ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF LINAGLIPTIN IN BULK AND DOSAGE FORM BY UV SPECTROSCOPY

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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. Linagliptin showed maximum absorbance at 295 nm by using distilled water as a solvent. The method shows linear relationship in the concentration range of (1-10µg/ml). The regression equation of linearity curve was found to be y = 0.0903x + 0.0278and correlation coefficient (R^2) was found to be0.998. The precision of the method was found to be within %RSD ≤ 2.Method accuracy was observed as 100.69±0.41.No interference was found in the absorbance of drug in the presence of common excipients and analysis conditions. This method was validated and applied to the determination of Linagliptin in pharmaceutical dosage form.***

KEYWORDS: UV Spectroscopy, Calibration curve method, Linagliptin.

INTRODUCTION:

Linagliptin is an orally-active inhibitor of the dipeptidyl peptidase-4 (DPP-4) enzyme. It is very slightly soluble in water (0.9 mg/ml). Linagliptin is soluble in distilled water (ca. 60 mg/ml), very slightly soluble in isopropanol (< 1 mg/ml) and very slightly soluble in acetone (ca. 1 mg/ml). Linagliptin is a white to yellowish, slightly hygroscopic but water uptake does not change the crystal modification. The chemical name is 8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1[(4methylquinazolin2yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione. Molecular formula is $C_{25}H_{28}N_8O_2$, molecular weight of 472.54 g/mol. Chemical structure of linagliptin is shown in Figure1. [1,2]

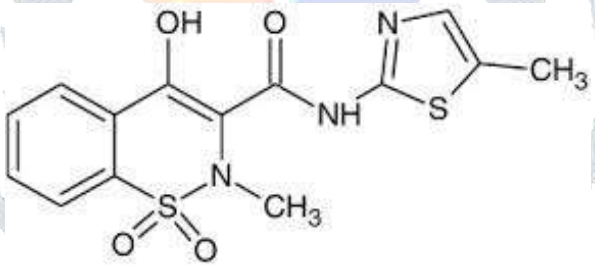

Figure1. Chemical structure of Linagliptin

Literature review reveals that there is few methods based on HPLC[1,2], UV method[3,4],RP-HPLC[6,7] and liquid chromatography[8,9]its estimation in bulk, dosage forms and biological samples . The present work describes the development and validation of UV spectroscopy method, which can quantify the linagliptin. An attempt was made to develop a simple, accurate, precise and rapid spectroscopy method for the estimation of linagliptin in bulk and pharmaceutical dosage form.

The method was validated as per International conference on Harmonization (ICH) guidelines.[10] The present work describes the development and validation of UV spectrophotometric method, which can quantify the Linagliptin. An attempt was made to develop a simple, accurate, precise and rapid spectrophotometric method for the estimation of Linagliptin in bulk and pharmaceutical dosage form. The method was validated as per International conference on Harmonization (ICH) guidelines.

MATERIALS:

Chemical and Reagents: Linagliptin was used as a standard. It was received as a gift sample from Lee Pharma Limited, Tilangana, India. Distilled Water and all other chemical reagents were of analytical grade. UV Spectrophotometer used was of make, Agilent Tech (Cry 60 UV-Visible).

Instrumentation and UV spectroscopy condition: UV-Vis. Spectroscopy and 1 cm Quartz cells; he measurement properties wavelength range (200-800) nm, scan speed: medium sampling interval: 2.0,scan mode: single, measuring mode: absorbance, slit width: 2.0 nm, light source change wavelength and digital electric balance and pipette.

METHOD:

Preparation of standard stock solution of Linagliptin

About 25mg of the drug was weighed accurately and transferred to 50ml volumetric flask and dissolved in about 25ml of distilled water. It was then sonicated for about 10 minutes in sonicator. The volume was than make up to the mark with distilled water and filtered.

Transferred 5ml of the solution to 25ml volumetric flask and diluted up to the mark with distilled water. This solution contained 100µg of drug per ml of the solution.

Determination of wavelength of maximum absorbance (λ_{max})

1ml of standard stock solution was pipette out and transferred to 10ml volumetric flask. The volume was made up to the mark with distilled water. This solution contained 10µg/ml of the drug. The absorbance of this solution was scanned in the UV range of 200-400nm against distilled water as blank. Absorbance detected at 295nm.

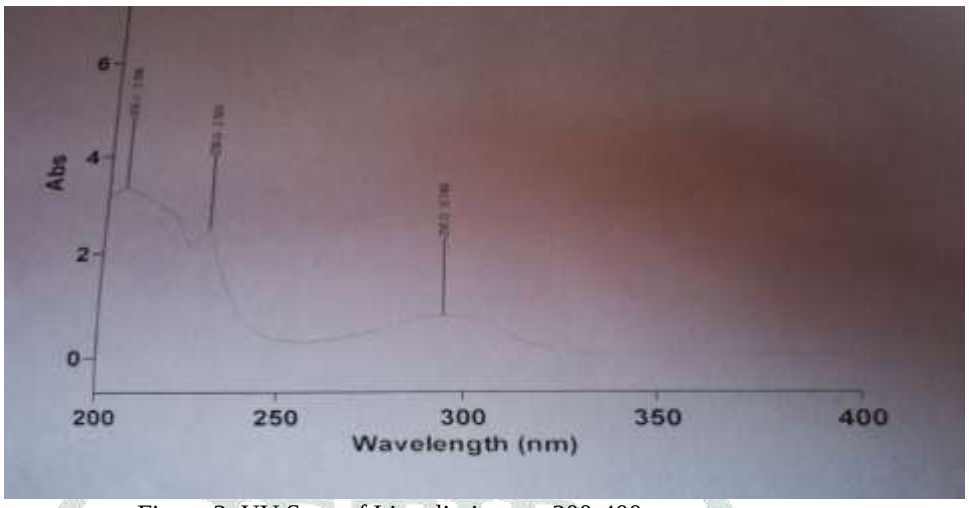

Figure 2: UV Scan of Linagliptin over 200-400 nm

Preparation of calibration curve for Linagliptin at 295nm:

0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 and 1.1 ml of the standard stock solution were pipette out in 10ml volumetric flask each containing distilled water. The final volume was made up to the mark with distilled water, to obtain solutions in the concentration range of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 µg/ml.

Table1. Concentration and absorbance of Linagliptin

Concentration(µg/ml)	Absorbance
1	0.1314
2	0.2094
3	0.2778
4	0.3895
5	0.472
6	0.5818
7	0.6648
8	0.7502
9	0.8454
10	0.9406
11	1.0054

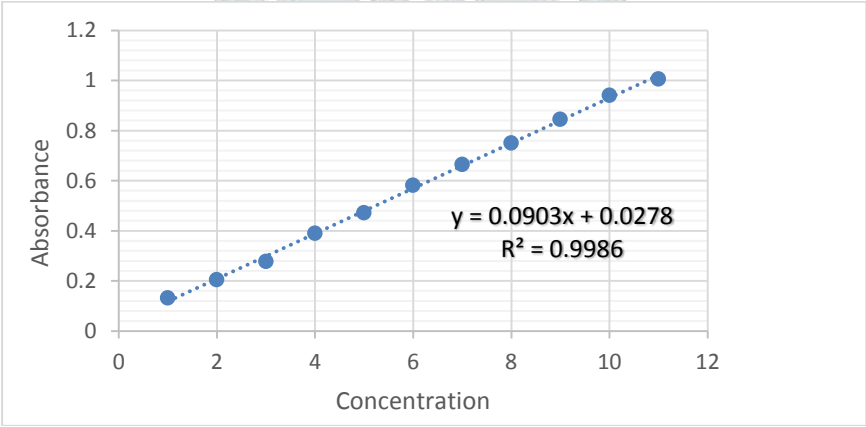

Figure 3: Calibration curve of Linagliptin

RESULT AND DISCUSSION:

Linearity& Range:

The linearity of the drug was performed in the concentration range of Linagliptin 1-10µg/ml as shown in Table1, the regression equation was found to be y=0.0903x +0.0278 as shown in figure 3. Correlation coefficient (R^2) was observed as 0.9986, the calibration curve was found to be linear in the above stated concentration range.

Precision:

The precision of analytical system was investigated by performing nine consecutive replicate solution of the same standard solution, and the standard deviation (SD) and relative standard deviation (RSD) obtained. It was performed over three levels as Repeatability, Intra-Day and Inter-Day Precision.

Repeatability- Transfer 1ml standard solution with distilled water in 10ml volumetric flasks and dilute it the same. Nine solutions of 10µg/ml concentration were prepared. Resultant solutions were scanned at 295nm using water as blank. The result obtained is shown in the table 2.

Table 2: Repeatability

Nominal Conc. (µg/ml)	Absorbance	Observed Conc. (µg/ml)	Mean Absorbance	SD	%RSD
10	0.8958 0.9083 0.9147 0.9347 0.9299 0.9295 0.9084 0.9215 0.9240	9.56 9.70 9.77 9.99 9.94 9.93 9.70 9.85 9.87	0.9185	0.0126	1.3816

Intra-day Precision- Transfer 0.2, 0.5 and 0.9 ml standard solution with distilled water in 10ml volumetric flasks and dilute it to get solutions of concentrations 2, 5 and 9 µg/ml respectively. Resultant solutions were scanned at 295nm using water as blank. Such three studies were performed within a day at 0, 3 and 6 hrs, interval. The result obtained is shown in the table 3.

Table3: Intra-day Precision

Nominal Conc. (µg/ml)	Absorbance			Observed Conc. (µg/ml)			Mean Absorbance	SD	%RSD
	0hr	3hr	6hr	0hr	3hr	6hr			
2	0.1756	0.1744	0.1716	2.23	2.63	2.08	0.1739	0.00205	1.18
5	0.4698	0.4712	0.4652	5.2	5.6	5.3	0.4687	0.00314	0.67
9	0.8295	0.8423	0.8295	8.66	9.06	9.4	0.8338	0.00739	0.89
								Mean	0.91

Inter-day Precision- Transfer 0.2, 0.5 and 9 ml standard solution with distilled water in 10ml volumetric flasks and dilute it to get solutions of concentrations 2, 5 and 9 µg/ml respectively. Resultant solutions were scanned at 295nm using water as blank. Such three studies were performed for three day at 0, 24, and 48 hrs interval. The result obtained is in the table 4.

Table 4: Inter-day Precision

Nominal Conc. (µg/ml)	Absorbance			Observed Conc. (µg/ml)			Mean Absorbance	SD	%RSD
	0hr	24hr	48hr	0hr	24 hr	48 hr			
2	0.2095	0.2069	0.2089	2.03	2.15	2.07	0.2084	0.0014	0.653
5	0.3812	0.3899	0.3867	5.03	5.1	5.21	0.3859	0.0044	1.14
9	0.8195	0.8298	0.8312	8.82	8.89	9.24	0.8268	0.0064	0.772
								Mean	0.855

Accuracy: The accuracy of the method was investigated by determination of Linagliptin at appropriate concentration, the %recovery and relative standard deviations(RSD) obtained.

Aliquot solution from (100µg/ml) solution and transfer it into 10ml volumetric flask with distilled water. Nine such transfers are made. Spike three of solutions with 0.2ml of standard solution and dilute each to 10 ml distilled water to get 2µg/ml solution. Spike three of solutions with 0.4ml of standard solution and dilute each to 10 ml distilled water to get 4µg/ml solution. Spike three of solutions with 0.6ml of standard solution and dilute each to 10 ml distilled water to get 6µg/ml solution. Absorbance of resultant solution was scanned at 295nm using distilled water as blank. The result is obtained is in table 5.

Table 5: Accuracy

Recovery at (%)	Nominal Conc. (µg/ml)	Absorbance	Observed Conc. (µg/ml)	% Recovery
80	2=1+1	0.2104	2.02	101.10

80	2=1+1	0.2102	2.01	100.99
80	2=1+1	0.2109	2.02	101.38
100	4=1+3	0.3910	4.02	100.55
100	4=1+3	0.3200	4.03	100.83
100	4=1+3	0.3911	4.02	100.58
120	6=1+5	0.5701	6.00	100.09
120	6=1+5	0.5711	6.01	100.27
120	6=1+5	0.5721	6.02	100.46
			Mean	100.69±0.41

Specificity: The specificity is investigated by observing interference in absorbance of drug in the presence of common excipients like starch, talc, lactose, mag. stearate. Prepare 2, 4, and 8 µg/ml of drug solution with and without excipients was measured at 295nm using distilled water as blank. The result obtained is in table 6.

Table6: Specificity

Nominal Conc.(µg/ml)	**Without Excipients**		**With Excipients**		**%Interference**
	Absorbance	**Observed Conc.(µg/ml)**	**Absorbance**	**Observed Conc.(µg/ml)**	
2	0.124	1.09	0.1674	1.56	0.48
2	0.1382	1.24	0.1896	1.81	0.56
2	0.1386	1.25	0.1945	1.86	0.61
4	0.3861	3.96	0.4417	4.57	0.61
4	0.3911	4.02	0.4501	4.67	0.65
4	0.3975	4.09	0.4547	4.72	0.63
8	0.7401	7.85	0.8039	8.56	0.70
8	0.7378	7.83	0.8173	8.71	0.87
8	0.7502	7.96	0.8019	8.53	0.57
				Mean	0.63

*Determination of Linagliptin in Pharmaceutical Dosage Form (Lintor 2.5 mg):*Weigh 20 tablets and calculate the average weight. Powder those tablets. Weigh accurately equivalent quantity of powdered tablet containing about 25 mg of Linagliptin and transfer into 50 ml of volumetric flask and dissolved in about 25ml of distilled water. It was then sonicated for about 10 minutes in sonicator. The volume was than make up to the mark with distilled water and filtered. Transferred 5ml of the solution to 25ml volumetric flask and diluted up to the mark with distilled water. This solution contained 100µg of drug per ml of the solution. 0.6, ml of the solution was pipette out in 10ml volumetric flask. The final volume was made up to the mark with distilled water, to obtain solutions 6µg/ml. Measure the absorbance of the resulting solution at 295 nm. The observed result is shown in table 7.

Table 7: Determination of Assay

S. No	**Absorbance**	**Conc. (µg/ml)**	**Dil. Factor**	**Content (mg)**	**Weight Taken(g)**	**Average Weight(g)**	**Label Claim**	**% Assay**
1	0.6227	6.58	4166.67	27.45	1.340	0.121	2.5	99.14
2	0.6216	6.57	4166.67	27.29	1.331	0.121	2.5	99.63
3	0.6209	6.56	4166.67	27.36	1.321	0.121	2.5	100.27
							Mean ± SD	99.68±0.56

CONCLUSION:

A simple and sensitive spectroscopy method for quantitative determination of Linagliptin in API and Pharmaceutical dosage form was developed.

Linagliptin showed maximum absorbance at 295 nm in distilled water solution. It has linear response in the entire range of 1-10 µg/ml with correlation coefficient of 0.998. The linear regression equation obtained is y = 0.0903x + 0.0278. The method has good precision within 2% RSD and average accuracy as 100.51±3.32. No significant interference was observed in the absorbance of the drug in presence of common excipients.

The method was statistically validated according to ICH. The summary of validation parameters are shown in the table 8.

Table 9: Summary of Validation

S. No.	**Validation Parameters**	**Observation**
1	**Range**	1-10 µg/ml
2	**Regression Equation**	y = 0.0903x + 0.0278
3	**Correlation Coefficient**	0.998
4	**Precision (%RSD)**	

	i. Repeatability **ii. Intra-Day Precision** **iii. Inter-Day Precision**	1.38 0.91 0.86
5	**Accuracy (% Recovery)**	100.69±0.41
6	**Specificity(% Interference)**	0.63

In conclusion, the developed spectroscopy method is simple, accurate, and precise can be used for routine analysis of Linagliptin in either API or in tablet dosage form.

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