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Original Article

Development and Validation of Five Simple UV-Spectrophotometry Methods for Estimation of Anagliptin in Bulk and *in-house* Tablets

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ABSTRACT

Anagliptin is a Dipeptidyl peptidase-4 (DPP-4) inhibitor used in the treatment of diabetes. Five simple, specific, sensitive, rapid and economical UV- Spectrophotometry methods have been established for the determination of Anagliptin in bulk and *in-house* tablets. All five methods of UV-Spectrophotometry based upon Zero Order, First Order and Second Order derivative Spectrophotometry have been established considering amplitude and Area under Curve of the spectrum. In all five methods, Anagliptin obeyed linearity in the concentration range of 2-8 µg/mL with correlation coefficient (r^2>0.999). The % amount of drug estimated in the developed methods was found to be good agreement with label claimed in *in-house* tablet formulation. All the methods were validated as per International conference on Harmonization (ICH) guidelines. All these proposed methods were proved to be linear, accurate, precise and rugged and also adequately sensitive.

Key words: Anagliptin, UV-spectro photometry, Area Under Curve technique, Derivative Spectro photometry, Validation.

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INTRODUCTION

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. UV-spectrophotometry methods acquire a significant place in pharmacopoeia.[1] the advantages of these methods are, low time and manpower consumption. The precision of these methods is also excellent. The use of UV–Vis Spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years.[2,3]

Derivative spectroscopy uses first or upper derivatives of absorbance with respect to wavelength for qualitative examinations and estimations. The use of derivative spectrometry is not limited to special cases, but may be of advantage whenever quantitative study of normal spectra is problematic. Disadvantage is also associated with derivative methods; the differential degrades the signal-to-noise ratio, so that some form of smoothing is required in conjunction with differentiation.[4,5]

The Area under Curve (AUC) method engages the calculation of the integrated value of AUC with respect to the wavelength between the two selected wavelengths $\lambda 1$ and $\lambda 2$. Selection of wavelength range is on the basis of repeated observations so as to get the linearity between AUC and concentration.[6]

Anagliptin (AGP), chemically N-[2-[[2-[(2S)-2-Cyanopyrrolidin-1-yl]-2-oxoethyl]amino]-2-methylpropyl]-2-methylpyrazolo[1,5-a]pyrimidine-6-carboxamide is a dipeptidyl peptidase-4 inhibitor. It is used in the treatment of type 2 diabetes mellitus.[7]

Dipeptidyl peptidase- 4 (DPP-4) inhibitors are a new class of oral anti hyper glycemic agent that enhance insulin secretion by reducing degradation of endogenous in cretins such as glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP).[8,9]

A review of literature revealed that one UV-Spectrophotometric method has been reported.[10] In this work, five simple, economical, and rapid spectrophotometric methods have been established for the quantification of anagliptin in bulk material and *in-house* tablets.

The developed methods were validated for accuracy, precision, ruggedness, and sensitivity as per ICH guidelines.[11]

MATERIAL AND METHODS

Chemicals and Reagents

Pharmaceutical grade Anagliptin working standards were obtained as generous gifts from Glenmark Pharm., Nashik, India.

Instrumentation

1. Spectrophotometer	UV-2450 and UV-1601 Shimadzu, Japan
Software	UV Probe 2.21
Sample cell	1 cm matched quartz cell
Lamp	Deuterium Lamp
Wavelength range	200 - 400 nm
Detector	Silicon Photodiode, Photomultiplier R-928
Scan speed	Medium
Spectral slit width	1.0 nm
2. Weighing Balance	Shimadzu AUX – 120

Preparation of Stock Standard Solution

The stock standard solution was prepared by dissolving 10 mg of AGP in 100 mL of water to acquire a concentration of 100 µg/mL. The working standards were prepared by dilution of the stock standard solution.

Selection of appropriate wavelength for analysis for AGP

For Method I (Zero order UV-Spectrophotometry using AUC technique) an appropriate concentration of 4 µg/mL from stock standard solution was prepared and scanned in the UV range 400–200 nm; AGP demonstrated a maximum absorbance at 248 nm. From the spectrum of AGP, the AUC between a wavelength range 240.50-253.50 nm was considered for the analysis. While, in Method II (First order derivative UV- spectrophotometry using amplitude), the zero order absorption spectrum of AGP was derivatized in first order using software UV Probe

2.21 with delta lambda 10 and scaling factor 10 and the amplitudes was recorded at 258 nm; For Method III (First order derivative UV- spectrophotometry using AUC technique); AUC between the two wavelengths 251.00-266.50 nm was selected for analysis.

For Method IV (Second order derivative UV- Spectrophotometry using amplitude); the zero order absorption spectrum of AGP was derivatized into second order using software UV Probe 2.21 with delta lambda 10 and scaling factor 10 and the amplitudes were recorded at 248 nm while for Method V (Second order derivative UV- spectrophotometry using AUC technique); AUC between the two wavelengths 241.50 and 255.00 nm was recorded for analysis.

The selection of wavelengths in all methods is shown in Figure 1.

Preparation of Sample Solution

Due to the unavailability of Anagliptin tablets in the local Indian market, *In-house* tablets were formulated *via* direct compression technique using commonly used excipients containing 100 mg of drug per tablet.

To determine the content of *in-house* prepared tablets of AGP; twenty tablets were weighed and powdered. An amount of powdered drug equivalent of 10 mg of AGP was weighed accurately, transferred into 100 mL volumetric flask containing 50 mL of water, sonicated for 20 min, and the solution was diluted up to 100 mL with the same solvent and filtered through Whatman filter paper (No. 41). From the filtrate, measured volume was taken and diluted with water to get the final concentration of 5 μg/mL for all the methods. The responses were measured as described above and concentrations in the sample were determined from respective linearity equations.

Validation of Methods

The proposed method was validated as per ICH guidelines.[11]

Linearity

For linearity study, seven solutions of Anagliptin of different concentrations (2, 3, 4, 5, 6, 7 and 8 μg/mL) were prepared using stock standard solution, analyzed by proposed methods and the obtained data were utilized to plot calibration curves.

Accuracy

The accuracy of all methods was evaluated through recovery experiments. To the pre-analyzed sample solutions of concentration 3 μg/mL; a known amounts of stock standard solutions were added at different levels, that is, 80%, 100%, and 120%. The solutions were re-analyzed by the proposed methods. The experiments were performed for three times at each level for each method.

Precision

The precision of the methods can be studied as repeatability; intra-day variation; inter-day variation studies.

Repeatability was studied by analyzing AGP (5 μg/mL) for six times. Intra-day precision was determined by analyzing the 4, 5 and 6 μg/mL of AGP for three times in the same day. Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days.

Sensitivity

The sensitivity of proposed methods was estimated in terms of Detection limit (DL) and Quantification Limit (QL) which were calculated using formulae "QL = 10 ×N/B" and "DL = 3.3 ×N/B," where "N" is standard deviation of the absorbance or amplitudes or peak areas of the anagliptin (n = 3), taken as a measure of noise, and "B" is the slope of the corresponding calibration curve.

Ruggedness

According to the USP definition of ruggedness, the method is repeatedly performed under different test conditions to examine the effects of some "non-procedure-related" factors, such as laboratories, instruments, analysts, reagents, and time, without changing the "procedure-related"

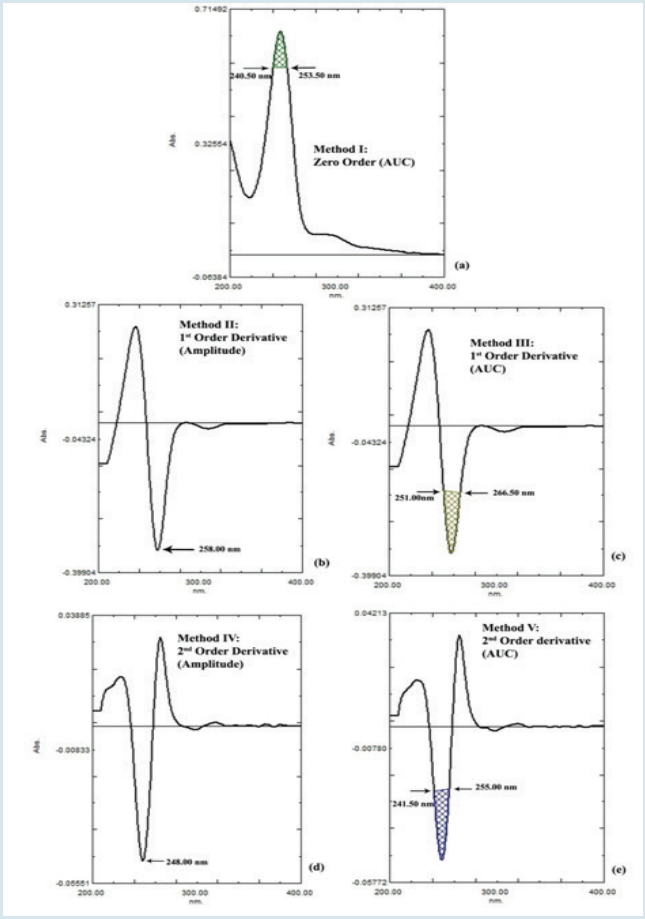

Figure 1: a) Zero order spectrum of Anagliptin showing AUC between selected wavelength range b) 1st order derivative spectrum c) 1st order derivative spectrum showing AUC between selected wavelength range d) 2nd order derivative spectrum e) 2nd order derivative spectrum showing AUC between selected wavelength range.

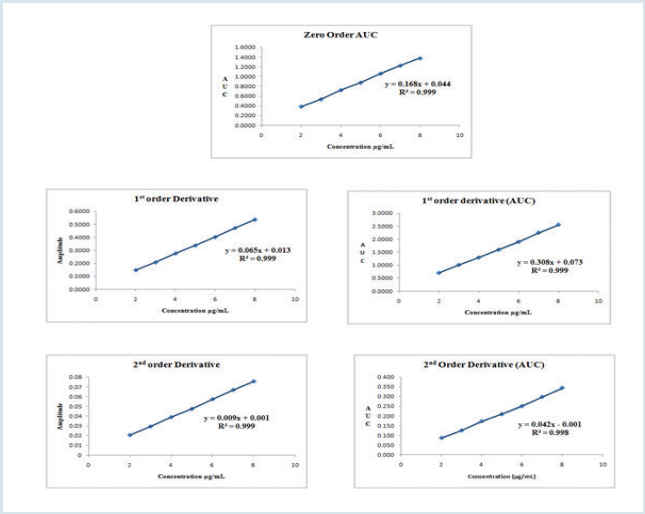

Figure2: Linearity curves.

Table 1: Accuracy Studies

Drug	Methods	Initial amount [µg/mL]	Amount added [µg/mL]	Amt recovered [µg/mL, n=3]	% Recovery	% RSD
		3	2.4	5.39	99.7	0.997
		3	3	5.97	99.15	0.902
	I	3	3.6	6.58	99.69	0.752
		3	2.4	5.37	99.08	0.819
		3	3	5.99	99.79	0.583
	II	3	3.6	6.59	99.72	0.369
		3	2.4	5.38	99.22	0.866
Anagliptin		3	3	5.97	99.14	0.996
	III	3	3.6	6.57	99.4	0.573
		3	2.4	5.37	98.92	1.504
		3	3	5.97	98.89	0.749
	IV	3	3.6	6.58	99.49	1.089
		3	2.4	5.38	99.17	0.824
		3	3	5.96	98.67	0.547
	V	3	3.6	6.58	99.44	0.742

n = number of estimations

Table 2: Precision studies

Drug	Methods	Concentration [µg/mL]	Intra-day *n=3*	%RSD	Inter-day *n=3*	%RSD
		4	100.27	1.155	99.73	1.144
	I	5	99.46	0.760	99.19	0.896
		6	99.83	0.837	99.40	0.774
		4	99.88	1.198	99.27	0.972
	II	5	99.78	0.731	99.38	0.846
		6	99.95	0.633	99.68	0.577
		4	99.65	1.149	99.35	0.944
Anagliptin	III	5	99.50	0.755	99.43	0.532
		6	99.26	0.580	99.56	0.435
		4	99.22	0.870	99.29	1.363
	IV	5	99.31	1.067	99.80	1.171
		6	100.56	0.656	99.38	0.868
		4	99.25	0.942	99.65	0.845
	V	5	99.65	0.742	99.42	0.564
		6	99.12	0.541	99.22	0.623

method parameters.[12]

Ruggedness of proposed methods was performed to examine effect of instruments and analysts. For this study AGP (5 µg/mL) was analysed by proposed methods using two different analyst and two different UV-spectrophotometers (UV-2450 and UV-1601, Shimadzu) restraining similar operational and environmental conditions.

RESULTS AND DISCUSSION

Method Validation

Developed methods were validated as for Linearity, accuracy, precision, ruggedness and sensitivity as per the ICH guidelines.

Linearity

From the linear regression data, it is clear that the calibration curves showed good linear relationship over the concentration range of 2-8 µg/mL for AGP. The calibration curves are shown in Figure 2.

Accuracy

The solutions were re-analyzed by proposed methods; results of recovery studies are reported in Table 1. The % RSD values that were determined and found to be less than 2 indicate that the methods are accurate.

Precision

The precision of the developed methods was expressed in terms of % relative standard deviation % RSD. These results showed reproducibility of the assay. The % RSD values were found to be less than 2, so this indicates that the methods are precise for the determination of the AGP in

Table 3: Ruggedness studies

Factors		Method				
		I	II	III	IV	V
Analyst I	% Amount Found	99.54	99.70	99.81	98.82	99.24
	% RSD *(n=6)*	0.655	0.979	0.541	0.961	0.751
Analyst II	% Amount Found	99.61	100.28	99.50	98.96	99.58
	% RSD *(n=6)*	0.704	0.683	0.764	0.949	0.654
UV-2450	% Amount Found	99.47	99.65	99.21	99.74	99.16
	% RSD *(n=6)*	0.541	0.632	0.744	0.455	1.023
UV-1601	% Amount Found	98.25	98.68	99.02	99.20	98.54
	% RSD *(n=6)*	0.654	0.986	0.532	0.624	0.844

n- Number of repetitions

in-house tablets. Results are shown in Table 2.

Sensitivity

In Method I, Method II, Method III, Method IV and Method V, DL for AGP was found to be 0.19 µg, 0.20 µg, 0.11 µg, 0.21 µg, and 0.24 µg, respectively While QL in Method I, Method II, Method III, Method IV and Method V, were found to be 0.58 µg, 0.63 µg, 0.34 µg, 0.66 µg, and 0.79 µg, respectively.

Ruggedness

Ruggedness was determined for solutions of AGP. The results are in the acceptable range that is % RSD values< 2 for all the methods as shown in Table 3. The results showed no statistical differences between different operators and instruments suggesting that the developed methods are rugged.

*Analysis of **in-house** Tablets*

The percentage amounts of AGP estimated from tablet formulation using Method I, II, III, IV and V were found to be 99.10%, 99.43%, 99.37%, 99.85%, and 99.32, respectively. The % amount estimated from tablet formulation indicates that there is no interference from excipients present in it.

CONCLUSION

The methods that were developed for the determination of Anagliptin are based on UV-Spectrophotometric absorbance, Derivative and Area Under Curve techniques. The methods were validated and found to be simple, sensitive, accurate, and precise. Hence, it can be used successfully for routine analysis of pharmaceutical dosage forms of Anagliptin.

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CONFLICT OF INTEREST

No conflicts of interest.

ABBREVIATION USED

AGP: anagliptin; **AUC:** Area Under Curve; **%RSD:** % Relative Standard Deviation; **QL:** Quantification Limit; **DL:** Detection Limit.

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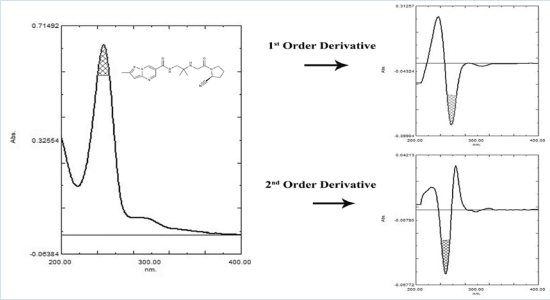
PICTORIAL ABSTRACT



SUMMARY

- Anagliptin is a Dipeptidyl peptidase-4 (DPP-4) inhibitor used in the treatment of diabetes.
- Five simple, specific, sensitive, rapid and economical UV- Spectrophotometry methods have been established for the determination of Anagliptin in bulk and *in-house* tablets.
- All the methods were validated as per International conference on Harmonization (ICH) guidelines.
- All these methods were proved to be linear, accurate, precise and rugged and also adequately sensitive.
- These methods can be used successfully for routine analysis of pharmaceutical dosage forms of Anagliptin.

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Dr. Atul A. Shirkhedkar: Is working as Vice-principal and Head of Department of Pharmaceutical Chemistry at R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule (MS), India. His scientific contribution to the field of drug analysis is globally recognized. He has published more than hundred research articles in international and national peer reviewed journals. He also authored few books on topics related to pharmacy field. He has more than 18 years of research experience in the field of drug analysis. So far 40 students have completed their M. Pharm. Thesis and supervising 4 students for doctoral program. He has also organized more 5 national conferences as a convener.