Journal of Chemical Health Risks

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JCHR (2023) 13(4s), 762-770| ISSN:2251-6727

AUC, 2nd order Derivative UV Spectrophotometry and RP-HPLC Assay of Vildagliptin

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KEYWORDS
Vildagliptin, Validation, Derivative Spectroscopy, RP-HPLC AUC

ABSTRACT:
There are three simple, new, accurate and precise methods developed and validated on the basis of ICH guidelines for estimation of Vildagliptin in pure and its tablet. **Method 1:** In UV–Spectrophotometric method two wavelengths 200nm to 210nm were selected for determination of Area Under Curve using scaling factor 1. Area was calculated between 200nm and 210nm by using LAB Solution UV-Vis software having version1.11. **Method 2:** 2nd Order derivative utilizing 215nm for estimation purposes. The linearity and range were found to be 5 to 40µg/ml for method 1 & 2. The sample and standard solutions were prepared in the double distilled water. **Method 3** comprises RP-HPLC method using Enable column C18 and mobile phase (Methanol & water in the ratio of 70 and 30). The flow rate of 1 ml/min, with detection at 198 nm and temperature 22^0 C were used for analysis purposes. The retention time was found to be 3.228 min. The linearity range was found to be 5-100µg/ml. % RSD is less than 1.5 in the precision, recovery, and robustness studies of the proposed method. So, the method was precise, accurate, sensitive, repeatability, reproducibility and robust in nature. The observed students 't' test values for all the three methods are within the acceptable range for tablet and recovery studies at 95 percent confidence level.

Introduction: Vildagliptin, a member of the class that enhances islet cell insulin secretion via an augmented incretin effect, is a high affinity dipeptidyl-peptidase-4 (DPP-4) inhibitor that improves glycaemic control.Vildagliptin (VLD) is chemically defined as [(2S)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile]. T2DM is another condition for which it is utilized. It is an orally anti-diabetic agent of DDP-4 class of drugs[1]. The chemical structure of Vildagliptinis shown in Fig-1. Literature review reflects that the HPTLC analysis[2], RP-HPLC analysis[3-10] for Vildagliptin, and in combination with other drugs like Remogliflozin Etabonate, and Metformin[11], Vildagliptin and Nateglinide for Spectro chromatography method[12], Vildagliptin with Metformin [13-18]. The proposed methods are simple, economy, highly sensitive, and robust method. Various reported method of Vildagliptin is shown in the Table 1.

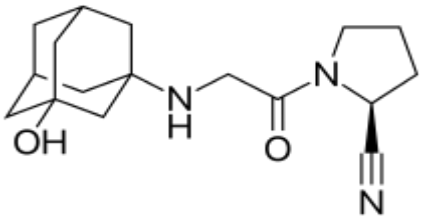

Fig 1: Chemical Structure of Vildagliptin.

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JCHR (2023) 13(4s), 762-770| ISSN:2251-6727

Table 1: Various reported method of Vildagliptin

Column	Mobile Phase	Flow rate (ml/min)	Retention time (Rt) minutes	Ref. No	Elution
Merck TLC plates Precoated with silica gel 60 F254 (10 cm × 10 cm with 250 µm layer thickness)	Chloroform: n-Butanol: CH_3 OH (5:2:3 v/v/v)	1	--	2	Isocratic
Jasco Crest Pack RP C18 (250 × 4.6 mm, 5µ)	ACN: Me OH (70:10:20 v/v) pH 6	1	7.21	3	Isocratic
Shimpack VP-ODS, Shimadzu (150mm x 4.6mm, 5µm) column	0.02M Phosphate buffer (pH 4.6) & ACN (80:20% v/v), 25ºC	0.7	3.6	4	Isocratic
Zorbax Eclipse Plus RP-C8 column (150 mm × 4.6 mm, 5 µm)	KH_2PO_4(pH 7): ACN (85:15, V/V)	1	7	5	Isocratic
Athena C18-column	Solution (A) & CH_3 OH (90:10), Solution (B) CH_3 OH, pH: 7.50 ± 0.05, 40°C	1	13.090	6	Gradient
Reversed phase [C18] column (4.6 x 150 mm id., 5 [micro]m)	10 mM Phosphate buffer (pH 4.6) and ACN (85:15, V/V)	1	3.380	7	Isocratic
Altima C18 column (150mm x 4.6 mm, 5µm)	Dil-O-Phosphoric acid solution & ACN (72:28 V/V) pH: 2.6±0.5, Ambient	1	3.25	8	Isocratic
ODS-4, C18 column (300 mm x 4.6 mm, 3 m) Swinnex type filter with a pore size of 0.45 m	Perchloric acid, ACN &CH_3 OH (87:10:3) (V/V/V), 50°C	1	8.8	9	Isocratic
Reversed phase C18 column (250 × 4.6 mm, 5- Hypersil Gold)	ACN and H_2O (40:60) & pH 7	1	5.3	10	Isocratic
Agilent Zorbax Eclipse Plus C18 (150×4.6mm, 5µm)	ACN & K di hydrogen phosphate buffer (80:20, v/v), pH: 4.2, 30°C	0.6	3.67, & 2.5	11	Isocratic
Xterra C18 column (250 mmL×4.6 mm I.D × 5µ)	ACN: Phosphate buffer (pH:6):water (65:20:15 v/v/v), Rm. Temp	1.0	2.32, & 4.29	12	Isocratic
Thermo Hypersil ODS C18 column (5 µm, 4.6 mm × 250 mm)	Me OH, ACN, and Phosphate buffer (pH 3.5) (5:30:65) (v/v/v), 35 °C	0.8	5.41, & 3.36	13	Isocratic
Chromolith ® C18 monolithic column (5µm, 50 mm × 4.6 mm i.d,)	ACN-Na dihydrogen phosphate (10 mM) and SDS (10 mM) (30/70, v/v) (pH 4.5), 30°C	2.5	2.155 & 1.291	14	Isocratic
Shim-pack solar C18 (250 mm × 4.6 mm, 5 µm)	ACN: CH_3 OH: H_2O (pH4.5 adjusted with O-Phosphoric acid (60:10:30%v/v/v)	1	Remo 4.497 & Vilda 7.304, Met 1.735	15	Isocratic
Kromstar® C18 (250 × 4.6 mm, 5 µm) Column	ACN: Phosphate buffer (70:30 % V/V) , pH3.2, 25^0C	1	Vilda 3.0min,Nate 6.0 min	16	Isocratic

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Method 1:

Instrument used-: A Shimadzu UV-Vis Spectrophotometer (UV-1900i) with 1cm matched quartz cells.

Preparation of SS: 10 mg of Vildagliptin was dissolved in 100ml volumetric flask containing double distilled water to produce 100µg/ml. The drug was sonicated for 2 to 3 minutes for complete dissolve purposes.

Preparation of WS: 0.5, 0.7, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 ml of ss solution was taken then volume was diluted up to 10 ml mark with double distilled water. In UV–Spectrophotometric method two wavelengths 200nm to 210nm were selected for determination of Area under Curve [AUC] using scaling factor 1. Calibration curve or linearity curve was prepared by utilizing Area vs Concentration. Area between 200nm to 210nm was calculated by using LAB Solution UV-Vis software having version1.11. The linear regression equation is Y = 0.218 x + 0.161 and Correlation coefficient r^2 = 0.996. The nine different concentrations and their corresponding absorbance for linearity curve of Vildagliptin is shown in the Table 2. and in the Fig 2. The AUC spectrum of the drug is shown in the Fig 3.

Table 2: Linearity Data of Vildagliptin by AUC

Sl. No	Concentration (µg/mL)	Area
1	0	0
2	5	1.078
3	7	1.787
4	10	2.25
5	12	3.059
6	15	3.325
7	20	4.795
8	30	6.844
9	35	7.74
10	40	8.66

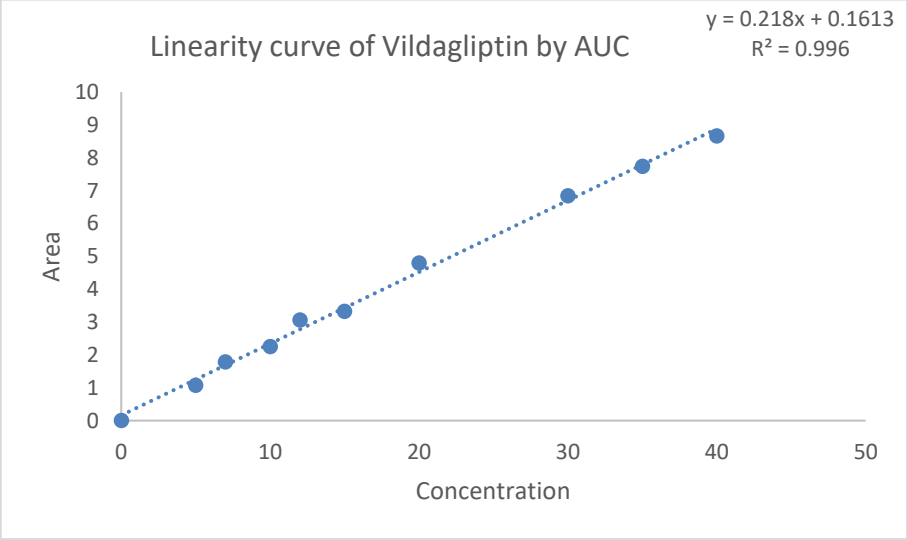

Fig.2: The linearity curve of AUC method

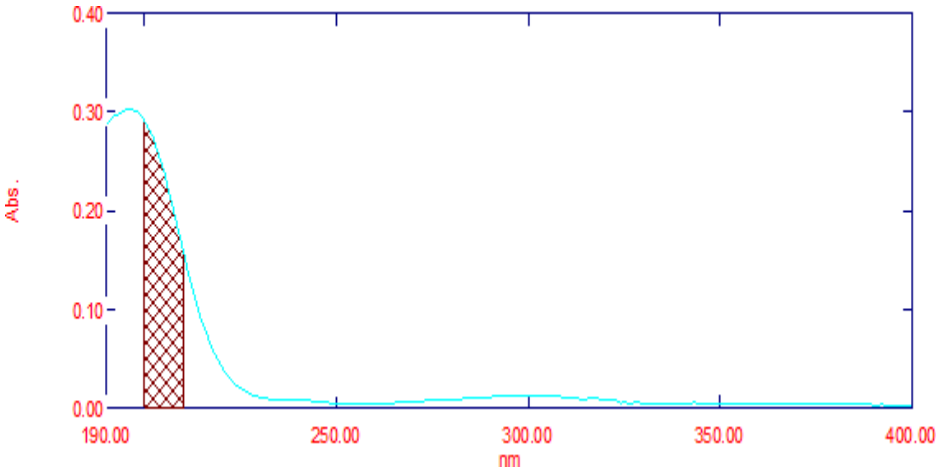

Fig.3: AUC UV Curve of Vildagliptin (12µg/ml)

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Method: 2: Estimation of Vildagliptin by Derivative Spectrophotometric method

Preparation of Standard Stock solution and Working standard solution were similar to the Method 1. The zero order overlay UV spectrum is shown in the Fig 4. Overlay UV 2nd order derivative spectrum of Vildagliptin is shown in the Fig 5. The linearity data of this 2nd order derivative method is shown in the Table 3. The linearity curve for this derivative method is shown in the Fig 6.

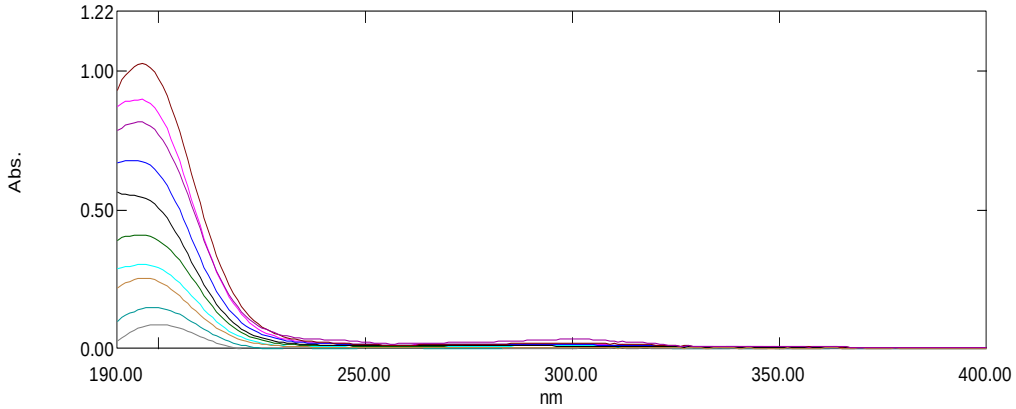

Fig.4: Zero Order Overlay UV spectrum of Vildagliptin

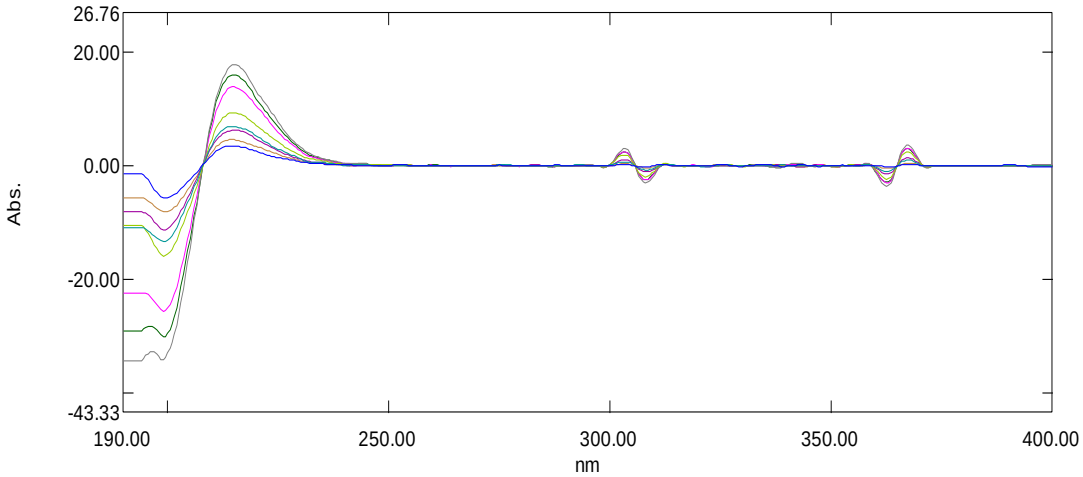

Fig 5: Overlay UV 2nd order derivative spectrum of Vildagliptin

Table. 3- The Linearity data on 2nd order derivative spectrum

Sl. No.	Conc.	Derv.Absorbance (dA/dλ) at 215nm
1	5	2.25
2	7	3.455
3	10	4.53
4	12	5.95
5	15	6.864
6	20	9.278
7	25	11.32
8	30	13.647
9	35	15.949
10	40	17.944

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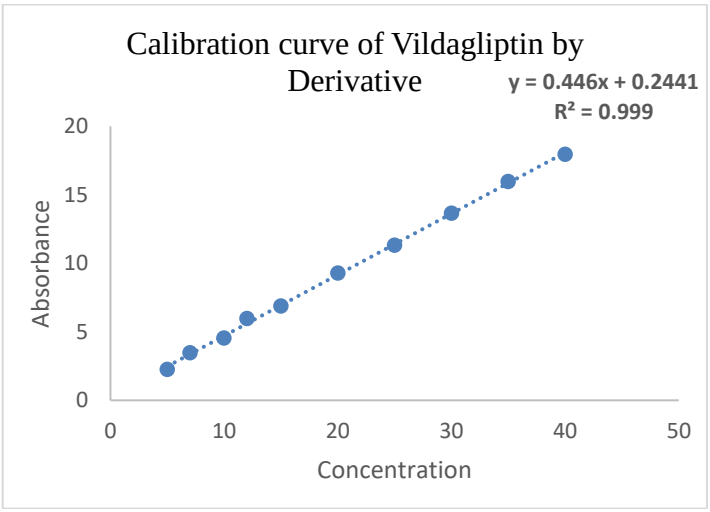

Fig 6: Linearity curve of Vildagliptin

Method 3: Estimation of Vildagliptin by RP-HPLC
Optimized chromatographic condition
RP-HPLC analysis was performed by isocratic elution with flow rate of 1ml/min.The mobile phase consisting of 70 ml of methanol and 30 ml of water ratio was used to obtain the best chromatographic peaks of Vildagliptin as shown in the Fig 7. The detection wavelength and chromatographic run times were selected at 198nm and 5min respectively. Injection volumes of 20µl each standard solution were injected into the column.

Analytical column: Enable column: C_{18}
Temperature: 22^0C.
Isocratic elution with single pump and manual injector

Diluent: Mobile Phase
Preparation of standard stock solution (SSS)10mg of Vildagliptin was accurately weighed and transferred in to a 100ml volumetric flask containing 40ml diluents and then it was shaken for 3minutes to dissolve the drug and then the volume was made up to the mark with the same diluent to produce 100µg/ml.
Then 0.5 ml of the stock solution was further transferred into an another 10ml volumetric flask and then the volume was made up to the mark with the diluent to measure maximum wavelength absorbance by UV-Visible spectrophotometer 1900i. The maximum wavelength was found to be **198nm**. The UV spectrum of the Vildagliptin is show in Fig 8.

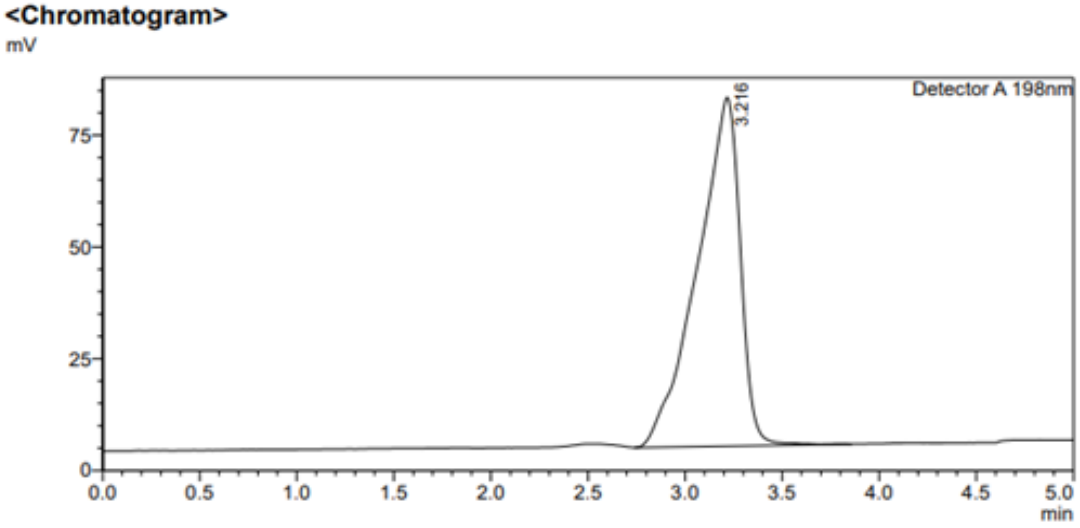

Fig 7: Representative chromatogram of Vildagliptin (60 µg/ml)

Preparation of standard Calibration curve for RP-HPLC:
0.5, 1, 2, 3, 4, 5, 6, 8ml of standard stock solution were taken in eight different 10ml volumetric flasks and diluted up to the mark with diluent to obtain final concentration of the drug which is given in the Table 4. The solutions were injected using a 25µl hemilton syringe and chromatograms were recorded. Calibration curve was formed by using Area on the Y-axis and conc. on the X-axis. Linear regression equation was

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found to be **Y = 21,897.8486 x + 8,300.63 and Correlation coefficient (r^2) = 0.9999.** The linearity curve of the Vildagliptin drug is shown in Fig.9. The chromatograms of the drug are shown in Fig : 7

Table 4: Linearity data of Vildagliptin

Sl. No.	Conc. (µg/ml)	Area
1	0	0.00
2	5	110541
2	10	230461
3	20	450975
4	30	671832
5	40	891944
7	60	1316263
8	80	1753324

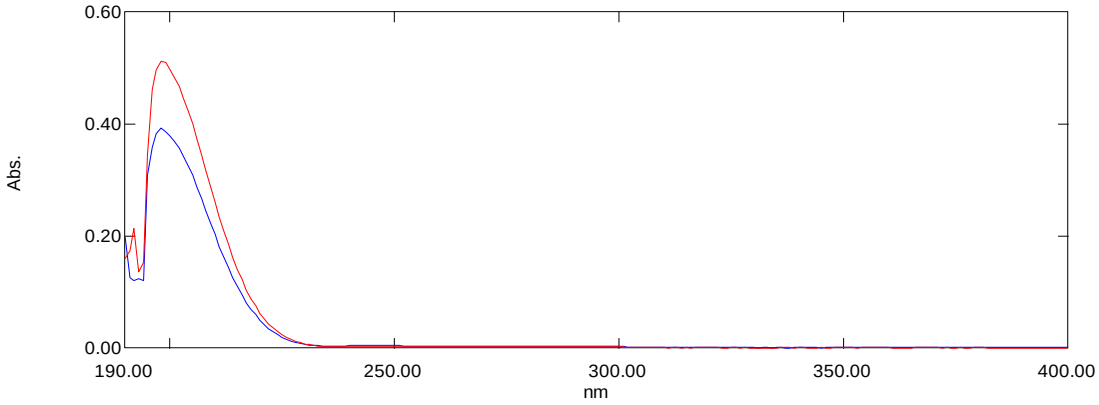

Fig 8: The UV spectrum of Vildagliptin is showing maximum wavelength absorbance

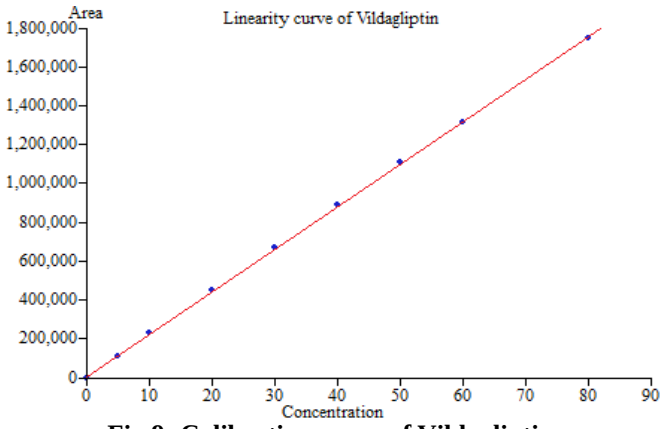

Fig 9: Calibration curve of Vildagliptin

Assay of Tablet: Assay of tablet was performed for all the three methods. Method 1 and 2 were used the tablet formulation concentration having 10µg/ml. The assay was performed by using the optimized spectroscopic conditions for Method 1 & 2. Actual API concentration of the tablet was calculated by putting the area and derivative absorbance values respectively of formulations in the corresponding linear regression equation of Method 1 and 2 respectively. The tablet formulation concentration having 20µg/ml was used for method 3. The formulation solution was injected under the optimized chromatographic condition by using the hemilton syringe and their areas were used in the linear regression equation to calculate the exact concentration of the Tablet. The observed values of the tablet for all the three methods are shown in the Table 5.

Table: 5 Observed value of Tablet analysis for three methods

Method	Formulation (µg/ml)	Label Claim (mg/tab)	Found. (mg/tab)	C.I.	%RSD	SE	t
1	10	50	49.725	99.45±1.16	0.736	0.366	1.5
2	10	50	49.932	99.86±1.07	0.676	0.337	0.399
3	20	50	50.042	100.08±1.09	0.684	0.342	0.248

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Method validation[18]: The proposed methods were validated as per ICH Q2B guidelines. The validation parameter for the determination of Vildagliptin bulk as well as tablet dosage form. The parameters are as follows:

System Suitability: Five replicate injections were used to acquire the results of the parameters for the system suitability test, which was conducted using a newly prepared working standard solution of Vildagliptin (60µg/ml). Table 6 presents the findings of system suitability analysis.

Table 6: Observed values of System Suitability Parameter

Parameter	Results	Limits
Asymmetry factor	1.21	NMT 1.5
Retention Time (mins)	3.218	____
Theoretical plates	3254.693	(NLT 3000)
Repeatability (%RSD)	0.694	< 1.5

Recovery study (Accuracy):
It was found out by recovery study using standard addition method. Tablet samples that had previously undergone analysis were added with known amount of pure drug at 50%, 100%, and 150% level, and they were subsequently exposed to the suggested RP-HPLC procedure. Table 7 displays the findings from recovery studies.

Robustness: It was performed by forced small changes in the chromatographic conditions and found to be unaffected by small changes like ±2% change in volume of the mobile phase, ±2 temperature and ± 2% flow rate, &± 2nm wavelength. % RSD were found to be less than 1 in all the parameter. So the proposed method is robust.

Specificity: By comparing the retention time (Rt) of a standard and a tablet sample containing the medication Vildagliptin, the peak purity of the drug was determined. The retention times of the drug's tablet and standard samples shown good correlations.

LOD & LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were used to determine the method's sensitivity. LOD and LOQ of the newly proposed method were calculated by using the equation of ICH guideline, Limit of detection = $3.3 \times \sigma/S$ & Limit of quantitation = $10 \times \sigma/S$. Where, "σ" is standard deviation of y intercepts of regression lines, "S" is Slope of calibration curve.

Table.7: Observed value for recovery study of Vildagliptin

Method	%Level of recovery	Formulation (µg/ml)	Amount pure drug added (µg/ml)	Amount of drug found (µg/ml)	C.I.	%RSD	SE	t
1	50	10	5	15.025	100.20±1.96	1.246	0.624	0.323
	100	10	10	20.222	101.11±2.36	1.472	0.744	1.494
	150	10	15	24.97	99.88±1.21	0.765	0.382	0.313
2	50	10	5	15.015	100.10±1.72	1.082	0.541	0.184
	100	10	10	20.122	100.612±1.22	0.764	0.384	1.59
	150	10	15	24.927	99.710±1.62	1.026	0.511	0.566
3	50	20	10	30.025	100.08±0.89	0.564	0.282	0.295
	100	20	20	39.975	99.937±1.40	0.881	0.440	0.141
	150	20	30	50.177	100.355±1.16	0.732	0.367	0.965

Standard deviation, standard error, confidence interval within which a real value may be discovered at a 95% confidence level, and mean percent result of the recovery analysis (n = 4) are represented by the symbols SD, SE, and C.I., respectively. Theoretical "t" values for n-1 degrees of freedom at the 95% confidence level: t (0.05, 3) = 3.182

Results and Discussion:
Method-1: The aim of the present work was to develop a simple, sensitive, accurate, and precise AUC method for routine analysis. AUC (Area under Curve) method by UV spectrophotometry is used calculate the concentration of drug in the tablet. The computation of the integrated absorbance with respect to wavelength between the two chosen wavelengths, λ_1 and λ_2 is involved. The processing item for area computation determines the region bounded by the horizontal axis and the curve. By placing the wavelength ranges over which the area needs to be calculated, the horizontal axis is chosen. In order to ascertain the linearity between areas under curve and concentration, this wavelength range is chosen based on a series of observations. The AUC was computed using the

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spectrums indicated above. Plotting Conc. versus AUC allows for the construction of the calibration curve.

Method 2: Zero order, 1st, 2nd, & 3rd order UV derivative spectrum were obtained by UV –Visible Spectrophotometer. Out of these four the 2nd order UV derivative spectrum was found precise for estimation of Vildagliptin in pure and tablet. The maximum wave length was utilized at 215nm for construction of linearity curve. SD was less than 1 in precision, accuracy and assay of tablet study by this method.

Method 3: Following multiple trials using different ratios of acetonitrile to water in the range of (60 + 40) and (50 + 40 + 10), the technique was selected. A mobile phase consisting of methanol and water in the ratio of (70 + 30) & (80 + 20) were performed and found that the 70 + 30 ratio best to achieve best chromatographic peak and highly sensitive. The experimental modalities used were validated satisfactorily in accordance with standard analytical procedures. By applying principle and process recovery studies, using a standard and sample, and applying these methods, all three approaches were shown to be reliable. Findings in the three distinct categories of recovery investigations ranged from 99 to 102%. The results of applying Excel and SSP statistical tools to the analytical method validation criteria. The tablet analysis results for each example were compared to the theoretical value of 100% using the students' t-test. Because the calculated "t" values are smaller than the theoretical "values," the tablet analysis results are in accord with 100% for each analyte. The typical excipients used in the tablets do not interfere with the analysis because there are no additional peaks in the chromatogram.

Table 8: Comparison table for all the three methods results

Sl. No.	Parameter	Method-1	Method-2	Method-3
1	λ max	200-210nm	215nm	198nm
2	Linearity &Range	5-40µg/ml	5-40µg/ml	5-80µg/ml
3	Slope	0.218	0.446	21852.43
4	r^2	0.996	0.999	0.9999
5	LOD (µg/ml)	3.45	2.15	1.95
6	LOQ (µg/ml)	9.57	6.47	3.67

Conclusion:

Simple, quick, selective, less expensive, and less time consuming compared to other published LC, TLC, and HPLC procedures; these are the scientific innovations of the present work. A search of the scholarly literature showed that there were no AUC and derivative spectroscopic methods published for the drug's detection. The purpose of this study was to create a straightforward derivative method that is sensitive, accurate, and exact enough to be used in everyday research. The proposed approach was validated according to ICH criteria. The newly developed RP-HPLC method is also simple, robust, and highly sensitive method. So all the methods can be used for quality control test for Vildagliptin in pure and dosage form.

Source of Conflict: No

Acknowledgement: Thanks to the Principal, Institute of Pharmacy & Technology, Salipur, Cuttack for providing necessary facilities to carry out this original research work.

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