Development of Stability indicating and RP-HPLC method for Linagliptin

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Abstract:

A new sensitive and rapid RP-HPLC method was developed for the development of stability indicating method for Linagliptin. Linagliptin is an anti-diabetic drug belonging to the category of DPP4 inhibitors. RP-HPLC analysis was performed on the Shimadzu model AY-120 with a Borwin software on a HiQ Sil C18 Column (250 mm × 4.6 mm, 5 µ) with a mixtue of Methanol:0.1%OPA (80:20 v/v) as the mobile phase ,at the constant flow rate of 1 ml/min with ambient column temperature .The calibration plot gave a linear relationship over the concentration range of 5-30 µg/ml. Linagliptin was exposed to forced degradation conditions i.e Acidic ,Alkaline, Thermal ,Photolytic and oxidative.Degradation peaks along with %degradation was found out for Linagliptin.

Introduction:

Diabetes mellitus (DM) is a metabolic disorder where in human body does not produce or properly uses insulin, a hormone that is required to convert sugar, starches and other food into energy. Absence or reduced insulin in turn leads to persistent abnormally high blood sugar and glucose in tolerance .Linagliptin is an inhibitor of DPP4 an enzyme that degrades the incretin hormones glucagon -like peptide-1(GLP-1) and glucose-dependent insulinotropic polypeptide(GIP). The molecular formula of Linagliptin is $C_{25}H_{26}N_8O_2$.

EXPERIMENTAL WORK

MATERIAL AND METHODS:

➢ **Reagents and chemicals:**

- Ortho Phosphoric Acid (AR Grade)
- Methanol (HPLC Grade)
- Acetonitrile (HPLC Grade)
- NaOH
- HCl
- 30% H_2O_2
- HPLC grade water.

All chemicals and reagents that is Methanol, Acetonitrile, Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Ortho Phosphoric acid, Hydrogen peroxide solution 30% w/v (H_2O_2) were purchased from LOBA CHEME PVT. LTD., Mumbai.

➢ **Instruments:**

1. HPLC:
   - Borwin chromatography software (version 1.50)
   - Model PU 2080 Plus Intelligent HPLC pump
   - Rheodyne sample injection port with 50 µl loop
   - HiQ Sil C18 Column (250 mm × 4.6 mm, 5 µ)
   - JASCO UV-2075 UV-VIS detector
2. Shimadzu (model AY-120) Electronic weighting balance
3. Sonicator: PRAMA solutions for laboratory
4. Extrapure lab link water purification system.
5. Photo stability chamber- Newtronic
6. Electronic pH meter
7. Calibrated Glassware's.

PREPARATION OF STANDARD STOCK SOLUTION

Standard stock solution of Linagliptin was prepared by dissolving 10 mg of the Linagliptin in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, 1 ml solution was pipette out to into 10 ml volumetric flask and volume made using methanol to get concentration 100 µg/ml. Further dilutions were prepared using stock solution. The retention time (RT) of Linagliptin was 3.689 min.

SELECTION OF ANALYTICAL WAVELENGTH:

The standard stock solution (1000 µg/ml) was prepared in methanol and further dilutions were prepared using mobile phase and solution was scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the 10 µg/ml Linagliptin solution showed maximum absorbance at 294 nm. Representative UV spectrum of Linagliptin. (Figure 1)

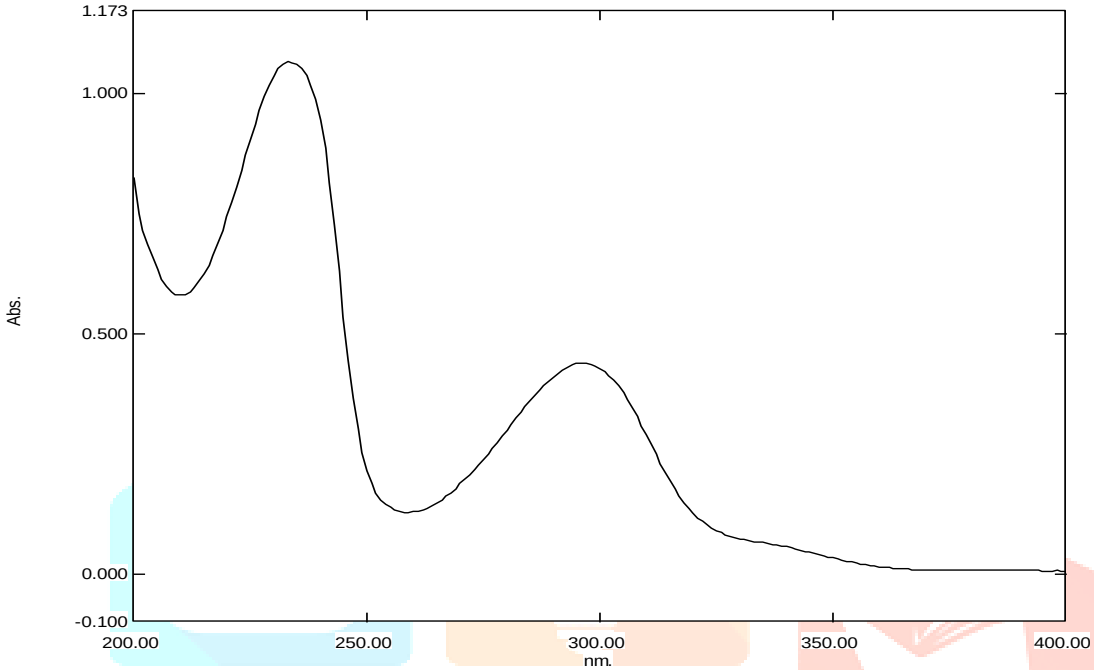
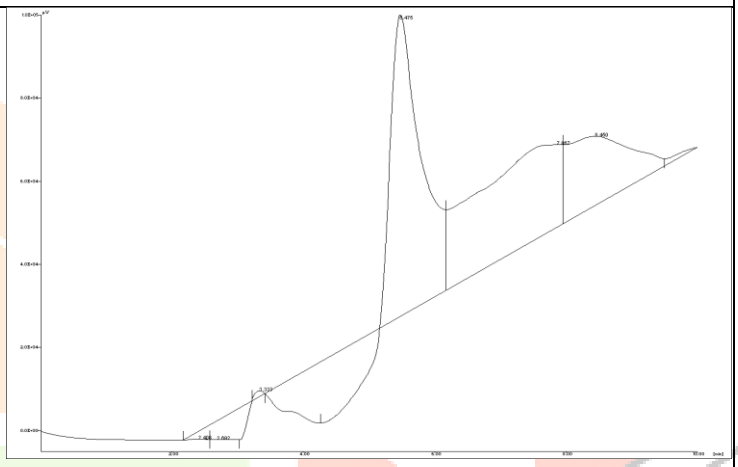
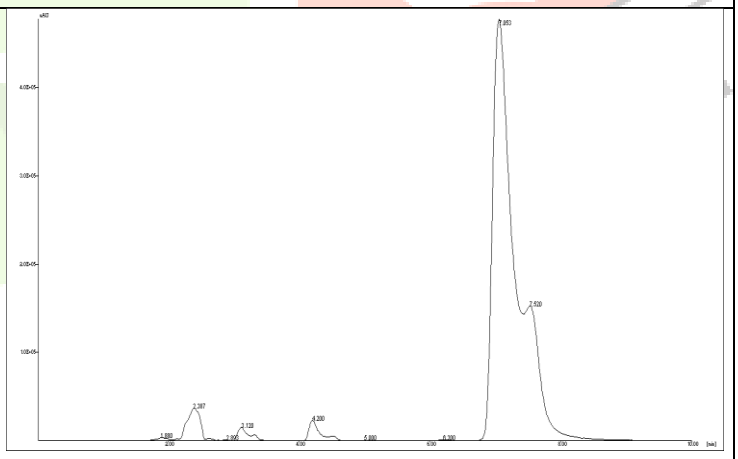
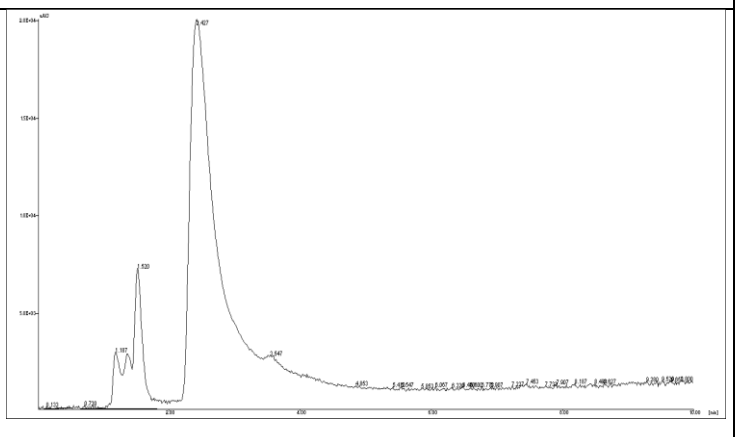


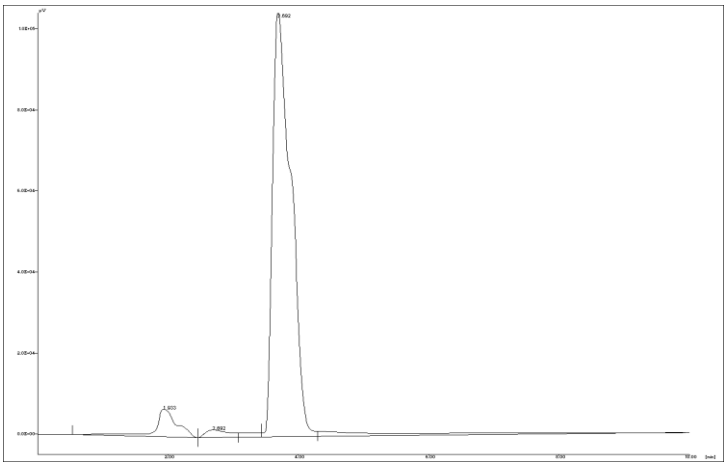
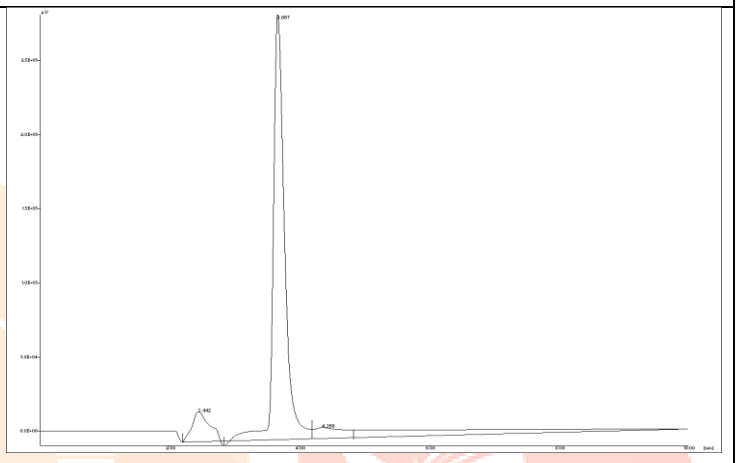
Fig 1.: UV- Spectrum of Linagliptin. (10 µg/ml)

MOBILE PHASE OPTIMIZATION

To achieve optimum chromatographic conditions various mobile phases were checked, The Acetonitrile: water, Methanol: water was tried at different proportions (70:30, 80:20, 90:10) system was initially tried but did not lead to appropriate peak shape. Using Methanol: 0.1 % OPA (80:20 v/v) a good peak shape was obtained with appropriate system suitability parameters. Table 1 shows all the optimized chromatographic conditions.

Table 1: TRIALS FOR MOBILE PHASE OF LINAGLIPTIN.

Sr. No.	M.P.	Observation	Chromatogram (294 nm)
1.	Acetonitrile: Water (70:30 v/v)	Peak was not found.	
2.	Acetonitrile: Water (80:20 v/v)	Peak Tailing observed	
3.	Acetonitrile: Water (90:10 v/v)	Peak Tailing	

4	Methanol:Water (80:30 v/v)	Poor Peak Shape	
5	Methanol: 0.1 % OPA (80:20 v/v)	Peak was observed having good shape with RT-**3.69 min**.	

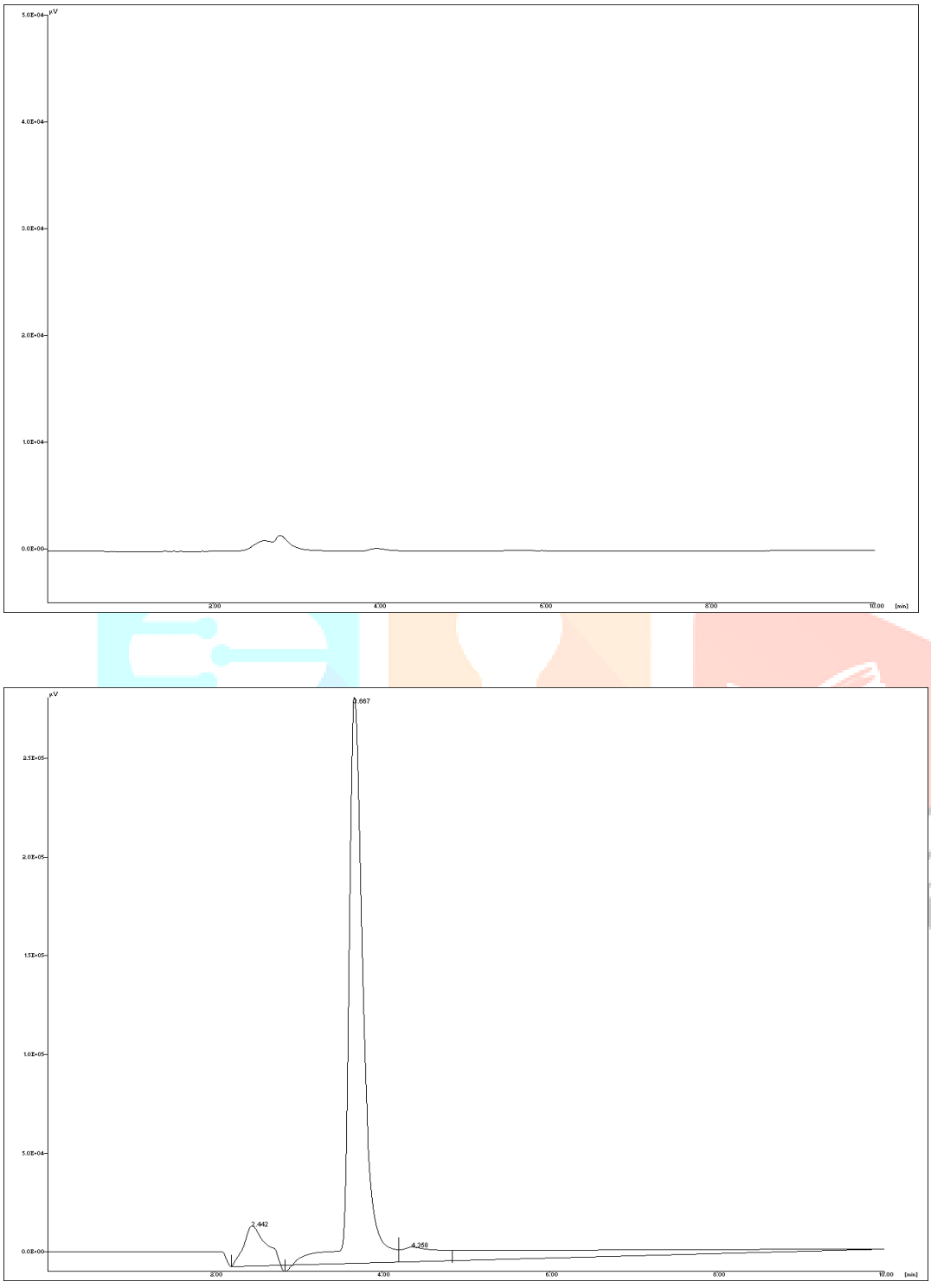


Fig. 2: Chromatogram of a) mobile phase blank b) Linagliptin

4.1 METHOD DEVELOPMENT AND LINEARITY

To saturate the column, mobile phase was pumped for about 30 min to get a stable base line. A series of dilutions were prepared from the standard stock solutions using mobile phase to get the concentrations of 5-30 µg/ml. Each concentration was injected to the HPLC system. The parameters noted after every injection were peak area,

retention time, asymmetry factor and theoretical plates. Calibration curve was plotted by taking peak area on Y-axis and concentration on X-axis, Regression equation was calculated from the calibration curve, this regression equation is used to calculate the content of Linagliptin. Using this mobile phase, the system suitability parameters are given in **Table 2**

Table 2: Optimized Chromatographic Condition:

Parameters	Conditions used
Column	HiQ Sil C18 (250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol: 0.1 % OPA (80:20)
RT (Retention Time)	3.692 ± 0.12 min
Flow Rate	1 ml/min
Sample Injector	50 µl loop
Column Temperature	Ambient
N (No of Theoretical Plates)	2894.74
Asymmetry Factor	1.08

Table 3: Linearity Results.

Concentration (µg/ml)	Area
5	429876.72
10	1157658.36
15	1992546.45
20	2898743.89
25	3757896.35
30	4698755.74

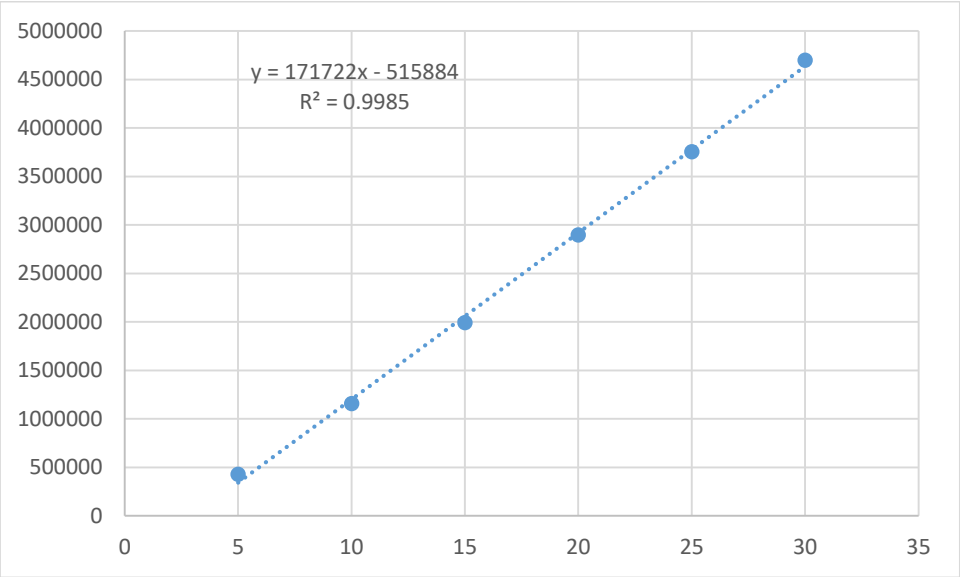


Figure 3: Linearity Curve of Linagliptin (5-30 µg/ml)

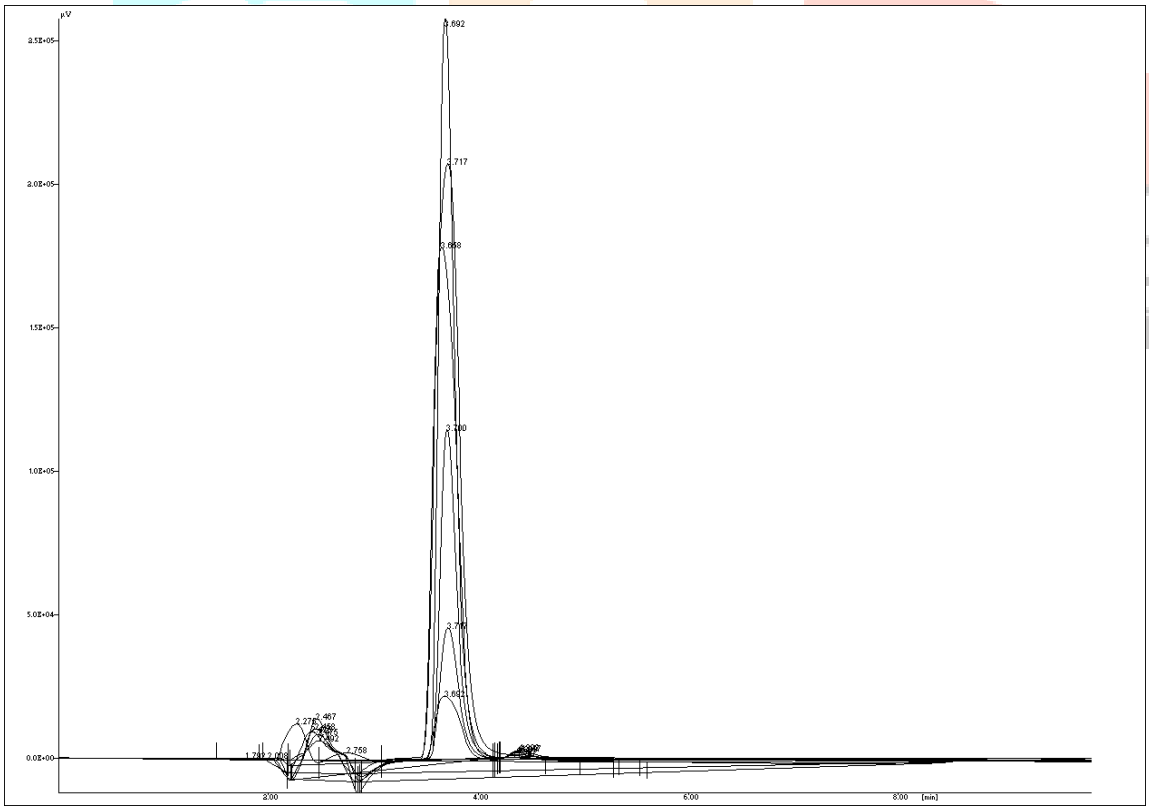


Figure 4: Overlain chromatogram of Linagliptin (5-30 µg/ml)

FORCED DEGRADATION STUDIES

The effect of different environmental factors on drug stability and quality must be checked. Thus, the drug was subjected to various stress conditions for varying periods of time, using various strength of reagent. Conditions

were managed to achieve recovery of 70- 90 per cent. The API was subjected to hydrolysis under acidic and alkaline pH, oxidation, thermal and photolytic degradation condition.

Degradation under Acid hydrolysis condition:

1 ml of standard stock solution of Linagliptin (1000 µg/ml) was mixed with 1 ml of 0.1N HCl and kept aside for 2 hours at room temperature. The resultant solution was neutralized using 0.1 N NaOH, volume made up to 10 ml with mobile phase to get 100 µg/ml solution and injected to the HPLC system and chromatogram was recorded. Under acidic stress condition 29.76 % degradation was observed along with the degradation peak at RT – 2.350 min as shown in Fig. 5.

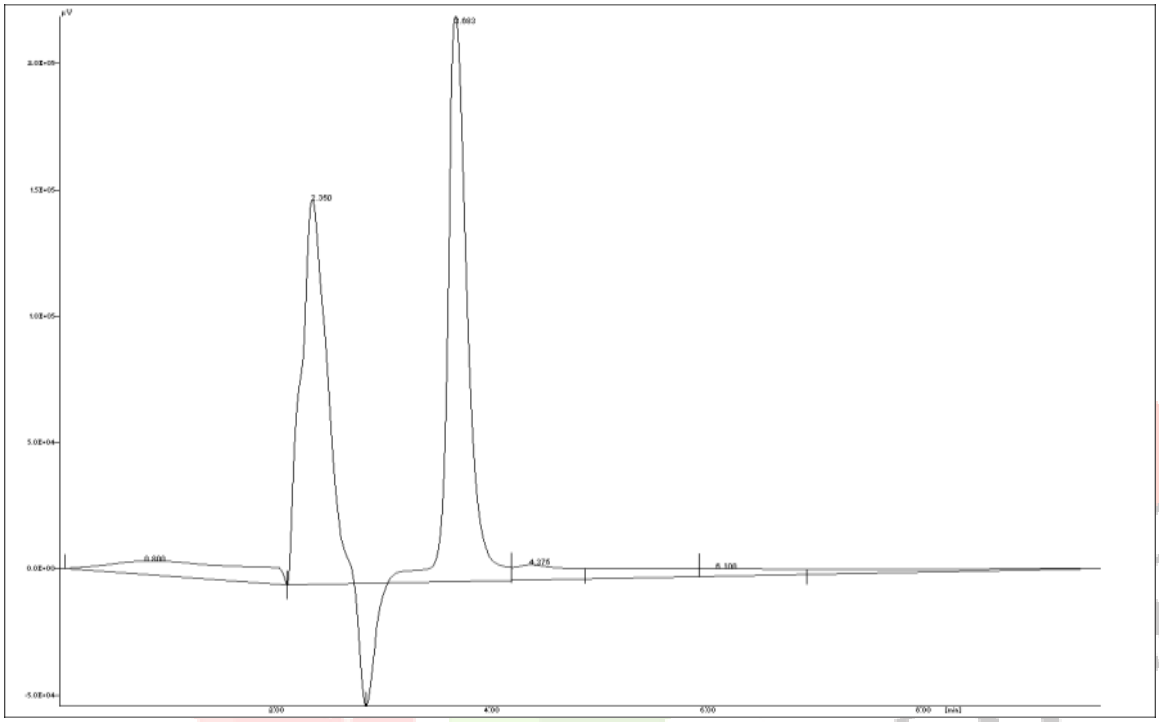


Fig. 5: Acid Hydrolysis Chromatogram

Degradation under Alkali hydrolysis condition:

1 ml of standard stock solution of Linagliptin (1000 µg/ml) was mixed with 1 ml of 0.1N NaOH and kept aside for 30 min at room temperature. The resultant solution was neutralized using 0.1 N HCl, volume made up to 10 ml with mobile phase to get 100 µg/ml solution and injected to the HPLC system and chromatogram was recorded. Under alkaline stress condition for 15 min 80.86 % degradation was observed along with the degradation peak at RT – 2.360 min as shown in Fig. 6.

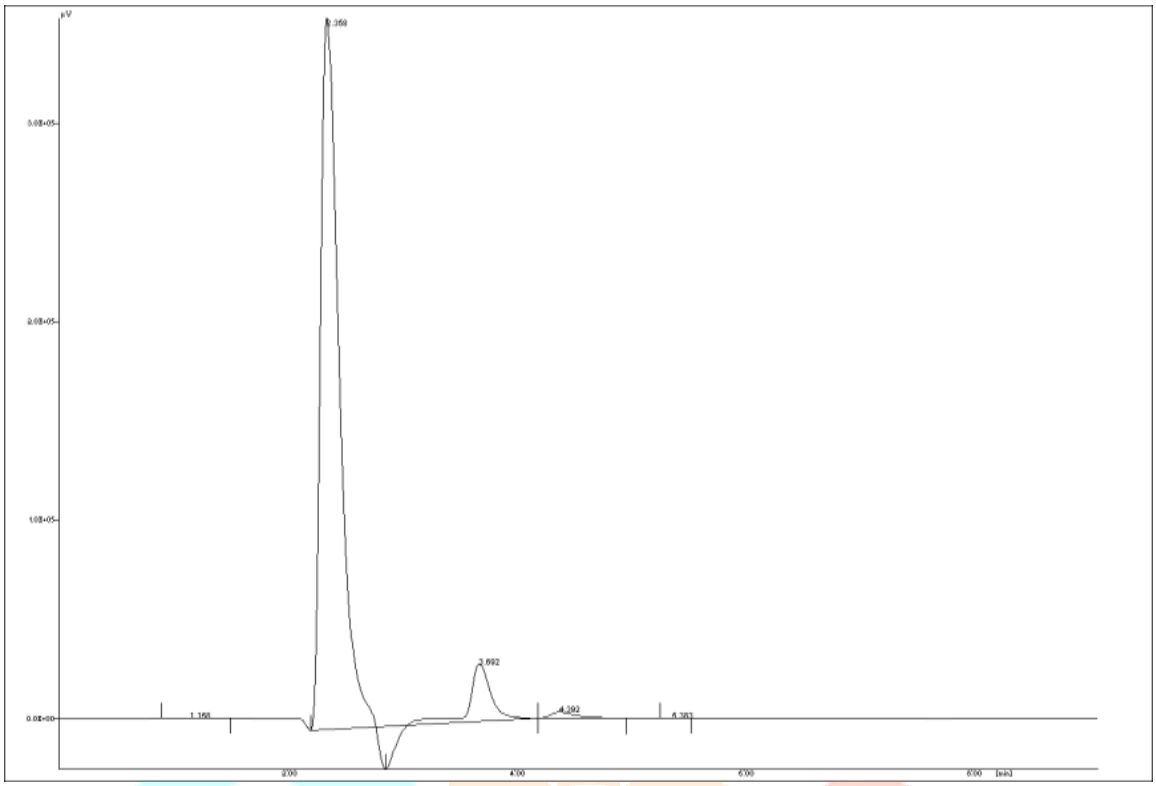


Fig. 6: Alkaline Hydrolysis Chromatogram (15 min).

Degradation under Oxidative condition:

1 ml of standard stock solution of Linagliptin (1000 µg/ml) was mixed with 1 ml of 3% H_2O_2 and kept aside for 60 min at room temperature. The volume made to 10 ml with mobile phase to get 100 µg/ml solution The resultant solution was then injected and chromatogram was recorded.

For oxidative stress; 25.39 % degradation was observed along with the degradation peak at RT- 2.675 min as shown in Fig. 7.

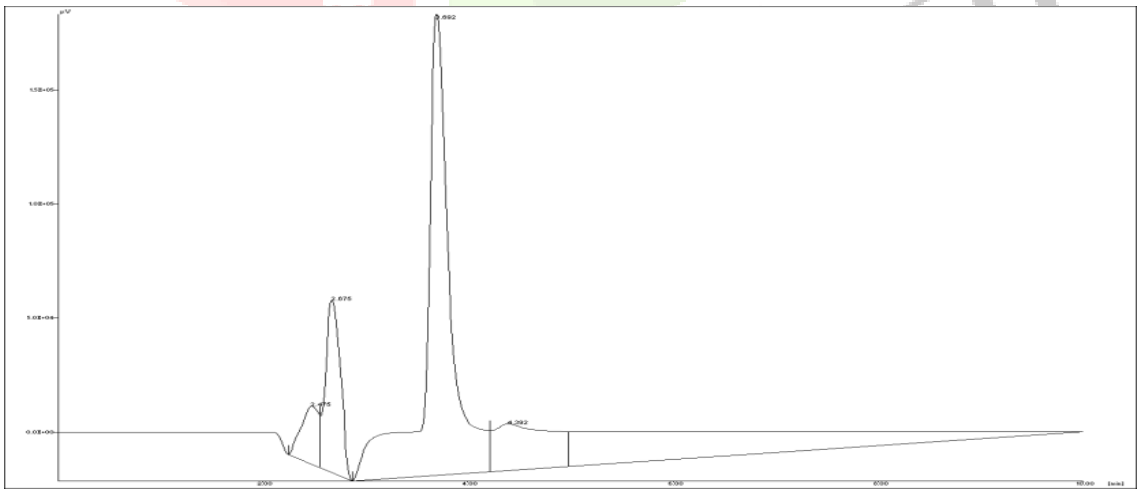


Fig. 7: Oxidative degradation chromatogram (60 min).

Degradation under Thermal condition:

Linagliptin API powder was kept in hot air oven at 100^0 C for 60 min. After exposure drug was dissolved in methanol to get 1000 µg/ml solution. From this solution 1 ml was diluted to 10 ml with mobile phase to get 100

µg/ml. The resultant solution was then injected and chromatogram was recorded. No degradation was observed as shown in Fig. 8

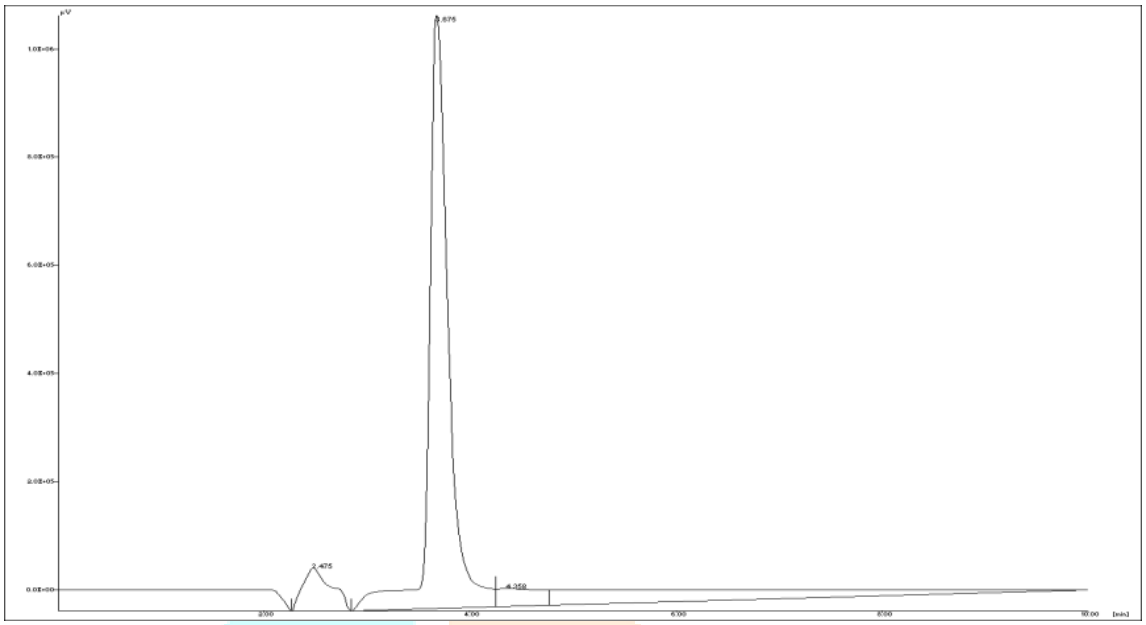


Fig. 8: Thermal Degradation (60 min)

Photo-degradation studies:

Photolytic studies were carried out by exposure of drug to UV light up to 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1.2 million Lux Hrs. After exposure accurately weighed 10 mg of drug was transferred to 10 ml of volumetric flask; the volume was made up with methanol. Further dilution made with mobile phase to get 100 µg/ml as final concentration and was injected to get chromatogram. No degradation was observed a seen in Fig. 9

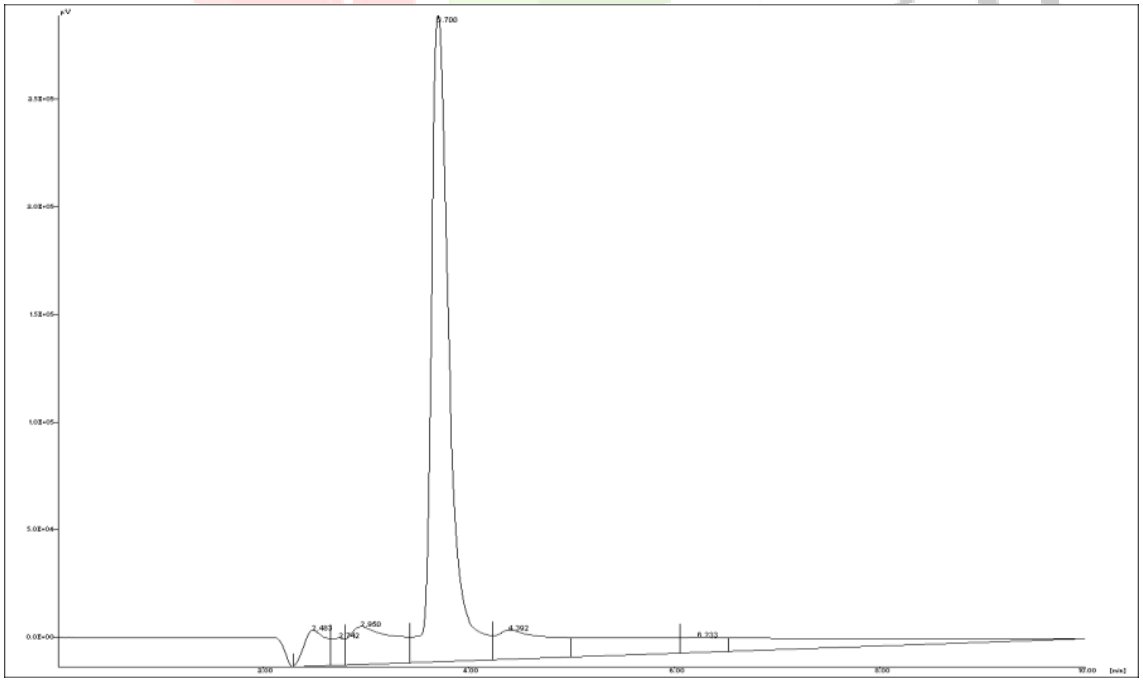


Fig. 9: UV and Fluorescent light exposure chromatogram.

Table: Summary of Degradation Study.

Parameter	Condition	% Recovery	% Degradation
Acid hydrolysis	0.1 N HCl 2 Hours	70.24	29.76
Alkaline Hydrolysis	0.1 N NaOH for 15 min	19.14	80.86
Oxidative Degradation	3% H_2O_2 for 60 min	74.61	25.39
Thermal degradation	100 ºC for 1 hour	96.80	3.20
Photo Degradation	UV Light – 200 Watt hours/square meter followed by fluorescent light upto 1.2 million Lux Hrs	97.55	2.45

CONCLUSION:

The method has been shown to be Linear withing the given range. Forced degradation studies were carried out in different conditions accordingly Linagliptin was most degraded in Alkaline degradation and was least Degraded in thermal degradation. This method can be used in quality control laboratories for stability studies and release of production batches.

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