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Development and Validation of Stability Indicating Rp-Uflc Method for The Estimation of Linagliptin in Active Pharmaceutical Ingredients

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Research Article

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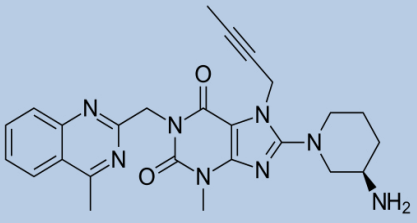
ABSTRACT

A simple, RP-UFLC method was established for determining Linagliptin in Active Pharmaceutical Ingredients. Linagliptin is a DPP-4 inhibitor developed by BoehringerIngelheim (German Pharmaceutical Company) for treatment of type II diabetes. Linagliptin was approved by the US FDA on 2 May 2011 for treatment of type II diabetes. Linagliptin and their degradation products were separated using C18 column with Acetonitrile: Methanol (50:50 (v/v) as the mobile phase. Detection was performed at 238 nm using a PDA detector and flow rate 1.5 ml/min and retention time was 4.4 min respectively. The method was validated using ICH guidelines and was linear in the range 2-10 µg/ml (r2=0.995) for Linagliptin. The method showed good linearity, accuracy, precision, ruggedness, robustness, and specificity. Good separation of the analytes and their degradation products was achieved using this method. The developed method can be applied successfully for the determination of Linagliptin. The present method was validated with respect to system suitability, accuracy (recovery) precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) and robustness according to the ICH Guidelines.

INTRODUCTION

Linagliptin is a DPP-4 inhibitor developed by BoehringerIngelheim (German Pharmaceutical Company) for the treatment of type II diabetes. Linagliptin was approved by the US FDA on 2 May 2011 for treatment of type II diabetes. It is being marketed by BoehringerIngelheim and Lilly. Linagliptin is an inhibitor of DPP-4 (dipeptidyl peptidase 4) an enzyme that degrades the incretion hormones, Glucagonlike peptide-1 (GLP-1) and Glucose dependent Insulinotropic 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. Linagliptin showed that the drug can effectively reduce blood sugar. 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[1].

Drug Profile:

Molecular structure:	
Synonyms	Tradjenta, Trajenta
Melting point	202°C
Solubility	Soluble in methanol; sparingly soluble in ethanol; very slightly soluble in isopropanol, alcohol
Physical appearance	White to yellow solid
Category	Antidiabetic agent

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Dose and Administration

Oral administration of a single 5-mg dose to healthy subjects, peak plasma concentrations of Linagliptin occurred at approximately 1.5 hours post dose (T_{max}); the mean plasma Area Under the Curve (AUC) was 139 nmol*h/L and maximum concentration (C_{max}) was 8.9 nmol/L.

Mechanism of Action

Linagliptin belongs to a class of drugs called DPP-4 inhibitors. DPP-4 inhibitors prevent the hormone incretin from being degraded, allowing insulin to be released from the pancreatic beta cells. While incretin remains in the blood stream, the pancreas is stimulated to produce more insulin. Meanwhile, glucagon release from the pancreas is staggered, preventing glucose level increase. In other words, Linagliptin, along with diet and exercise, can help the body produce more insulin and lower blood glucose. Managing blood sugar can mean a lower HbA1c, an index for Glycaemia control that theoretically correlates with glucose level in the blood [2-4].

MATERIALS AND METHODS

Chromatographic Conditions

Column	Kinetex 5 µ C18 (250 x 4.6 mm. 5 µ)
Flow rate	1.5 mL/min
Run time	10 min
Wavelength	238 nm
Injection Volume	10 µL
Detector	PDA Detector
Elution	Binary Gradient
Mobile Phase	Methanol and Acetonitrile (50:50) (v/v)
Column oven temperature	25 ± 5ºC

Preparation of Diluent

The diluent is a mixture of 50 parts of methanol and 50 parts of acetonitrile.

Preparation of Mobile Phase

Mobile phase is Methanol and Acetonitrile was used in the ratio of (50:50) (v/v).

Preparation of Standard Stock Solution for Linagliptin

100 mg of Linagliptin was taken into 100 mL volumetric flask. To this add 50 mL of diluent and sonicate to dissolve and the volume was made up to the mark with diluent (1000 µg/ml). Pipette 1ml of the above solution into 10 ml volumetric flask and make up the volume using diluent (100 µg/ml).

Preparation of Solutions for Linearity

The solutions for linearity were prepared from the stock solution by diluting with diluent. The concentration ranging from 2, 4, 6, 8, 10 µg/mL were prepared for Linagliptin. Pipette 0.2, 0.4, 0.6, 0.8, 1.0 mL in 10 ml volumetric flasks and make up the volume using diluent to get the above concentrations.

Preparation of Calibration Curve

From the stock solution (100 µg/mL) aliquots of Linagliptin were pipetted into a series of 10 mL volumetric flask. The volume was made up to the mark by using Acetonitrile and Methanol as diluent so as to obtain concentration range of 2-10 µg/mL and filtered through membrane filter of 0.45 µ pore size. 10 µL solutions were injected and peak areas were recorded. The calibration curve was established. The Beer's law is obeyed in the concentration range of 2-10 µg/mL

Selection of Wavelength for Analysis (λ max)

The standard solutions of Linagliptin were scanned in the range of 200-400 nm against mobile phase as blank. Linagliptin showed maximum absorbance at 238 nm. Thus the wave length selected for the determination of Linagliptin is 238 nm.

Method Development

The RP-UFLC strategy created in this examination was gone for finding the chromatographic framework fit for eluting and resolving Linagliptin and its degradation product with fulfilling framework appropriateness conditions. To build up the conditions different parameters, for example, versatile stage, pH, stream rate and dissolvable proportion were changed and reasonable chromatographic condition has been created for routine investigation of medication tests. Beginning trails were done by utilizing same segment taking Methanol and Acetonitrile in different extents with stream rate of 1.5 ml/min. The column was kept up with gradient phase. The chromatograms got subsequent to injection drug tests and kept up with run time of 10 min detailed in separation and peaks were watched wide with thick peak heads and high retention time.

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Method Validation

Method validation is the process used to confirm that the analytical method used for a particular test is reasonable for its expected utilize. Results from method validation can be utilized to reliability, judge the quality and consistency of analytical results; it is a necessary piece of any great diagnostic practice. It is the way toward characterizing a scientific necessity, and affirms that the technique under thought has execution abilities steady with what the application requires.

The different approval parameters incorporate linearity, precision, accuracy, Selectivity and Specificity, range, robustness and LOD, LOQ [5-10].

RESULTS AND DISCUSSION

In developing the method, systematic study of the effects of various parameters is carried out. Initially the solubility of the Teneligliptin drug is determined. In UFLC method, chromatographic conditions stand advanced to obtain great peak. Initially, various mobile phase compositions were tried to elute the drug. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or asymmetry factor), run time and resolution. The system with mobile phase containing Mobile Phase A (Methanol): Mobile Phase B (Acetonitrile): Mobile Phase C (Potassium dihydrogen ortho phosphate of pH 4.6) is used in the ratio of 40:20:40 (v/v) with 1 mL/min flow rate is very strong. The ideal wavelength for identification is 246 nm at which better detector response for the drug is acquired. The chromatogram for blank and Teneligliptin with retention time at 3.308 min were shown in **Figures 1** and **2** respectively.

From the standard chromatograms various system suitable parameters were recorded.

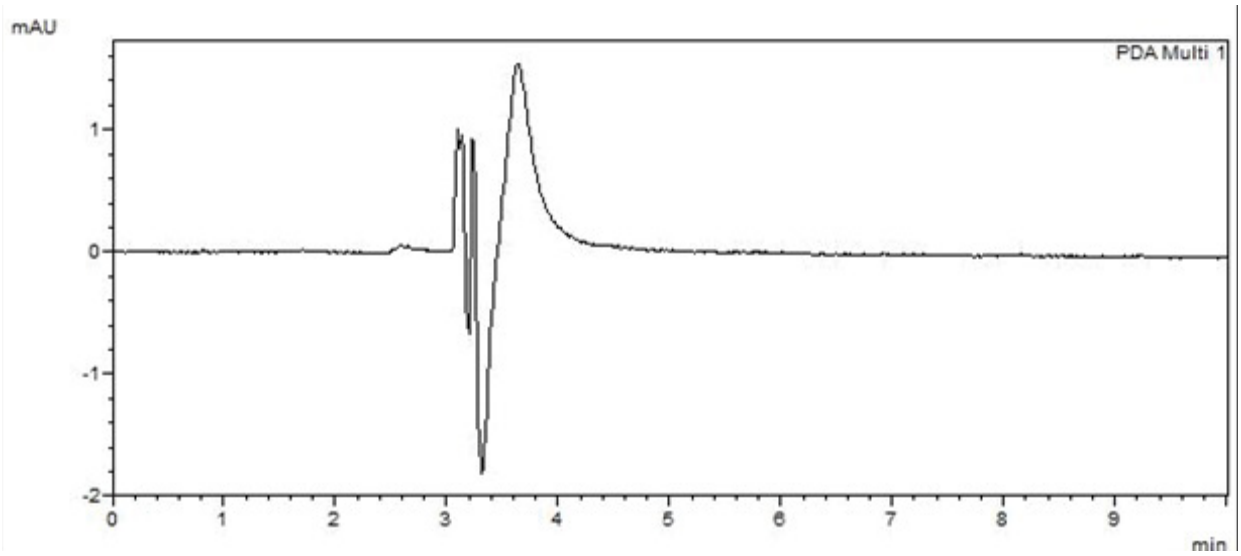

Figure 1. Chromatogram of Blank (Diluent).

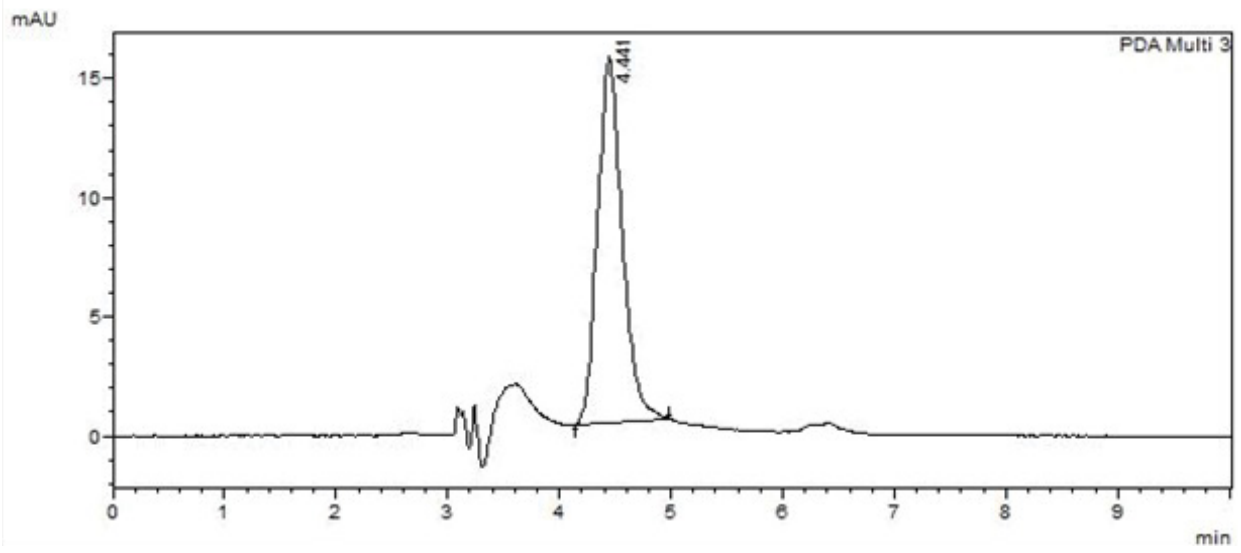

Figure 2. Chromatogram for linagliptin.

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System Suitability

System suitability tests are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions.

Parameters	Acceptance criteria	Results
Tailing factor	NMT 2.0	1.215
Theoretical plates	NLT 2000	4078.495

Table 1. Results for System Suitability

Data Interpretation

From the above tabulated data **Table 1**, it was observed that the system suitability parameters were within the acceptance criteria **(Figures 3-10, Table 2).**

Linearity

Level	Concentration (µg/mL)	Peak Area of Linagliptin
1	2	88571
2	4	125745
3	6	156769
4	8	190716
5	10	214721
Regression Equation		y = 15864x + 60123
Correlation Coefficient (R^2)		R^2=0.995
Slope		15864
Intercept		60123

Table 2. Results for linearity.

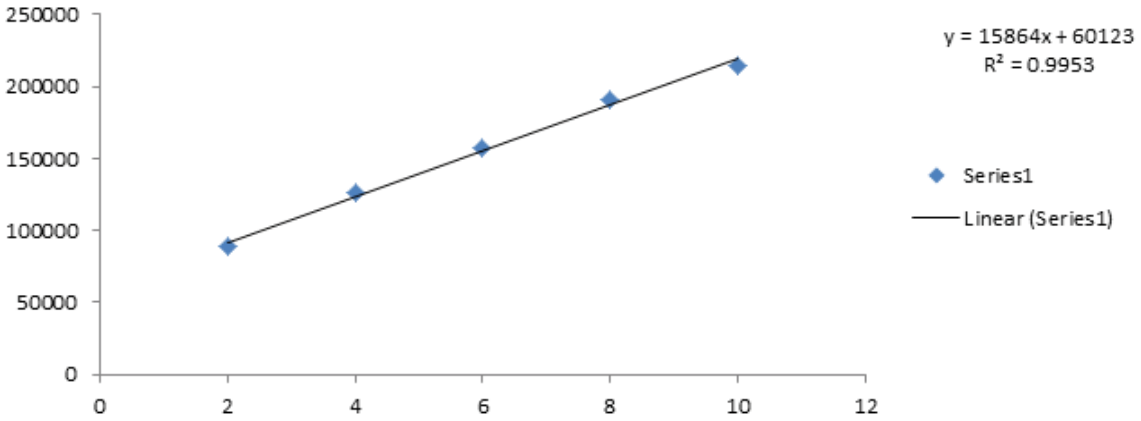

Figure 3. Calibration curve of linagliptin.

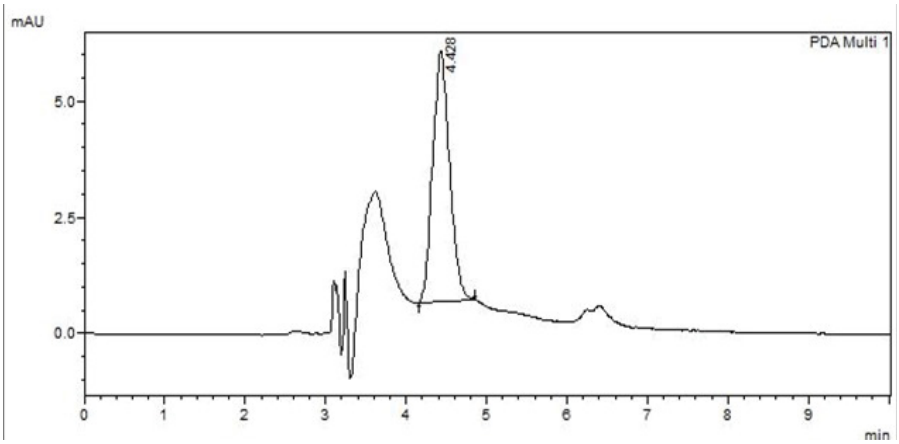

Figure 4. Chromatogram for standard linagliptin (2 µG/ML).

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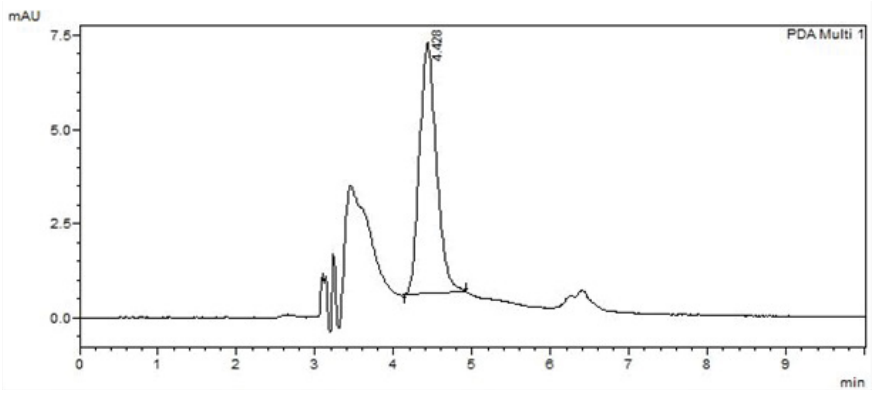

Figure 5. Chromatogram for standard linagliptin (4 µG/ML).

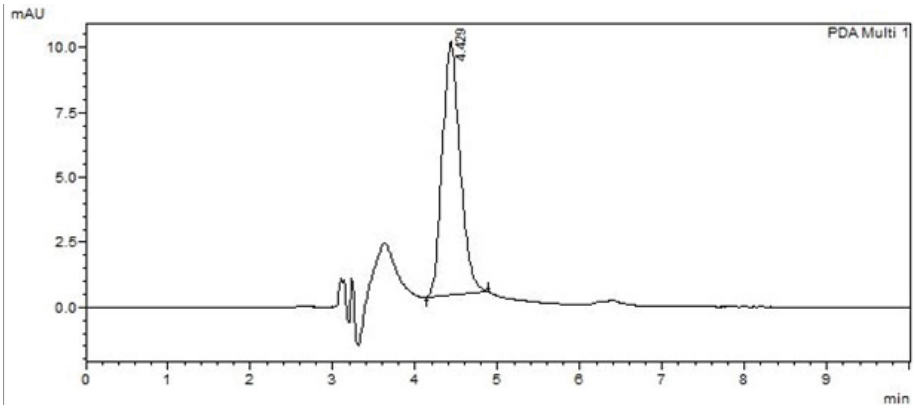

Figure 6. Chromatogram for standard linagliptin (6 µG/ML).

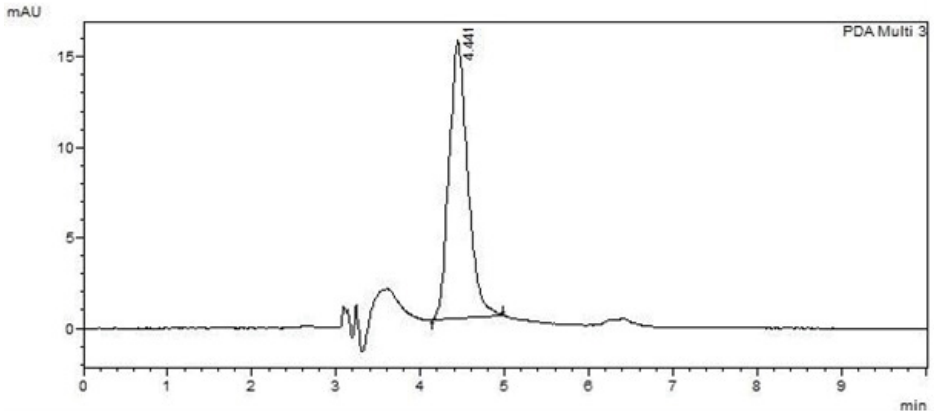

Figure 7. Chromatogram for standard linagliptin (8 µG/ML).

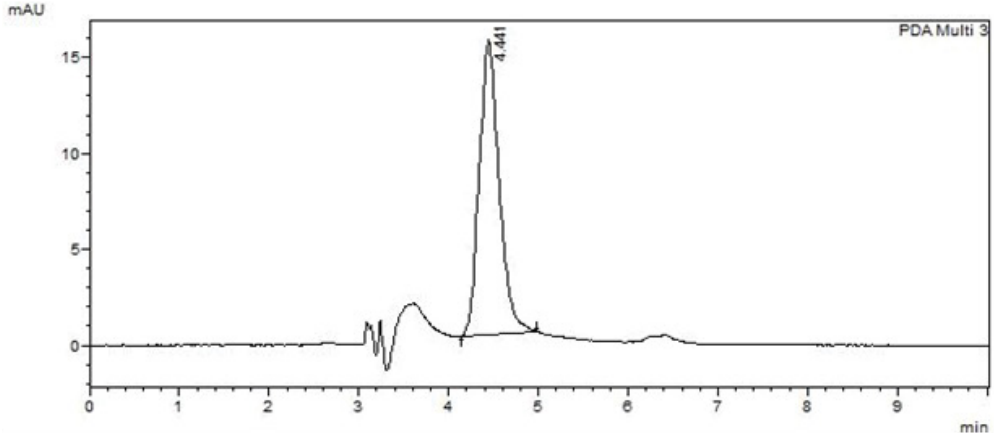

Figure 8. Chromatogram for standard linagliptin (10 µG/ML).

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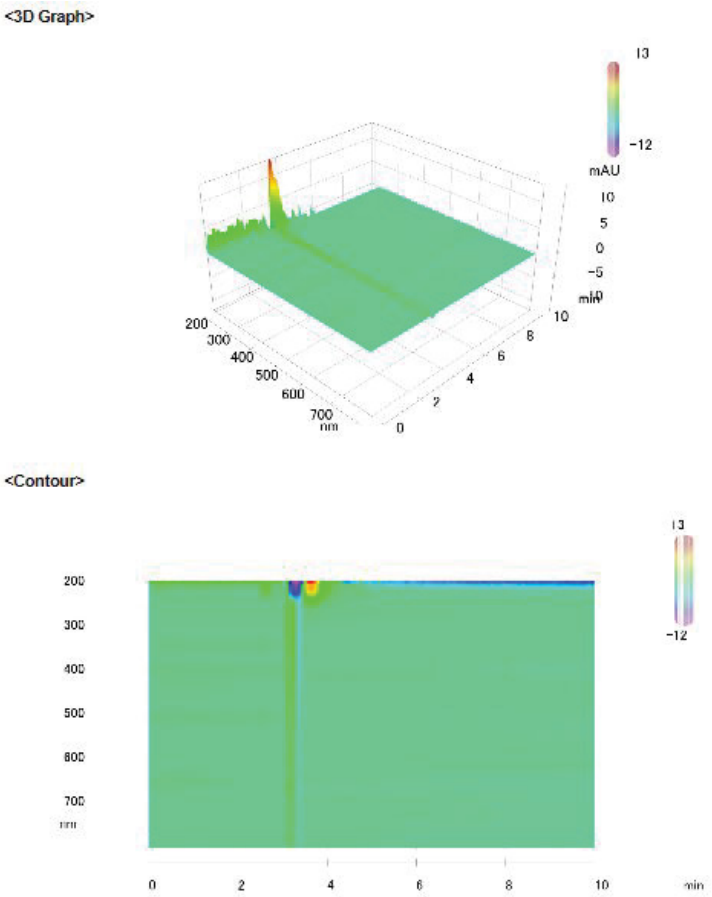

Figure 9. Graph for blank linagliptin.

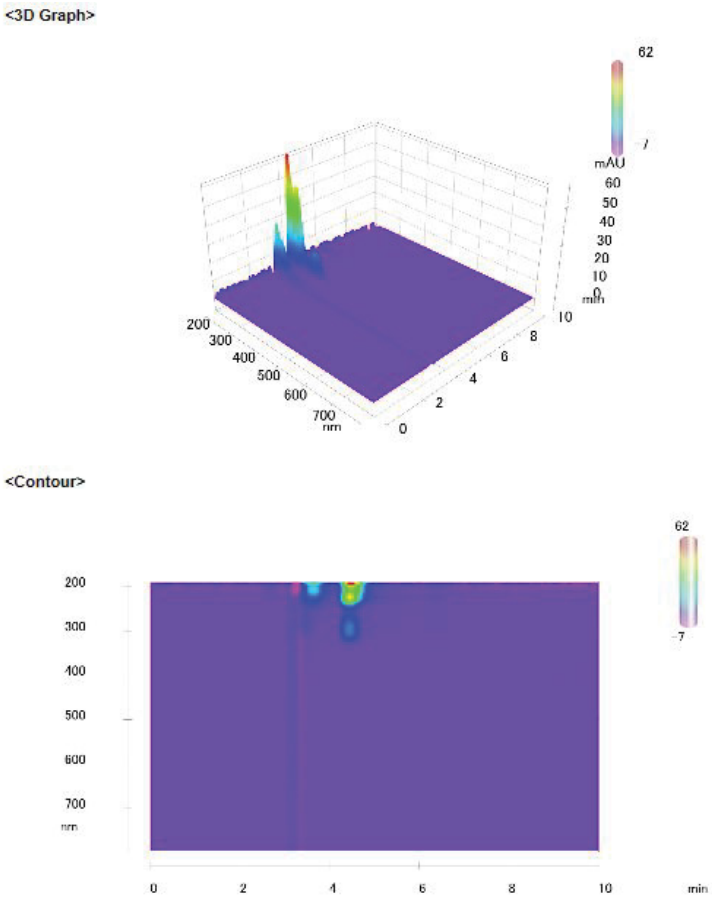

Figure 10. Graph for standard linagliptin (10 MG/ML).

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Acceptance Criteria

Correlation coefficient should be NLT 0.95.

Data Interpretation

From the statistical treatment of linearity data from **Table 2** of Linagliptin it is clear that the response of Linagliptin is linear between 15% to 50% level of working concentration. The correlation was not less than 0.95, so linearity parameter was within the acceptance criteria.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample.

System Precision

The system precision is to ensure that the analytical system is working properly **(Table 3).**

Sl.No	2µg	6 µg	10 µg
1	88571	156769	214721
2	88575	158999	215780
3	88756	158950	216330
4	88780	159795	218690
5	88570	158989	219800
6	85811	159790	218547
Average	88177.16667	158882	217311.3
Standard Deviation	1061.829776	1012.633859	1809.184
% RSD	1.204200379	0.637349643	0.832531

Table 3. Results for system precision studies, SD: Standard Deviation RSD: Relative Standard Deviation.

Acceptance Criteria

The %RSD of the area response for Linagliptin peak obtained from 6 injections of Standard preparation should be NMT 2.0%.

Data Interpretation

From the above Table 3, it can be concluded that area response are consistent as evidenced by relative standard deviation.

Method Precision

Method precision indicates whether a method is giving consistent results for a single material or not **(Table 4).**

Sl.No	2µg	6 µg	10 µg
1	88571	156769	214721
2	89678	159658	215858
3	89751	153658	215455
4	89652	158954	215885
5	90581	158655	215888
6	91874	158666	225858
Average	90017.83333	157726.6667	217277.5
Standard Deviation	1014.590133	2018.733899	3859.338
% RSD	1.127099038	1.279893845	1.776225

Table 4: Results for method precision studies, SD: Standard Deviation RSD: Relative Standard Deviation.

Acceptance Criteria

The % RSD calculated on 6 determinations should be NMT 2.0%.

Data Interpretation

From the above **Table 4**, it can be concluded that the method is precise.

Limit of Detection & Limit of Quantitation

Limit of Detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

Limit of Quantitation (LOQ) is the lowest amount of analyte in a sample that can be quantitated with acceptable accuracy and precision, under the stated experimental conditions.

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The LOD & LOQ is calculated according to slope, intercept and correlation coefficient and the relative standard deviation from the linearity curve.

LOD	0.097 (µg/mL)
LOQ	1.023 (µg/mL)

Table 5. Results of limit of detection & limit of quantitation.

Data Interpretation

From the above **Table 5**, it can be concluded that distinct visible peaks were observed at LOD level concentration. The LOD and LOQ for Linagliptin were found to be 0.097 and 1.023 µg/mL respectively.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value (Standard value).

$$\% \text{ Recovery} = \left(\frac{\text{Amount of drug recovered}}{\text{Amount of drug added}}\right) \times 100$$

Sl.No	Level of % Recovery	Amount of std formulation (µg/mL)	Amount of drug added (µg/mL)	Total amount of drug (µg/mL)	Total amount of drug found	% Recovery
1.	50	4	2	6	5.9	98.3
					5.8	96.6
					6.2	103.3
					Mean	*99.4*
2.	100	4	4	8	8.3	103.7
					7.8	97.5
					8.1	101.2
					Mean	*100.8*
3.	150	4	6	10	9.8	98
					9.7	97
					10.4	104
					Mean	*99.6*

Table 6. Results for method accuracy studies.

Acceptance Criteria

Individual and Mean % recovery at each level should be between 98.0% and 102.0%.

Data Interpretation

From **Table 6**, it can be concluded that the recovery is well within the limit. Hence the method is accurate.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Condition		Tailing	%RSD	Theoretical plates	%RSD
As such condition (optimized method)		1.215	--	4478.4	--
Mobile phase ratio as such (50:50)	25:75	1.341	0.9789	4417.8	0.6811
	45:55	1.358	1.6086	4415.6	0.7060
Change in pH	Decreased (-0.2 units)	1.328	0.4918	4419.3	0.6642
	Increased (+0.2 units)	1.322	0.2654	4421.3	0.6415
Flow rate	Decreased (-0.2 mL/min)	1.358	1.6086	4425.9	0.5896
	Increased (+0.2 mL/min)	1.366	1.9022	4430.5	0.5376
Column temperature	Decreased (-5°C)	1.354	1.4612	4435.6	0.4801
	Increased (+5°C)	1.333	0.6797	4440.9	0.4204
Wave length	Decreased (1 nm)	1.348	1.2392	4445.3	0.3709
	Decreased (2 nm)	1.352	1.3873	4447.9	0.3416
	Increased (1 nm)	1.356	1.5350	4449.9	0.3192
	Increased (2 nm)	1.358	1.6086	4321.9	1.7783

Table 7. Results of robustness.

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Acceptance Criteria

- The Tailing factor should be NMT 2.0.
- The Theoretical plates should be NLT 2000
- The relative standard deviation should be NMT 2.0%

Data Interpretation

From **Table 7**, it can be concluded no significant changes were observed due to change in above said chromatographic conditions, hence the method is robust.

Forced Degradation Studies

The stress studies were performed for Linagliptin drug at 50 µg/ml concentration. Here the bulk drug is exposed to acidic stress by addition of 1.0 ml of 0.1M HCl to drug solution and counteracted with 1.0 ml of 0.1M NaOH, at 0 min, 30 min, 1 hrs, 2 hrs, 4 hrs, 8 hrs, 6 hrs and 32 hrs respectively. Similarly, the basic stress studies were performed by adding 1.0 ml of 0.1 M NaOH and neutralized with 1 ml of 0.1M HCl. Oxidation studies were achieved on bulk drug by addition of 1.0ml of 3% H_2O_2, Thermal studies were performed by heating the sample at 60^{0}C and UV studies were also carried out by sample at UV- Lamp 450ºC respectively.Entire samples were placed in different volumetric flask (10 ml) and dissolved in HPLC grade methanol. Final drug concentration for assay was made up with methanol and injected in the chromatographic system. For all these stability study, the development of degradable item was affirmed by contrasting and the chromatogram of the arrangement kept under ordinary unstressed conditions. Every stressed sample was analyzed by improved UFLC method. The degradation data for Linagliptin was shown [11-16].

Acid Stress

For 2 ml sample add 2 ml 0.1N HCl keep aside for 5 min and then add 2.ml of 0.1N NaOH, then inject this sample for 36 hours at intervals 30 min, 1 hr., 1.30 min respectively **(Figure 11).**

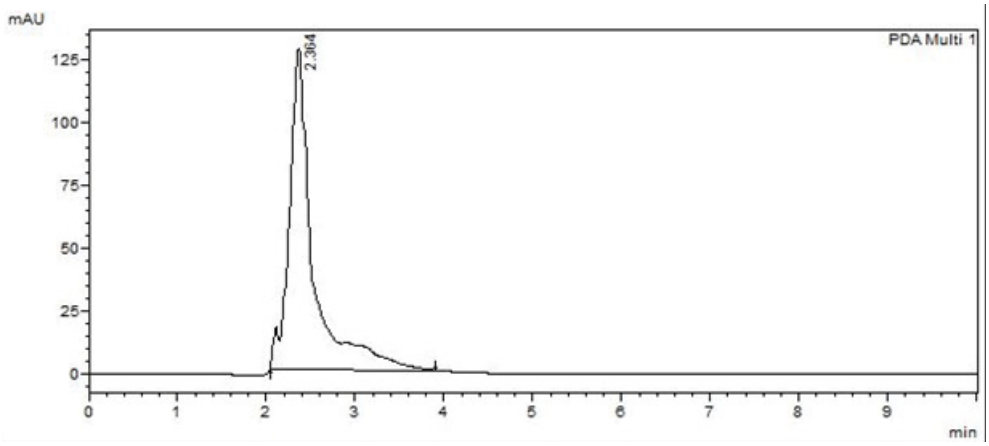

Figure 11. Chromatogram for acid stress.

Basic Stress

For 2 ml sample add 2 ml of 0.1N NaOH keep aside for 5 min and then add 2 ml of 0.1N HCL, and inject the sample **(Figure 12).**

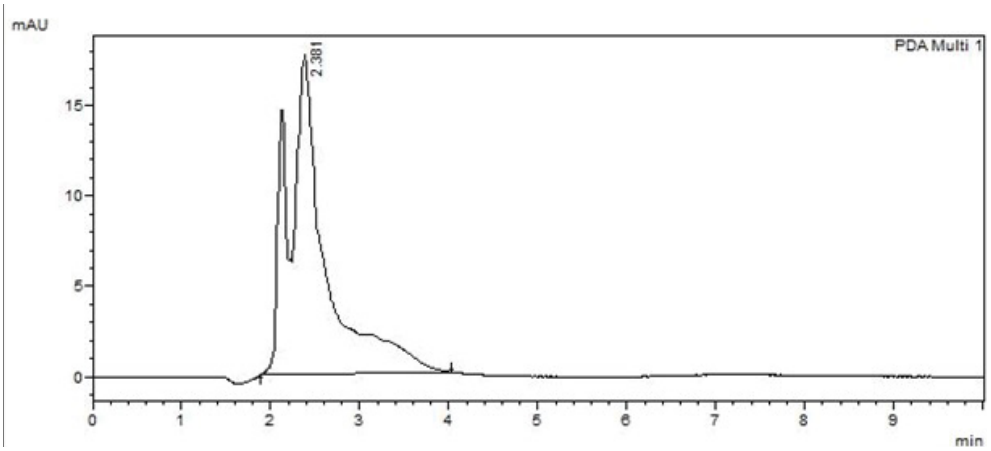

Figure 12. Chromatogram for basic stress.

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Peroxide Stress

For 2 ml sample add 1 ml of 3% peroxide solution and inject this sample **(Figure 13).**

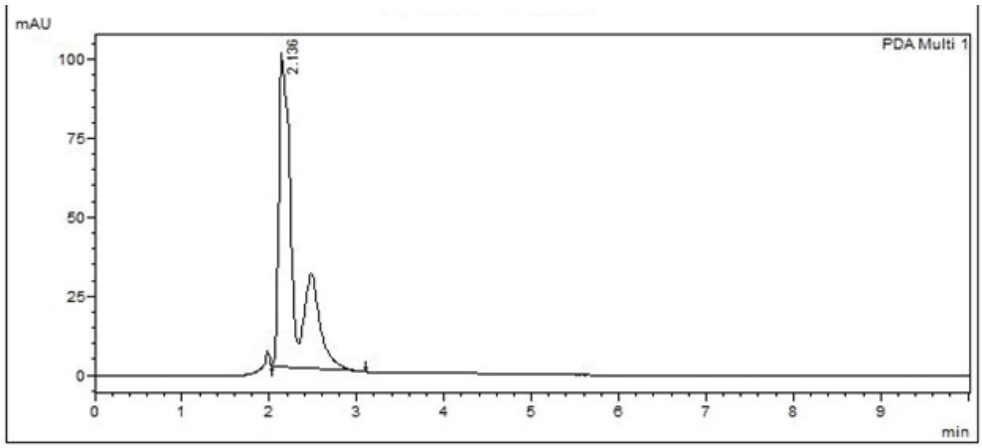

Figure 13. Chromatogram for peroxide stress.

Heat Stress

Take 2 ml sample and heat for 1 hr. at 80 c and inject the sample **(Figure 14).**

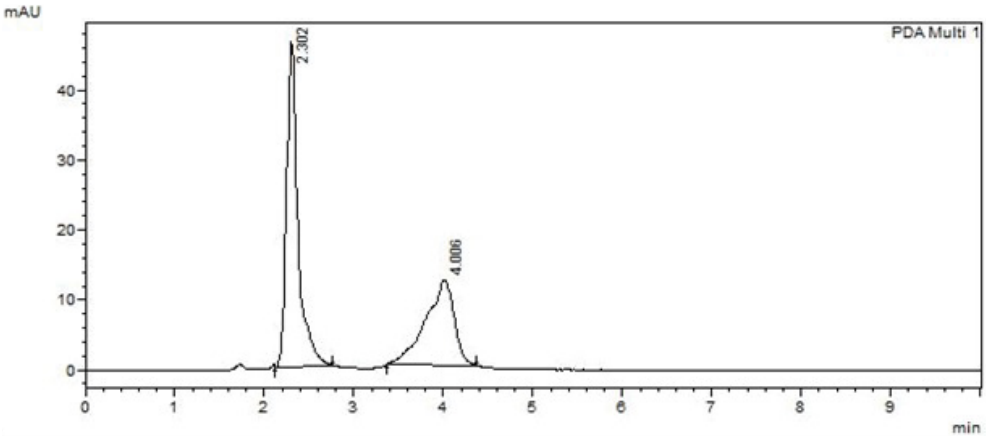

Figure 14. Chromatogram for heat stress.

Photolytic Stress

Take 2 ml sample and place in a UV chamber for 1 hr. UV- Lamp 450^{oC}respectively and then inject the sample **(Figure 15, Table 8).**

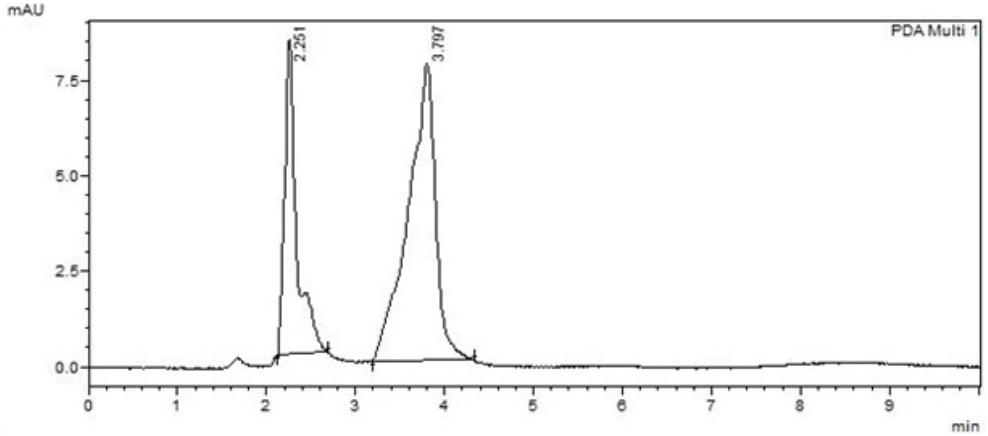

Figure 15. Chromatogram for photolytic stress.

Time	Drug	UV	THERMAL	0.1N HCL	0.1N NaOH	3%H_2O_2

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0 Min	**Linagliptin**					
		89.23%	80.76%	87.79%	89.35%	81.34%
30 Min	**Linagliptin**					
		85.34%	77.31%	84.14%	87.34%	74.34%
1 hr	**Linagliptin**					
		82.43%	60.16%	78.86%	80.34%	68.23%
2 hr	**Linagliptin**					
		77.34%	57.14%	74.78%	78.38%	60.87%
4hr	**Linagliptin**					
		69.34%	31.69%	67.27%	70.34%	44.34%
8hr	**Linagliptin**					
		52.23%	20.15%	59.65%	57.23%	32.62%
16hr	**Linagliptin**					
		43.87%	19.6%	44.64	43.24%	22.23%
32hr	**Linagliptin**					
		34.24%	---	--	---	--

Table 8. Results for recovery studies linagliptin after the stress conditions (% recovery of drug).

CONCLUSION

The above RP-UFLC analytical method satisfies all validation parameters like accuracy, precision, system suitability, specificity, linearity of detector response, ruggedness and robustness. At the same time the method satisfies the forced degradation study. Hence, the validated method can be used for routine determination of stability studies in quality control laboratories in the pharmaceutical industry.

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