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Analytical Method Development and Validation of RP-HPLC Method for Estimation of Linagliptin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise, and specific spectroscopy method for quantitative determination of Linagliptin in API and in pharmaceutical dosage form was developed and validated. The development was performed on a Zorbax SB Phenyl column (150 x 4.6 mm, 5 µm). The pH was adjusted to 3.2 with buffer solution: acetonitrile (75:25% v/v) at a 1.0 mL/min flow rate. Samples werelyzed using a UV-visible 2487 detector at 295 nm. The developed method complied with the system suitability study with an acceptable asymmetric factor and number of theoretical plates. The linearity was observed between 10–30 µg/mL concentrations (r^2 = 0.999). Method accuracy was observed as 99.7 %. The drug content was found within the acceptable limit in the intermediate precision study. The retention time was obtained at 2.73 min. This method was validated and applied to the determination of Linagliptin in pharmaceutical dosage form. [(1)]

Keywords: *Linagliptin, RP-HPLC, antidiabetic, Acetonitrile, Validation, Accuracy.*

1. INTRODUCTION

An antidiabetic medication called Linagliptin is used to treat and regulate the plasma sugar levels of people with type 2 diabetes. It is recommended to be taken alongside other antidiabetic medications, as well as with a healthy diet and frequent exercise. The dipeptidyl peptidase-4 (DPP-4) enzyme is inhibited by Linagliptin, an oral medication. It functions by preventing the breakdown of GLP-1 and GIP, two naturally occurring hormones that aid in blood sugar regulation, by blocking the dipeptidyl peptidase-4 (DPP-4) enzyme. The primary excretion of Linagliptin taken orally occurs in the feces. Ninety percent of an oral dosage is eliminated unaltered in the stools and urine. Very slightly soluble in alcohol and isopropanol; soluble in methanol; sparingly soluble in ethanol. Because Linagliptin is slightly hygroscopic and white to yellowish, water absorption does not alter the crystal modification. Linagliptin, 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl) methyl]-3,7-dihydro-1H-purine-2,6-dione, is a xanthine-derived DPP-4 inhibitor. [(5,6)] Its molecular weight is 472.5422 g/mol, and its formula is $C_{25}H_{28}N_8O_2$. Linagliptin (LNG) has different pharmacokinetic (PK) characteristics, which may have some advantages or benefits in therapeutic practice. On May 2, 2011, the USFDA approved this medication to treat the patients with type II diabetes. [(7,8,9)]

Chemical structure of Linagliptin is shown in Figure1. [(2,3)]

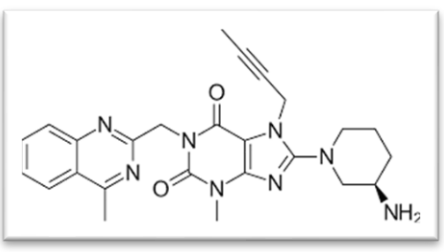

Fig.1: Structure of Linagliptin

Linagliptin is a reversible, competitive inhibitor of DPP-4. The breakdown of glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 is slowed when this enzyme is inhibited. GLP-1 and GIP limit the release of glucagon from pancreatic beta cells while stimulating the release of insulin from these cells. When combined, these effects enhance the release of insulin in response to glucose and decrease the breakdown of glycogen in the liver.

2. MATERIAL AND METHOD

Chemical and Reagents:

The standard was linagliptin. Lupin Pharmaceutical PVT LTD, Chikalthana, Chhatrapatti Sambhajinagar-431210, Maharashtra, supplied the gift sample. A local pharmacy sold the commercial form of linagliptin (Dynaglipt L tablet 5 mg). Mankind Pharma, Ltd. The distillation Every chemical reagent, including water, was of analytical quality. Analysis tools include methanol, water, acetonitrile, a 0.45 µ nylon membrane disc filter, and a.45µ PVDF syringe filter.

Instrumentation and Chromatographic Condition:

We used an Agilent Infinity II system and an auto sampler to perform High-performance Liquid Chromatography (HPLC). A UV/PDA detector was used as a chromatographic system, and Open Lab EZ Chrome on a Windows computer system was used to register chromatograms for data collection and processing. Linagliptin concentration was determined using a Zorbax SB Phenyl column (150 x 4.6 mm, 5µ).

Reverse Phase High Performance Liquid Chromatography Method Development and Optimization.

The standard solution of Linagliptin was used for method development trials to optimize the method for determination of Linagliptin 5 mg. The mobile phase is containing of pH was adjusted 3.2 with buffer solution: Acetonitrile (75:25% v/v) at 1.0 mL/min flow rate. Samples were analysed Uv visible 2487 detector at 295 nm and injection volume is 20 µL. Test samples were prepared by optimizing sample preparation which was used for method development trials to optimize the method as a Specific, Accurate, Precise and robust.

Selection of Wavelength for Linagliptin:

After baseline correction, the UV spectrophotometer scanned with 10 µg/mL working standard solution between 400 to 200 nm against methanol as a blank. The optimised maximum wavelength is 295 nm.

Preparation of Buffer solution: Weigh and transfer about 1.36 gm of potassium dihydrogen phosphate was added to 1000 mL HPLC water mix well. The pH was adjusted to 3.2 ± 0.05 with dilute orthophosphoric acid. Filter through 0.45µ nylon

Preparation of Mobile phase: A mixture of Buffer Solution pH 3.2 and Acetonitrile in the ratio 75:25 *v/v*, was prepared, mixed and sonicated to degas used as mobile phase.

Preparation of Diluent: Prepare mixture of water and Methanol in the ratio of 20:80 v/v respectively, mix well.

Preparation of Blank: Use diluent as blank.

Preparation of Standard solution:

Weighed and transferred accurately about 25 mg of Linagliptin working standard into 100 mL clean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 30 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 4.0 mL of this stock solution to 50 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Linagliptin standard solution: 20 ppm)

Preparation of Sample solution:

Weighed and transferred 10 Linagliptin tablets in to 250 mL clean and dry volumetric flask. Added about 150 mL of diluent, sonicate for 60 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Further diluted above stock solution 5.0 mL of this sample stock solution to 50 mL volumetric flask make up with Diluent and mixed well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Sample Solution: 20 ppm)

3. RESULT AND DISCUSSION

Table 1: Final reversed phase High performance liquid chromatographic condition.

Column	Zorbax SB Phenyl, 150 x 4.6 mm, 5µ,
Mobile Phase	Buffer Solution pH 3.2 and Acetonitrile(75:25 v/v)
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Wavelength	295 nm
Column Temp	35ºC
Sample Temp	25ºC
Run Time	8.0 minutes
Retention Time	2.73 min

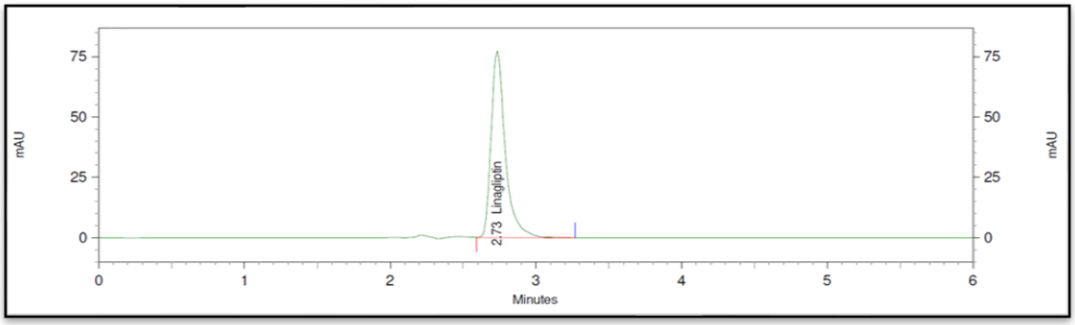

Fig. 3: Typical chromatogram for linagliptin

Method Development:

A regulatory requirement for the development and validation of analytical methods ensures that the method can effectively quantify the drug from dose forms of bulk medicines. (4) The analytical method for linagliptin was established using the RP-HPLC method to estimate the concentration from marketed tablets and bulk. To maximize the simple and reliable analytical technique, a number of factors were examined, including mobile phase, flow rate, wavelength, etc. Buffer solution: Acetonitrile (75:25% v/v) at a flow rate of 1.0 ml/min was found to optimize the mobile phase's parameters to 3.2. At 295 nm, samples were studied using a UV visible 2487 detector. Table 1 shows the HPLC conditions that have been optimized.

ANALYATICAL METHOD VALIDATION OF RP-HPLC.

The established method for estimating linagliptin for the following parameters was validated using ICH Q2(R1) recommendations. Specificity, linearity, accuracy, precision, and robustness were among these criteria.

1.System Suitability:

The various system suitability parameters tested are summarised in Table 2 as follows. The peak was observed at a retention time of 2.73 min for sample of linagliptin which is acceptable and indicates the applicability of the developed method.

Table.2: system suitability test of Linagliptin

Tailing Factor	**1.34**
Theoretical plates	**5244**
Injection No.	**Area**

1	8536201
2	8532149
3	8533109
4	8542597
5	8540123
Mean	**8536836**
%RSD	**0.05**

The tests were performed by collecting data from Single injection of blank (Diluent) and five replicate injections of Standard solution were injected into the chromatograph. The data obtained is summarized in Table

Conclusion:

The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

2. Specificity (Identification, Interference & Peak Purity)

2.1 Identification

2.2 Interference

2.3 Peak purity

To ensure the absence of interference from blank and placebo, those are likely to be present in linagliptin drug product.

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table

Table 3: Specificity (Identification and Interference)

Solution	**Specificity data**		
	Retention time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	2.73	Purity angle	Purity threshold
		1.63	2.32
Sample solution	2.72	1.24	2.11

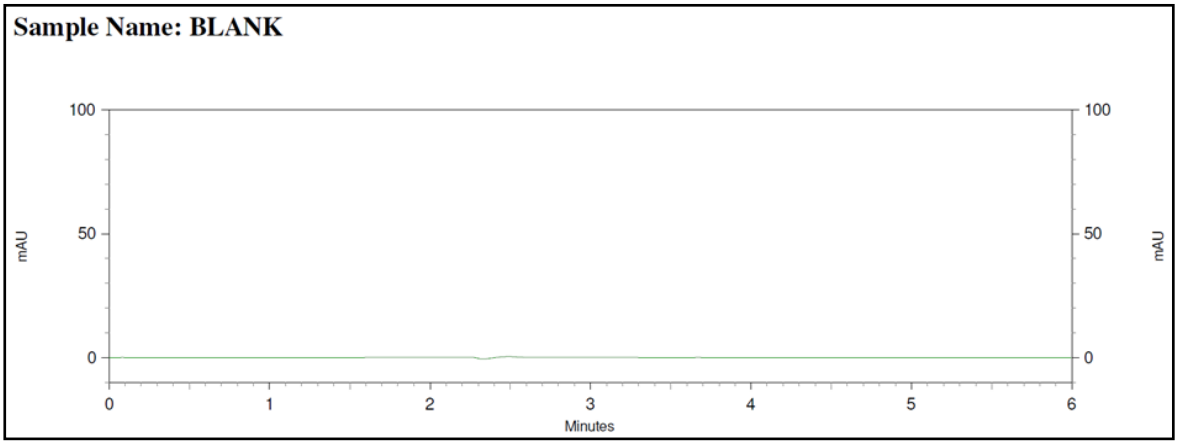

Fig 4: Chromatogram of Blank

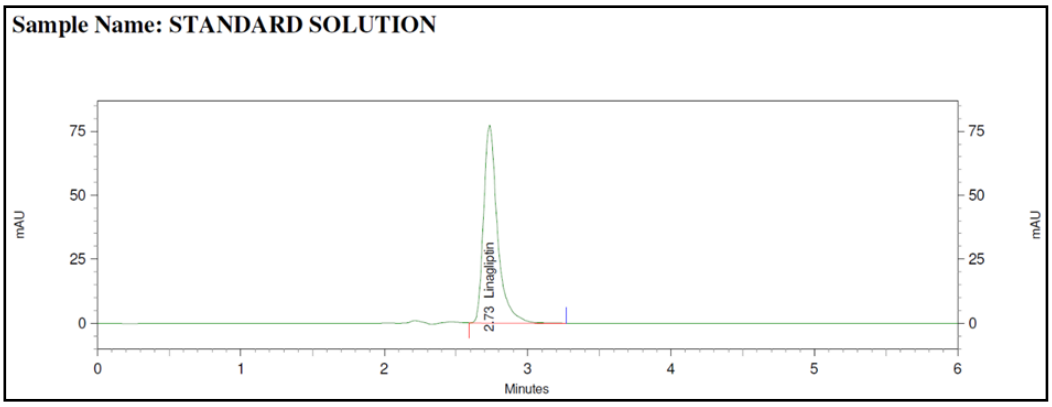

Fig 5: Chromatogram of Standard

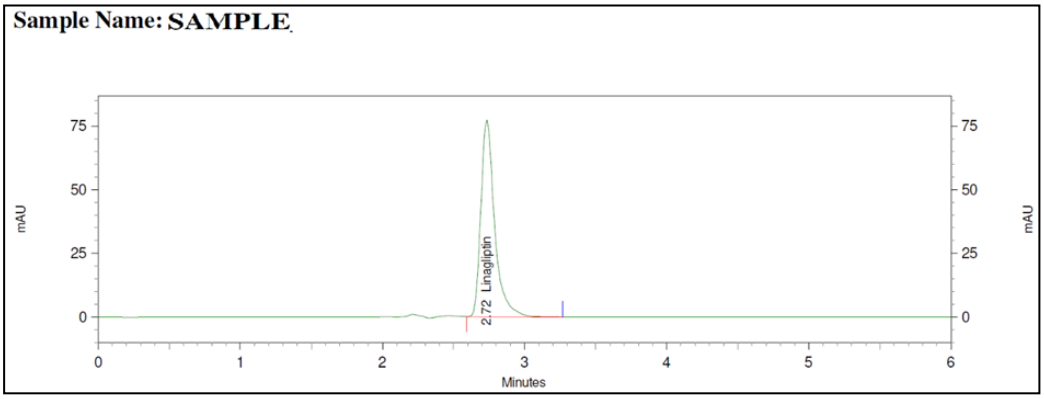

Fig 6: Chromatogram of Sample

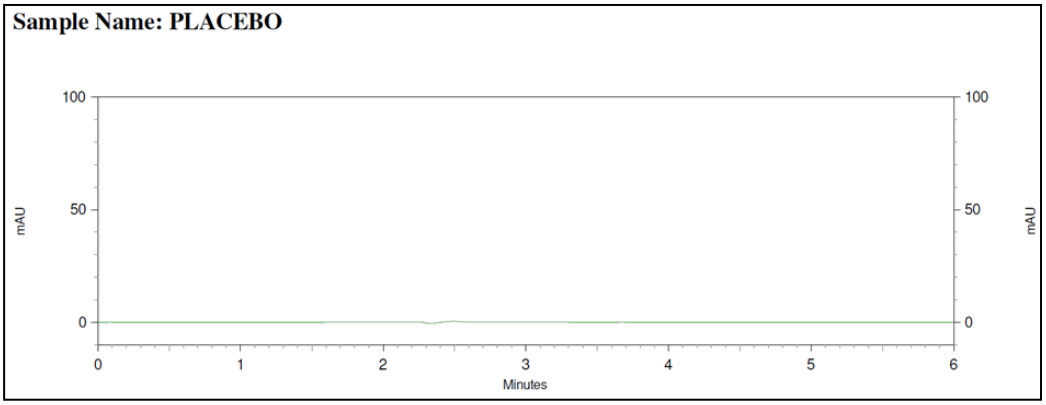

Fig 7: Chromatogram of Placebo

Conclusion:

- The data demonstrates that retention time in standard and sample is same for Linagliptin peak.
- The data demonstrates that there is no interference in blank and placebo at the retention time of Linagliptin peak. Peak Purity match in both chromatograms obtained from Standard and Sample solution.

3. Linearity:

Linearity was evaluated in the range of 50 % to 150 % of Linagliptin for working concentration. The working concentration of Linagliptin in solution is 20 µg/mL. The data summarized in Table.

Table 4: Linearity of Linagliptin

Level	Conc. (µg/mL)	Area	Mean
50%	10.0	4210259	4213121
		4216248	
		4212857	
75%	15.0	6439517	6445016
		6442591	
		6452941	
100%	20.0	8534251	8535505
		8532109	
		8540156	
125%	25.0	10625419	10655826
		10639541	
		10702519	
150%	30.0	12896524	12891165
		12890446	
		12886524	
Corr. Coeff.			0.9997
Intercept			50353
Slope			422775
% Y-intercept			0.59

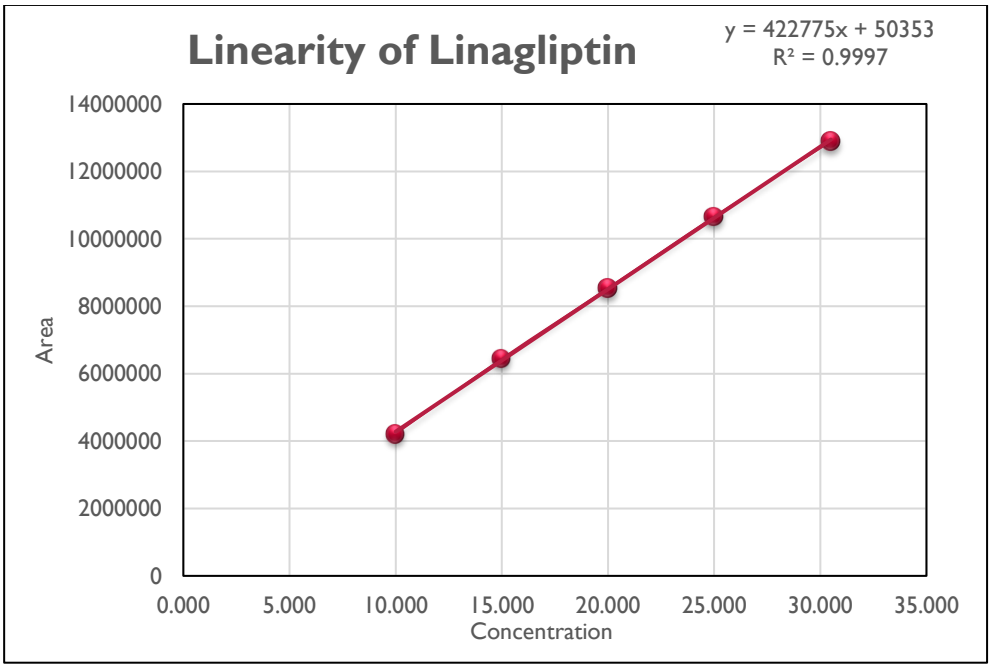

Fig 8: Linearity plot of Linagliptin

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that the response is found to be linear.
- Co-relation coefficient (r^2 was found 0.9997).

4. ACCURACY (BY RECOVERY STUDY)

Evaluated accuracy from 50% to 150% of Linagliptin tablet, working concentration level. Each level prepared in triplicates.

Table 5: % Recovery for Linagliptin

Level (%)	Linagliptin Added Conc (µg/mL)	Linagliptin Recovered conc.	Area	% Recovery	Mean % Recovery
50	10.24	10.16	4336521	99.21	99.38
	10.30	10.14	4326996	98.42	
	10.10	10.15	4332540	100.50	
100	20.32	20.05	8560129	98.69	99.48
	19.96	20.18	8612967	101.09	
	20.24	19.97	8522691	98.65	
150	30.46	30.14	12865024	98.95	99.85
	29.92	30.28	12923652	101.19	
	30.32	30.14	12863259	99.39	

Conclusion:

The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 95.0% - 105.0%.

5. PRECISION:

5.1 Method Precision: (Repeatability)

Method precision was carried out by analysing six replicate injections of assay concentration of standard and sample solutions.

Single injection of blank (Diluent), Standard solution (Five replicates) and sample solution (six preparations) was injected on the system.

Table 6: Method precision

Sample	Area	% Assay
Sample 1	8320149	97.42
Sample 2	8336524	97.69
Sample 3	8296555	97.19
Sample 4	8425963	98.93
Sample 5	8365014	98.29
Sample 6	8395241	98.04
Mean		**97.93**
STD DEV		**0.6335**
% RSD		**0.647**

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

5.2 Intermediate Precision: (Ruggedness)

Intermediate precision was assessed by analyzing the standard solution and sample solution on different days. The % assay and % RSD were calculated**.**

six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 7: Intermediate Precision

Sample	**Area**	**% Assay**
Sample 1	8396522	98.17
Sample 2	8296524	96.89
Sample 3	8410268	98.10
Sample 4	8359421	97.58
Sample 5	8265904	96.83
Sample 6	8365009	97.84
Mean		**97.57**
STD DEV		**0.5886**
% RSD		**0.603**

Table 8: Intermediate Precision Pool Data

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**AD/HPLC-31**	**AD/HPLC-19**
Column No.	**HPLC-24**	**HPLC-30**
Sample No.	**%Assay**	
1	97.42	98.17
2	97.69	96.89
3	97.19	98.10
4	98.93	97.58
5	98.29	96.83
6	98.04	97.84
Mean	**97.93**	**97.57**
Mean of Precision % Assay	**97.75**	
Absolute Mean difference % assay	**0.6**	

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.
-

6. ROBUSTNESS

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table 9: Robustness for linagliptin

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	8320149	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.1 ml/min	8212519	1.3
	0.9 ml/min	8286521	0.4
Change in wavelength (±2 nm)	297 nm	8173500	-1.8
	293 nm	8416128	1.2

Conclusion:

- System suitability criteria were fulfilled.
- The difference of % assay value in each modified condition is within acceptance criteria.

7. CONCLUSION

RP-High Performance Liquid Chromatography (HPLC) Method:

1.HPLC Advantages: HPLC is valued for its ease of use, specificity, sensitivity, and suitability for analyzing complex samples.

2. Purpose of Study: Used to estimate Linagliptin in tablet formulations.

3. Equipment Used:

HPLC.

Zorbax SB Phenyl column (150 x 4.6 mm, 5µ).

UV/PDA detector.

Openlab EZ Chrome workstation software.

4. Sample Preparation: Standard and sample solutions of Linagliptin were prepared in a diluent.

5. Mobile Phase Optimization:

Various solvent combinations were tested.

Best mobile phase: Buffer solution (pH 3.2) and acetonitrile.

6.Wavelength Selection:

295 was chosen based on λ max from UV scanning in water: methanol mixture.

7.Chromatographic Performance:

Provided good resolution and retention time.

Tailing factor was within acceptable range (<2).

8.Results:

The method was accurate and reliable.

Successfully applied to analyze Linagliptin in tablet form.

8. ACKNOWLEDGEMENT

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