Development and Validation of a UV-Spectroscopic Analytical Method for Estimating Vildagliptin in Bulk and Pharmaceutical Formulations

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Abstract:

A simple, sensitive, specific, spectrophotometric, and cost-effective analytical method has been developed to estimate vildagliptin in both pure and pharmaceutical formulations quantitatively. The stock solution and subsequent dilutions were prepared using 0.1% NaOH, and the absorption maximum was observed at 216 nm. The drug adhered to Beer-Lambert's Law within a 5-10 µg/ml concentration range, demonstrating a correlation coefficient (R^2) of 0.996. The method's precision and accuracy were assessed through replicate analyses of standard solutions and with pharmaceutical preparations. The developed method was validated according to ICH guidelines, and the method proved to be precise, accurate, and robust, making it suitable for routine analysis of vildagliptin in pharmaceutical products.

Keywords: *Vildagliptin, Estimation, Validation, Pharmaceutical formulations*.

1. INTRODUCTION

Vildagliptin chemically [(2s)-1-{2-[3-hydroxyadamantan-1-yl)-amino]-acetyl}-pyrolidine-2-carbanitrile] [Indian pharmacopoeia, 2022] Fig. 1, is a dipeptidyl peptidase -4 (DPP-4) [Sagar Kishor Savale, 2017]. (DPP-4) inhibitor represents a new therapeutic approach to the treatment of type 2 diabetes mellitus [Mohammed Ishaq, 2012]. It improves glycemic control by increasing the levels of incretin hormones, thereby enhancing insulin release and decreasing glucagon levels in the circulation [Herman, et al., 2005].

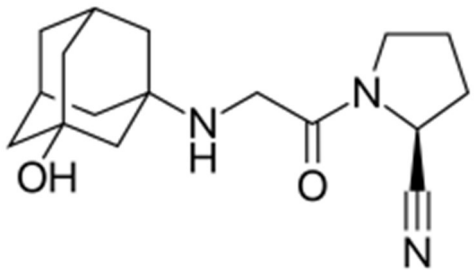

Fig. 1: Structure of vildagliptin

Vildagliptin is rapidly absorbed by following oral administration, with a maximum plasma concentration (C max) achieved in a time (T max) of 1.5h [He & Sadler, 2007] to 1.7h [Kasid & Ghorpade, 2013]. Vildagliptin exhibits low plasma protein binding of 9.3% and has an absolute bioavailability of 85 %. Vildagliptin is metabolized to pharmacologically inactive cyano (57%) and amide (4%) hydrolysis products in the kidney [Ghorpade, 2013]. A few methods were reported

for the determination of vildagliptin, in plasma [Pharne & Santhakumari, 2012], in the tablet dosage form in combination with metformin [Nandipati & Reddy, 2012], reports regarding the determination of vildagliptin impurities and synthetic intermediates [Ramzia & Bagary, 2011].

Literature review for vildagliptin analysis revealed that few methods were found based on different techniques, such as the UV-visible spectroscopic method [Usharani Gundala, 2013, Sheetal Vishnudas et al., & Tadikonda Rama Rao et al., 2023], LC-MS [Uber CP et al.; Abdul Shakoor et al., 2009 & Amanda Thomas Barden., 2011], RP-HPLC method [Santhosha, Ravindranath, 2012 & Thangabalan Boovizhikannan, 2013], HPTLC method [Santosh R. Butle, Padmanabh B. Deshpande], and UPLC- MS/MS [EI- Badary RI, EI Kady EF& Chinta Srinivas et al., 2021].

The present work aimed to develop a simple, sensitive, specific, precise and accurate UV-visible spectrophotometric method for the determination of vildagliptin in its pure form and pharmaceutical formulations.

2. MATERIALS

Instruments:

Absorption spectral measurements were carried out with a Systronics 2202 UV-visible spectrophotometry and sonication, a Branson 2510 sonicator was used.

Chemicals:

Vildagliptin was obtained as a gift sample from Industrial Estate, Bangalore which was used as such for further analytical development. Vildagliptin tablet formulations (50 mg) were procured from local pharmacies. Sodium hydroxide (AR grade) and in house prepared distilled water were used for the study.

3. METHODS

3.1. Preparation of Stock Solution:

100 mg of vildagliptin was accurately weighed and transferred to a clean 100 ml volumetric flask. Approximately 20 ml of 0.1 N NaOH was added along the sides of the flask. The solution was mixed thoroughly until complete solubilization was achieved. Then, the solution was diluted to a final volume of 100 ml with 0.1 N NaOH to obtain a concentration of 5 mg/ml.

3.2. Determination of absorbance maxima:

The absorbance maximum was determined by transferring 1 ml of the standard stock solution of vildagliptin to a 100 ml volumetric flask. 20 ml of 0.1 N NaOH was added, and the flask was filled to 100 ml with 0.1 N NaOH to achieve the concentration of 5 µg/ml. The UV spectrum was then obtained by scanning from 400 nm to 200 nm.

3.3. Linearity and range:

Aliquots of 5, 6, 7, 8, 9, and 10 ml of the standard stock solution were transferred into separate clean 100 ml volumetric flasks. Then, 10 ml of 0.1 N NaOH was added to each flask, mixed well, and subsequently diluted and made up to the mark with 0.1 N NaOH to achieve concentrations of 5, 6, 7, 8, 9, and 10 µg/ml of vildagliptin. The absorbance of each solution was measured at 216 nm, and a calibration curve was constructed by plotting drug concentration against absorbance.

3.4. Precision:

Twenty tablets were accurately weighed and ground into a fine powder using a clean mortar and pestle. A quantity of the tablet powder equivalent to 100 mg was weighed and transferred to a 100 ml volumetric flask. Then, 20 ml of 0.1 N NaOH was added, and the mixture was sonicated for 5

minutes. The solution was diluted to 100 ml with 0.1 N NaOH and filtered using Whatman filter paper. Subsequently, 10 ml of the filtrate was pipetted into another 100 ml volumetric flask, diluted, and mixed. The absorbance was measured at 216 nm using a UV-visible spectrophotometer. This procedure was repeated six times.

3.5. Accuracy:

An amount of tablet powder equivalent to approximately 50 mg of vildagliptin was weighed and transferred to a 100 ml volumetric flask. Then, 20 ml of 0.1 N NaOH was added, and the mixture was sonicated for 3 minutes. Aliquots of 25, 75, and 100 ml of the stock solution were added separately to achieve varying accuracy levels. The mixture was well mixed, and the volume was made to 100 ml with 0.1 N NaOH. The solution was filtered, and then 5 ml of the filtrate was pipetted into another 100 ml volumetric flask, diluted, and mixed well. The absorbance was measured at 216 nm. The procedure was repeated in triplicates to perform accuracy levels of 50%, 100%, and 150%.

3.6. Limit of detection (LOD) and Limit of quantitation (LOQ):

The limit of detection (DL) is defined as the lowest concentration of analyte that can be detected but not quantified. It can be calculated using the formula: $DL=3.3\sigma/S$.

The limit of quantitation (QL) is the lowest concentration of analyte that can be quantitatively determined with acceptable precision and accuracy. It is calculated as: $QL=10\sigma/S$

where σ is the standard deviation of the response and S is the slope of the calibration curve. The slope can be determined from the calibration curve, and σ can be estimated through various methods, such as the deviation of the blank.

3.7. Ruggedness:

Ruggedness refers to the reproducibility of test results under varied but controlled conditions, such as different laboratories or analysts. It is assessed by having different analysts perform the analysis on aliquots of the same sample.

3.8. Robustness:

Robustness is a measure of an analytical method's ability to remain unaffected by small, intentional variations in method parameters, indicating its reliability during normal use.

3.9. Stability of the sample solution:

Stability studies are considered critical in many analytical procedures, with a focus on the integrity of the equipment, electronics, analytical operations, and samples. Parameters for stability tests should be established based on the specific procedure being validated.

4. RESULTS AND DISCUSSION

4.1. Determination of absorption maximum:

Vildagliptin is a UV-absorbing compound with specific chromophores that enable its quantification using UV spectroscopic methods. The absorbance maximum was determined by dissolving 5 µg/ml of vildagliptin in 0.1 N NaOH and the solution was scanned from 400 to 200 nm using a UV-visible spectrophotometer. It was revealed by the absorption spectrum (Fig. 2) that the λ max for vildagliptin is 216 nm in 0.1 N NaOH.

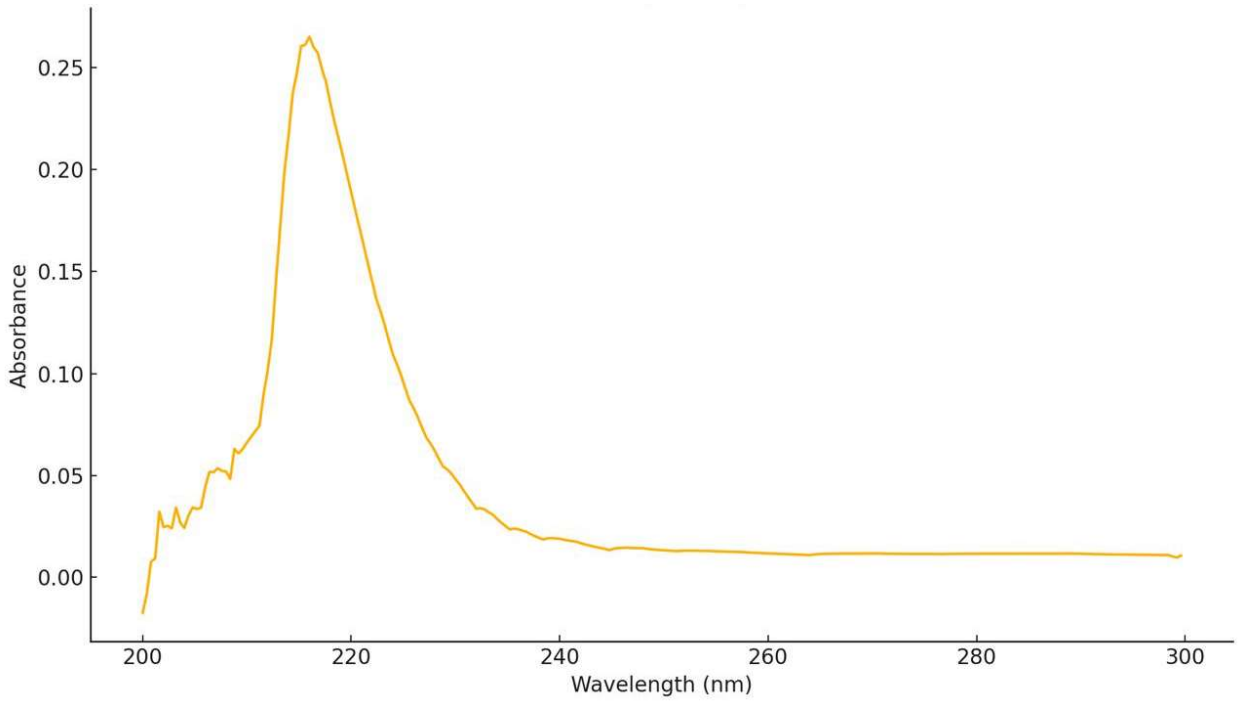

Fig. 2: UV-Spectrum of vildagliptin

4.2. Linearity and range:

A calibration curve was established for vildagliptin in the concentration range of 5-10 µg/ml using a series dilution method with 0.1 N NaOH. The absorbance for each concentration was measured at 216 nm, and the resulting graph (Fig. 3) plotted concentration against absorbance. The regression equation was determined to be y=0.0409x, with a correlation coefficient (R^2) of 0.995. The data are summarized in Table 1, confirming the linearity of the method, and making it suitable for estimating vildagliptin.

Table 1: Linearity of vildagliptin

S. No.	Concentration (µg/ml)	Absorbance (nm)
1	5	0.177
2	6	0.230
3	7	0.270
4	8	0.360
5	9	0.379
6	10	0.408

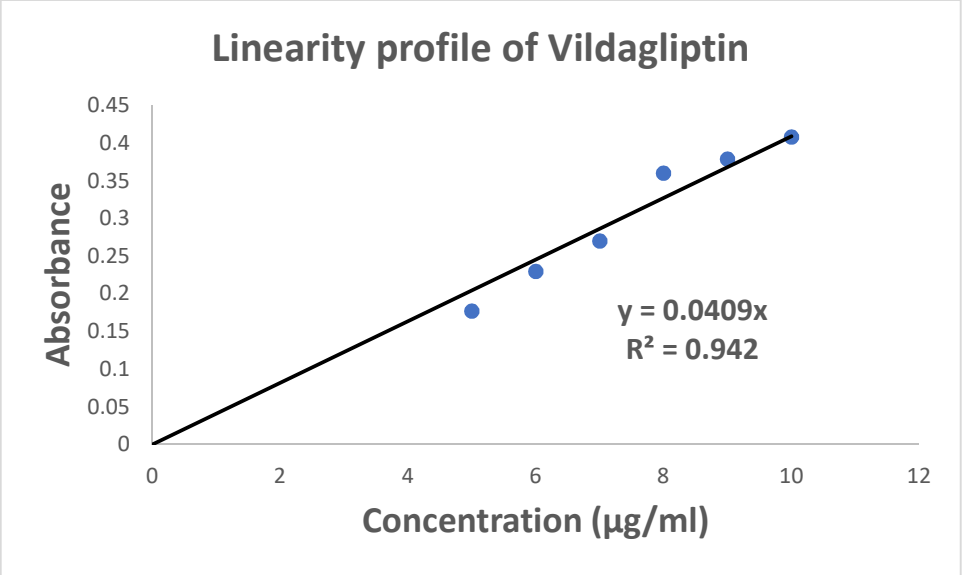

Fig. 3: Calibration curve for vildagliptin at 216 nm

4.3. Precision:

The precision data for UV spectrophotometry are presented in Table 2. The repeatability results indicate no significant differences in precision values, confirming the method's reliability for analysing vildagliptin in tablet formulations. No evidence of interference from excipients was found. The mean precision value was found to be 99.91 ± 1.03%, with values ranging from 98.49% to 100.55%.

Table 2: Precision study of vildagliptin

S. No.	Weight of the table powder (mg)	Absorbance	Drug content present (mg)	Percentage found (%)
1	361.0	0.403	49.24	98.49
2	361.0	0.409	49.98	99.96
3	362.0	0.413	50.27	100.55
4	363.0	0.416	50.62	101.24
5	362.0	0.412	50.16	100.30
6	361.0	0.405	49.46	98.92
			MEAN±SD	99.91±1.03

4.4. Accuracy:

Accuracy was assessed through recovery studies, with results detailed in Table 3. No interactions between the drug and excipients or solvents were observed. The standard deviation was found to be less than 2%, and the mean recovery was found to be above 100%, confirming the absence of interference from excipients. The percentage recovery was determined to be 100.12 ± 1.83%.

Table 3: Recovery of vildagliptin

S. No.	Percentage level (%)	Sample weight (mg)	Drug in the tablet powder (mg)	Drug added (mg)	Total drug (mg)	Absorbance	Amount found (mg)	Amount recovered	Percentage found (%)	Mean ± S. D
1	50	361	50	25	75	0.617	75.38	25.43	50.9	
2	50	362	50	25	75	0.620	75.46	25.51	51.07	51.02± 0.11
3	50	361	50	25	75	0.618	75.48	25.53	51.1	
4	100	360	50	50	100	0.820	100.1	50.21	100.52	
5	100	362	50	50	100	0.824	100.3	50.35	100.80	100.45 ±0.40
6	100	360	50	50	100	0.818	99.91	49.96	100.02	
7	150	361	50	75	125	0.220	123.8	73.9	149.29	
8	150	362	50	75	125	0.223	125.0	75.14	150.43	148.89 ±1.78
9	150	361	50	75	125	0.219	123.3	73.4	146.94	
										100.12 ±1.83

4.5. LOD and LOQ:

The limit of detection (LOD) was calculated to be 1.2952 µg/ml, while the limit of quantification (LOQ) was found to be 3.9247 µg/ml, with both being measured at 216 nm using 0.1 N NaOH.

4.6. Ruggedness:

Ruggedness data are summarised in Table 4, with no significant deviations in absorbance being demonstrated, indicating that the developed method is robust across different conditions.

Table 4: Ruggedness of vildagliptin

S. No	Absorbance	
	Analyst-1	Analyst-2
1	0.228	0.230
2	0.232	0.227
3	0.231	0.232

4.7. Robustness:

No significant differences in absorbance were revealed by robustness studies when minor variations were introduced, such as changes in wavelength (215 nm and 217 nm) and NaOH concentration (0.05 N and 0.15 N). The results are presented in Tables 5 and 6 respectively.

Table 5: Absorbance at a different wavelength

S. No.	Absorbance (215nm)	Absorbance (217nm)
1	0.228	0.229
2	0.229	0.230
3	0.227	0.228

Table 6: Absorbance at different strength of the solvent

Wavelength	0.05 N	0.15 N
216nm	0.228	0.229

4.8. Stability studies:

No significant changes in absorbance were indicated by stability data (Table 7) within the first 30 minutes. However, after one hour, a notable increase in absorbance was observed, likely due to chemical degradation or the formation of chromophoric products under alkaline conditions. Therefore, the sample solution is considered stable for up to 30 minutes post-preparation.

Table 7: Stability of the sample solution

S. No.	Time (min)	Absorbance (nm)
1	0 min	0.344
2	15 min	0.345
3	30 min	0.355
4	60 min	0.374
5	90 min	0.398

5. VALIDATION PROFILE

Replicate analyses of standard solutions were performed to assess accuracy, precision, and reproducibility. Vildagliptin concentrations within the calibration range were prepared in 0.1 N NaOH and analysed against the calibration curves to determine intra-day and inter-day variability.

Precision, LOD, and LOQ were also calculated, with data presented in Table 8. A broad linearity range is demonstrated by this method, making it effective for quantifying vildagliptin in both formulations and pure forms.

Table 8: Validation profile of vildagliptin

Parameters	Observation
Coefficient of co-relation (R^2)	0.995
Linearity range (µg/ml)	5-10
Precision (%)	99.91±1.03
Accuracy (%)	100.12±1.83
LOD (µg/ml)	1.295
LOQ (µg/ml)	3.924

This method shows a wide range for linearity and most effective method to determine the said that the drug vildagliptin in the formulation and as pure form.

6. CONCLUSION

A validated spectrophotometric method for quantifying vildagliptin in pure and pharmaceutical products has been developed. The developed method is selective, precise, accurate, and linear across a concentration range of 5-10 µg/ml, with a precision of 99.91 ± 1.03%. The LOD and LOQ were determined to be 1.295 µg/ml and 3.924 µg/ml, respectively, using 0.1 N NaOH. The recovery percentage was found to be 100.12 ± 1.83%. This method is straightforward and suitable for the estimation of vildagliptin in both pure and pharmaceutical formulations.

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