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Research Article

Simultaneous Estimation Of Vildagliptin And Pioglitazone In Bulk And Pharmaceutical Dosage Form By UV

Aishwarya Ubale[1]*, Monika Gopal Shinde[2], Punam Nivrutti Bandgar[3], Pradnya Prashant Shinde[3], Baban Subhash Satpute[3], Abhishek shashikant Kambale[3]

[1]Department-Pharmaceutical Chemistry. Sahyadri college of Pharmacy, Methwade Tal. Sangola, Dist. Solapur
[2]Associate professor at Sahyadri college of Pharmacy, Methwade Tal. Sangola, Dist. Solapur
[3]Sahyadri college of Pharmacy, Methwade

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ABSTRACT

A simple, precise, and accurate UV-Spectrophotometric simultaneous equation method for estimating Vildagliptin and Pioglitazone has been developed and validated in accordance with ICH criteria. The method requires solving simultaneous equations based on measuring absorbance at two wavelengths: 210 nm and 226 nm (λmax of Vildagliptin and Pioglitazone) in Water: Acetonitrile (50:50, v/v). Beer's law applies to both Vildagliptin and Pioglitazone at concentrations of 40-60 and 12-18 µg/ml, respectively. The percent recovery for both medications ranged from 98.88 to 100.42%, suggesting good accuracy. The procedures were accurate, with a relative % RSD of less than 2% for both medicines. The developed methods were validated in accordance with ICH requirements, and accuracy, precision, and other statistical analysis results were determined to be within acceptable limits. Thus, the method can be utilized for routine drug monitoring in industry, including bulk drug assays and commercial formulations.

INTRODUCTION

Vildagliptin, sold under the trade mark of Galvus, is a novel oral hypoglycaemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. It also available in combination – Galvus Met consists of Vildagliptin with Metformin are the active constituents. Vildagliptin (Galvus) was first synthesized in May 1998 and was named after Edwin B. Villhauer. It was discovered when researchers at Novartis examined adamantly derivatives that had proven to be very potent. The 'vil' in Vildagliptin was in recognition of Ed Villhauer's contribution. (https://en.wikipedia.org/wiki/Vildagliptin).

Vildagliptin is chemically (2R)-1-[2-[(3-Hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile (Fig.1) and CAS Number is 274901-16-5. Vildagliptin is used as monotherapy or in

***Corresponding Author:** Aishwarya Ubale
Address: *Department-Pharmaceutical Chemistry. Sahyadri college of Pharmacy, Methwade Tal. Sangola, Dist. Solapur*
Email : aishwaryaubale20@gmail.com

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combo with other drugs for the treatment of type 2 diabetes. [1, 2] This drug is well tolerated and is weight-neutral. Dipeptidyl-peptidase IV (DPP-4) inhibitors prevent the degradation of the incretins, glucagon- like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). This article gives a highlight on the activity of DPP-4 inhibitors in the human body and targets on their development and their significant physiological actions with related to the treatment of type 2 diabetes. [3, 4]

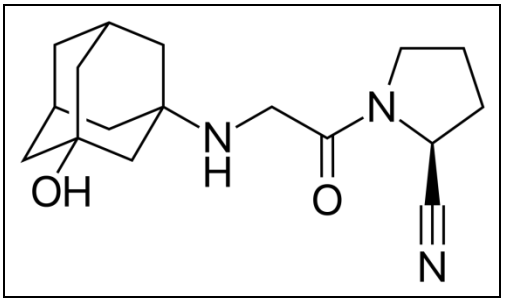

Figure 1. Structure of Vildagliptin

Pioglitazone is a member of the class of thiazolidenediones that is 1,3-thiazolidine-2,4-dione substituted by a benzyl group at position 5 which in turn is substituted by a 2-(5-ethylpyridin-2-yl) ethoxy group at position 4 of the phenyl ring. It shows hypoglycemic activity. It has a role as an insulin-sensitizing drug, an EC 2.7.1.33 (pantothenate kinase) inhibitor and a xenobiotic. It is a member of thiazolidenediones, aromatic ether and a member of pyridines. [5] Pioglitazone triggers the nuclear peroxisome proliferator activated receptor-γ (PPAR-γ), which assist to the increased transcription of various proteins regulating glucose and lipid metabolism. [6,7] These proteins escalates the post-receptor actions of insulin in the liver and peripheral tissues, which assist to improved glycemic control with no increase in the endogenous secretion of insulin. [8, 9] The drug has been well tolerated by adult patients of all ages in clinical studies. Oedema has been reported with mono therapy, and pooled data have shown hypoglycemia in 2 to 15% of patients after the addition of pioglitazone to sulphonylurea or insulin treatment. There have been no reports of hepatotoxicity. [10] Pioglitazone is an orally administered insulin sensitizing thiazolidinedione agent that has been developed for the treatment of type 2 diabetes mellitus. [11]

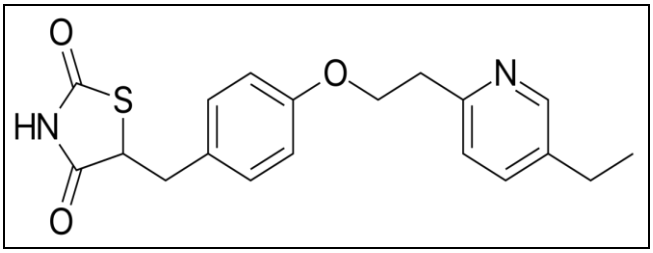

Figure 2 Structure of Pioglitazone

MATERIALS & METHODS:

Chemicals and reagents:

Dalton Pharma Chem, located in Vadodara, Gujarat, India, generously supplied us with a gift sample of Vildagliptin and Pioglitazone as our reference standard throughout the course of our study. Loba Chemie. Pvt. Ltd., Mumbai, India supplied all other solvents, chemicals, and excipients (specificity) made used in this research.

UV Instrumentation:

For the experiment, a Shimadzu UV visible spectrophotometer (double beam) with a paired quartz cell having a 1 cm path length (UV-1800, UV Probe, Shimadzu Corporation, Kyoto Japan) was utilized. Weighing was carried out using the Digital electronic balance Citizen & Contact (CY 220 & CY 223).

Method Parameters:

Diluent: Water : Acetonitrile (50: 50%, v/v)

Wavelength: λ1 = 210 nm; λ2 = 226 nm

Standard Preparation:

Vildagliptin Standard Stock Solution-I (VSSS-I):

Initially Prepare a Standard Stock Solution (VSSS-I) of by adding 5 mg of Vildagliptin in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Vildagliptin = 500µg/ml).

Pioglitazone Standard Stock Solution-II (PSSS-II):

Then prepare a Standard Stock Solution (PSSS-II) of Pioglitazone by adding 15 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Pioglitazone = 1500 µg/ml). Then add 1.0 ml of VSSS-I & 0.1 ml PSSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Vildagliptin= 50µg/ml &Pioglitazone = 15µg/ml).

Selection of Wavelength:

50µg/ml of VDG Working Standard and 15µg/ml of PIO Working Standard were scanned in the UV range of 190-400 nm. Both the spectrum was recorded. From the spectra wavelengths 210 nm (λmax of VDG) and 226 nm (λmax of PIO) were selected for analysis of both drugs using simultaneous method. (λ1-210 nm and λ2-226 nm). The absorbance at λ1 and λ2 was measured and the concentration was calculated using following formula;

Cx= (A2ay1-A1ay2)/(ax2ay1-ax1ay2)

Cy= (A1ax2-A2ax1)/(ax2ay1-ax1ay2)

Where,

Cx and Cy are the concentrations of Vildagliptin and Pioglitazone, respectively,

A1 and A2 are the absorbances of sample at λ1 and λ2, respectively,

ax1 and ax2 are the absorptivity of Vildagliptin at λ1 and λ2, respectively,

ay1 and ay2 are the absorptivity of Pioglitazone at λ1 and λ2, respectively.

Validation:-

Linearity:

i. 5 samples of varying concentrations ranging from 80% to 120% were made.
ii. The concentrations are given below

Table 1: Concentration for linearity Study.

X ml of VSSS-I	Y ml of PSSS-II	Diluted to	Conc. of VDG (µg/ml)	Conc. of PIO (µg/ml)
0.8	0.08	10 ml	40	12
0.9	0.09	10 ml	45	13.5
1.0	0.10	10 ml	50	15
1.1	0.11	10 ml	55	16.5
1.2	0.12	10 ml	60	18

iii. The sample preparations are given as below;

X ml of VSSS-I and Y ml of PSSS-II was diluted to 10 ml.

LOD/ LOQ:

Can be calculated by using AVONA Technique.

LOD= (3.3× Std Error of Intercept)/(Coefficient of X variable 1)

LOD= (10× Std Error of Intercept)/(Coefficient of X variable 1)

c. Repeatability :

A single sample was prepared as described and 6 Measurements were made from same sample; checked for RSD.

d. Accuracy:

i. Samples were made of 80%, 100% and 120% concentration as per Table 1.
ii. Samples were measured in triplicate to calculate % RSD.
iii. % recovery was also calculated.

% Conc	VDG Conc. (µg/ml)	PIO Conc. (µg/ml)
80	40	12
100	50	15
120	60	18

e. Intra- & Inter-day Precision:

- The working standard and drug product samples were freshly prepared and analysed in morning and evening for Intra-day precision.

- The same working standard and drug product were used for analysis on 2nd day for inter-day precision.
- % RSD for Assay was calculated for the confirmation of precision.

RESULTS AND DISCUSSION:

Selection of analytical wavelength:

Wavelength of maximum absorption λmax was determined by scanning 50µg/ml of VDG Working Standard and 15µg/ml of PIO by UV-Visible double beam spectrophotometer from 200 to 400 nm using diluent as blank (λ1-210 nm and λ2-226 nm).

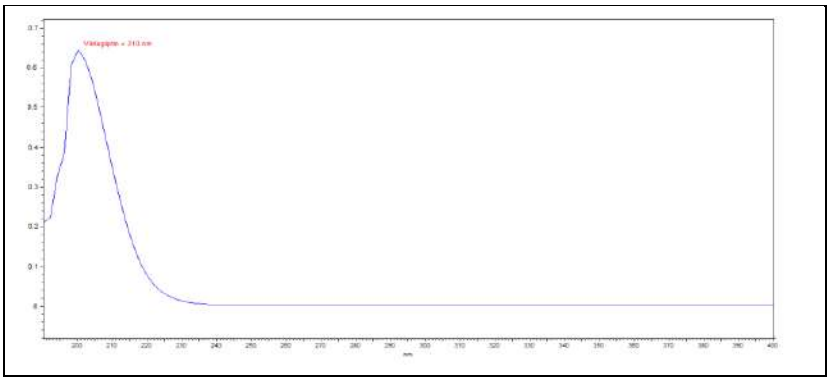

Figure 3: Vildagliptin showed λmax at 210 nm.

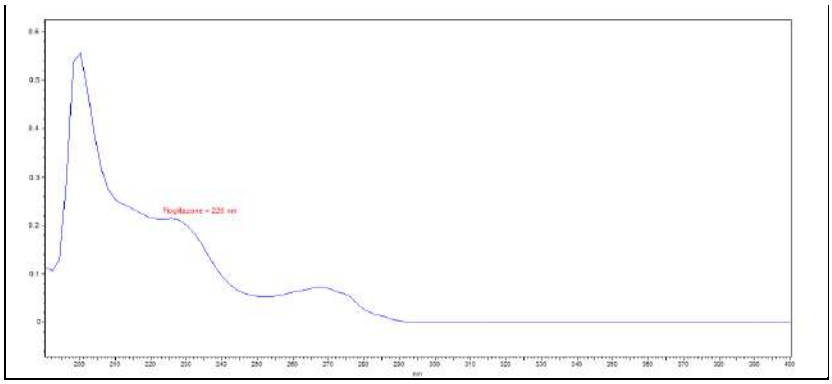

Figure 4: Pioglitazone shows λmax at 226 nm.

Procedure for Analysis of Tablet Formulation:

Then add 1.0 ml of VSSS-I & 0.1 ml PSSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Vildagliptin = 50µg/ml & Pioglitazone = 15µg/ml).

The method was validated as per the ICH Q2 (R1) guidelines.

Table 2: Analysis of Tablet Formulation by proposed methods:

Sample	Vildagliptin		Pioglitazone	
	Conc. (µg/ml)	% Assay	Conc. (µg/ml)	% Assay
DP-1	49.83	99.66	14.74	98.27
DP-2	49.62	99.24	14.85	99.00
DP-3	50.03	100.06	14.68	97.87
DP-4	49.88	99.76	14.79	98.60
DP-5	49.78	99.56	14.89	99.27
AVG		99.66	**AVG**	98.60
STDEV		0.30	**STDEV**	0.56
RSD		0.30	**RSD**	0.57

Validation:-

The proposed method was validated according to ICH guidelines for validation of analytical procedures in order to determine the linearity, Range, LOD and LOQ. precision and accuracy. [12-15]

1. Linearity:

Its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The calibration curve was constructed between concentration verses absorbance. Different concentrations of Vildagliptin& Pioglitazone varying concentrations ranging from 40 to 60 µg/ml and 12-18 µg/ml were made. The sample preparations are given as below; X ml of Montelukastand Y ml of Fexofenadine was diluted to 10 ml.

Table 3: Different concentration of Vildagliptin.

Vildagliptin		
% Level	**Concentration (µg/ml)**	**Absorbance**
80	40	0.286
90	45	0.320
100	50	0.353
110	55	0.387
120	60	0.426

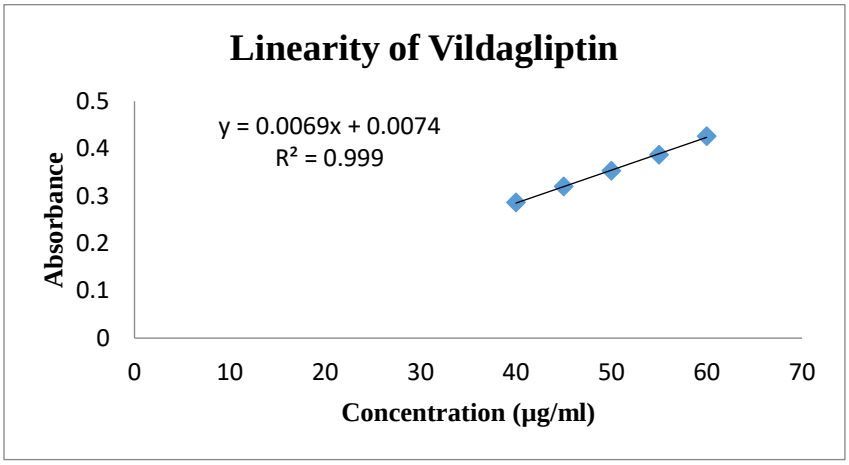

Figure 5: Linearity of Vildagliptin

Table 4: Different concentration of Pioglitazone.

Pioglitazone		
% Level	**Concentration (µg/ml)**	**Absorbance**
80	12	0.176
90	13.5	0.199
100	15	0.222
110	16.5	0.242
120	18	0.265

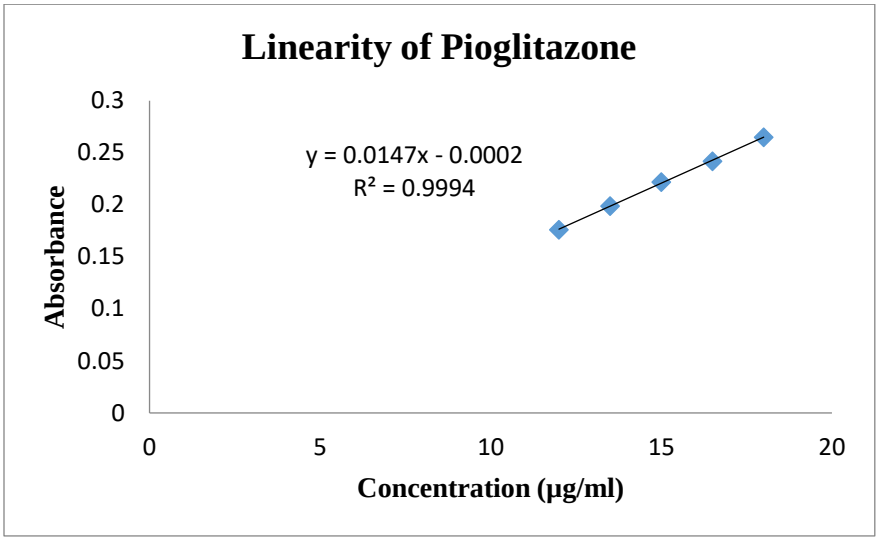

Figure 6: Linearity of Vildagliptin

The proposed method was found to be linear in the range of 40-60 and 12-18 µg/ml with correlation coefficient 0.999 and 0.999 for Vildagliptin & Pioglitazone respectively. The calibration curve was constructed between concentration verses absorbance. It is shown into the Figure 5 and 6 resp. and result of linearity study shown in Table 3, 4.

2. Range:

40-60 and 12-18 µg/ml for Vildagliptin& Pioglitazone.

3. Limit of detection (LOD) and Limit of quantification (LOQ):

The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determination of detection of limit and quantification limits.

Detection limit= 3.3 σ/s

Quantification limit= 10 σ/s

Where, σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) data are given in Table 5.

The LOD and LOQ for Vildagliptin& Pioglitazone were determined according to ICH guideline

LOD = 3.3 σ / S LOQ = 10 σ / S

Where,

σ = Standard deviation of the y intercept of calibration curves

S = Slope of the calibration curve

Table 5: LOD and LOQ of Vildagliptin & Pioglitazone.

Drug	LOD (µg/ml)	LOQ (µg/ml)
Vildagliptin	3.08	9.323
Pioglitazone	0.68	2.06

The LOD and LOQ of Vildagliptin were discovered as 3.08 and 9.323 µg/ml, individually, whereas for Pioglitazone was 0.68 and 2.06 µg/ml, separately.

Limit of Detection and Limit of Quantitation was calculated on the basis of Slope and Standard deviation of response.

4. Repeatability:

Table 5: Repeatability of Vildagliptin & Pioglitazone

Sample ID	Vildagliptin ABS	Pioglitazone ABS
100% Rep 1	0.356	0.219
100% Rep 2	0.352	0.222
100% Rep 3	0.354	0.221

100% Rep 4	0.357	0.217
100% Rep 5	0.359	0.219
100% Rep 6	0.358	0.218
AVG	0.356	0.219
STDEV	0.003	0.00
RSD	**0.73**	**0.85**

5. Precision:

The precision of an analytical method is expressed as % RSD of a series of measurements which should be less than 2 %.(Table 6).

Table 6: Intraday and Interday precision for Vildagliptin & Pioglitazone

Condition	Sample ID	Interval	Vildagliptin		Pioglitazone	
			Conc. (µg/ml)	% Assay	Conc. (µg/ml)	% Assay
Intraday	WS	Mrng	50.00	-	15.00	-
	DP	Mrng	49.83	99.66	14.74	98.27
	WS	Evng	49.92	-	14.95	-
	DP	Evng	49.75	99.66	14.63	97.86
Interday	WS	Day 2	49.93	-	14.91	-
	DP	Day 2	49.62	99.38	14.55	97.59
			% RSD	0.16	% RSD	0.35

The % RSD (< 2.0) values obtained in the intraday precision study indicate the repeatability of the data and the interday precision results were also found to be satisfactory. The results of the precision trials, which were expressed as a percentage RSD and ensured that ICH recommended limits, were met (<2). The outcome demonstrates that all of the proposed methods had great repeatability and reduced intra-and inter-day changeability

6. % Recovery (Accuracy):

Accuracy of the methods was determined at three different concentration levels i.e.80%, 100% and 120% in triplicate for each drug as per ICH guidelines. Method accuracy was evaluated as the percentage of recovery of known amounts of Vildagliptin & Pioglitazone. It is performed at spike concentration that was 80%, 100% and 120%.

Table 7. % Recovery data of the Vildagliptin.

Vildagliptin								
% Level	Reps	Spiked Conc. (µg/ml)	Abs	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	RSD
80	Rep 1	40.00	0.286	40.17	100.42	100.07	0.35	0.35
	Rep 2	40.00	0.284	39.89	99.72			
	Rep 3	40.00	0.285	40.03	100.07			
100	Rep 1	50.00	0.353	49.58	99.16	99.16	0.28	0.28
	Rep 2	50.00	0.352	49.44	98.88			
	Rep 3	50.00	0.354	49.72	99.44			
120	Rep 1	60.00	0.426	59.83	99.72	100.11	0.36	0.36
	Rep 2	60.00	0.428	60.11	100.19			
	Rep 3	60.00	0.429	60.25	100.42			

Table 8. % Recovery data of the Pioglitazone.

Pioglitazone								
% Level	**Reps**	**Spiked Conc. (µg/ml)**	**Abs**	**Amount Recovered (µg/ml)**	**% Recovery**	**AVG**	**STDEV**	**RSD**
80	Rep 1	12.00	0.176	12.05	100.46	99.51	0.87	0.88
	Rep 2	12.00	0.174	11.92	99.32			
	Rep 3	12.00	0.173	11.85	98.74			
100	Rep 1	15.00	0.222	15.21	101.37	101.22	0.26	0.26
	Rep 2	15.00	0.222	15.21	101.37			
	Rep 3	15.00	0.221	15.14	100.91			
120	Rep 1	18.00	0.265	18.15	100.84	100.71	0.58	0.58
	Rep 2	18.00	0.266	18.22	101.22			
	Rep 3	18.00	0.263	18.01	100.08			

The proposed procedures' accuracy was determined using the standard addition method for recovering analyte's. For each medicine, recovery rates in the experiments ranged from 98.88 % to 100.42 % for Vildagliptin and 98.74 to 101.37 % for Pioglitazone, proving the efficacy of the established protocols (Table 7, 8) Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of Vildagliptin and Pioglitazone in marketed formulations.

CONCLUSION:

The results and statistical parameters show that the suggested UV spectrophotometric Method is simple, fast, specific, accurate, and precise. Furthermore, the devised UV-spectrophotometric method requires minimal sample preparation, has a wide concentration range, and is sensitive. There is no statistically significant variation between the methods. As a result, this method can be used to determine Vildagliptin and Pioglitazone in bulk or dosage formulations that are free of commonly used excipients and related compounds.

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