Development and Validation of a UV-Spectrophotometric Method for Determination of Vildagliptin and Linagliptin in Bulk and Pharmaceutical Dosage Forms

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Abstract

The present study was undertaken to develop a spectrophotometric method for determination of vildagliptin and Linagliptin in pharmaceutical dosage forms. This paper describes a simple, rapid, accurate and precise UV-spectrophotometric method for the assay of vildagliptin and linagliptin in bulk and marketed tablet dosage forms. The validation of the developed method was carried out according to ICH guidelines with respect to linearity, precision, accuracy, specificity, limit of detection and limit of quantification. Calibration curves were obtained in the concentration range of 8-32 µg/ml for vildagliptin and 5-25 µg/ml for linagliptin with good correlation coefficients (r=0.999). The precisions of the new method for both drugs were less than the maximum allowable limit (%RSD < 2.0) specified by the USP, ICH and FDA. Therefore, the method was found to be an accurate, reproducible and sensitive for analysis of vildagliptin and linagliptin in pharmaceutical dosage forms.

Key words: Type2 diabetes, vildagliptin, linagliptin, UV-spectrophotometer, method validation.

Introduction

Inhibitors of dipeptidyl peptidase 4, also known as DDP-4 inhibitors or gliptins, are a class of oral hypoglycemic agents (anti-diabetic drugs) that block DPP-4 enzyme. They can be used to treat type 2 diabetes mellitus. Type 2 diabetes mellitus (also called as non-insulin dependent diabetes mellitus, NIDDM) is characterized by insulin resistance in peripheral tissue and an insulin secretary defect of the beta cell (Mayfield, 1998). Vildagliptin and linagliptin are DPP-4 inhibitors which are used in the management of NIDDM (Mentlein, 1999). While vildagliptin is still not approved for use in the US, it was approved in February 2008 by European Medicines Agency for use within the EU and is also listed on the Australian PBS with certain restrictions (Balas *et al.,* 2010). Linagliptin is also a DPP-4 inhibitor developed by Boehringer Ingelheim, which is used the management of NIDDM. It was approved by the U.S. Food and Drug Administration (FDA) on 2 May, 2011 for management of type2 diabetes (Spreitzer, 2008).

Chemically, vildagliptin is (2S)-1-[N-(3-Hydroxy-adamantan-1-yl) glycyl]-2-pyrrolidinecarbonitrile with the empirical formula of $C_{17}H_{25}N_3O_2$ (Figure 1). The molecular weight of vildagliptin is 303.3993 and is soluble in water (Gupta *et al.*, 2009). Linagliptin is 1H-Purine- 2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1-piperidinyl)-3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl) methyl] derivate with empirical formula of $C_{25}H_{28}N_8O_2$ and molecular weight 472.5 (Figure 2). Linagliptin is a white to yellowish compound and is slightly hygroscopic. It is slightly soluble in water (0.9 mg/ml), isopropanol (<1 mg/ml) and acetone (ca. 1 mg/ml) but soluble in methanol (ca. 60 mg/ml) and sparingly soluble in ethanol (ca. 10 mg/ml) (Spreitzer, 2008; WHO, 2011).
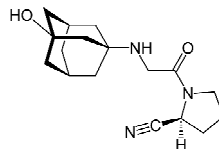

Figure 1. Structure of vildagliptin.

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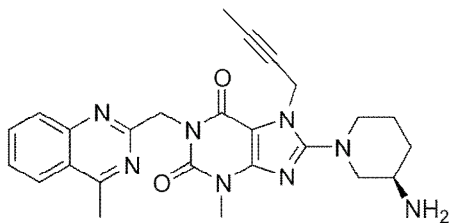
Figure 2. Structure of linagliptin.

Literature survey revealed that there are very few references or no official method available for the determination of vildagliptin and linagliptin in oral formulations. Vildagliptin and linagliptin in pharmaceutical formulations (tablets) can be estimated by liquid chromatography (Verma *et al.,* 2013; Kavitha *et al.,* 2013; Swamy and Baba, 2013). Although various analytical methods have been reported for determination of vildagliptin and linagliptin in bulk as well as in pharmaceutical formulations, most of them require sample pretreatment steps prior to analysis of the drug. Furthermore, these methods require of expensive equipments and considerably skilled personnel.

Our present study has been designed to describe a simple, accurate, rapid and precise spectrophotometric method for the determination of vildagliptin and linagliptin in bulk and marketed dosage forms. This method has been optimized and validated as per as the ICH guidelines.

Materials and Methods

Instruments: A UV-Visible spectrophotometer (Shimadzu UV-1800 spectrophotometer, Shimadzu, Japan) with a pair of matched quartz cells was used for measurement of absorbance. An analytical balance was used for weighing and an ultrasonic bath (POWER SONIC 405, China) was used for sonicating the drugs and sample solutions.

Materials and reagents: Vildagliptin and linagliptin were obtained as gift samples from NIPRO JMI Pharma Ltd., Dhaka, Bangladesh. Tablets of Vilda and Lijenta were purchased from our local market; which contain 50 mg and 5 mg of vildagliptin and linagliptin, respectively. All chemicals and reagents used were of analytical grade and used without further purification.

Determination of λ_{max}: An ultra violet spectrophotometric scanning (190 nm -400 nm) was carried out to select the λ_{max} for the detection of Vildagliptin and Linagliptin.

Preparation of standard stock solution: Distilled water was selected as the solvent for the analysis of vildagliptin and methanol was selected for the analysis of linagliptin. For vildagliptin, 20 mg of vildagliptin pure drug was accurately weighed and transferred into a clean and dry 100 ml volumetric flask. Fifty milliliter of distilled water was added to the flask and the flask was shaken well. Then the volume was made up to the mark using distilled water and mixed well. From this 5 ml of solution was transferred by a pipette into a clean and dry 50 ml volumetric flask and then diluted up to the mark using distilled water to obtain a solution of the concentration of 20 µg/ml which was termed as standard stock solution (A). For linagliptin, 25 mg of pure drug was weighed and taken into a clean and dry 50 ml volumetric flask, diluted with 25 ml of methanol and shaken well. Then the volume was made up to the mark using methanol and mixed well. Two milliliter of this solution was taken into another clean and dry 50 ml volumetric flask and then diluted up to the mark using methanol to obtain a solution with a concentration of 20 µg/ml which was standard stock solution (B).

Preparation of sample solution: Twenty tablets were weighed accurately and their average weight was determined and crushed to powder. Then accurately 70 mg of powder sample (equivalent to 20 mg of vildagliptin) was taken into a clean and dry 100 ml volumetric flask. The drug was diluted with 50 ml of distilled water and the flask was shaken ultrasonically for 10 minutes. Then the volume was made up to 100 ml using distilled water and mixed well. The solution was filtered through Whatman No. 42 filter paper (Whatmann International Ltd., Ket, UK). After filtration an aliquot of 5 ml of this solution was transferred into a clean 50 ml volumetric flask and the volume was adjusted up to the mark with distilled water. Then the absorbance of this solution was recorded at 197 nm. For linagliptin, twenty tablets were weighed accurately and their average weight was determined and crushed to powder. Then accurately 400 mg of powder sample (equivalent to 25 mg of linagliptin) was taken into a clean and dry 50 ml volumetric flask which was diluted with 25 ml of methanol and the flask was shaken ultrasonically for 10 minutes. Then the volume was made

up to 50 ml using methanol and mixed well. The solution was filtered through Whatman No. 42 filter paper (Whatmann International Ltd., Ket, UK). After filtration an aliquot of 2 ml of this solution was transferred into a clean 50 ml volumetric flask and the volume was adjusted up to the mark with methanol. Absorbance of this solution was recorded at 294 nm.

Preparation of calibration curve: Standard stock solutions of both A and B were serially diluted with distilled water and methanol respectively to obtain varying concentrations of stock solutions as (8.0, 12.0, 16.0, 20.0, 24.0, 28.0 and 32.0 µg/ml) for Vildagliptin and (5.0, 10.0, 15.0, 20.0 and 25.0 µg/ml) for Linagliptin to prepare calibration curves. Then the contents of each flask immediately transferred to the spectrophotometric cell and the absorbance was recorded at λ_{max} nm of both drugs respectively.

Method validation : The proposed method was validated for specificity, linearity, limit of detection, limit of quantification, precision, and accuracy as per International Conference on Harmonization (ICH) guidelines (ICH: 2003, 2005).

Specificity: A study conducted to establish specificity of the proposed method using UV-Spectrophotometer for the reference standard and quality control samples of vildagliptin and linagliptin. Both the standard solutions and samples solutions of vildagliptin and linagliptin were shown maximum wavelengths of absorbance at 197 nm and 294 nm, respectively.

Linearity: The linearity of an analytical procedure is its ability of produce results that are directly proportional to the concentrations of an analyte in the samples. The determination was repeated three times at each concentration level. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Limit of detection (LOD): Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. LOD was determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected. LOD was calculated using the following equations (Banik *et al.*, 2013).

$LOD = 3.3 \times S_o/b$, where S_o and b are the standard deviation of the response and the slope of the calibration curve.

Limit of quantification (LOQ): Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be quantified with acceptable accuracy and precise. LOQ was calculated using the following equations (Banik *et al.,* 2013).

$LOQ = 10 \times S_o/b$, where S_o and b are the standard deviation of the response and the slope of the calibration curve.

Accuracy: The accuracy is the measure of how close the experimental value is to the true value. The recovery study was carried out as 80%, 100% and 120% of the test concentration as ICH guidelines. The recovery study was performed three times at each level. Accuracy was determined and expressed as percent recovery.

Precision: Method repeatability was determined by six times repetitions of assay procedure. The reproducibility of the proposed method was determined by analyzing tablet at different time intervals on same day in triplicates (Intra-day assay precision). Precision of analyst was determined by repeating study by another analyst working in the laboratory. Standard deviation and percent RSD was determined.

Potency test: The proposed validated method has been applied for the determination of potency of the tested tablets of vildagliptin and linagliptin.

Results and Discussion

In the present work, vildagliptin and linagliptin showed good solubility in distilled water and methanol, respectively. A linear relationship was obtained when the absorbance was plotted against the concentrations in the range of 8-32 µg/ml for vildagliptin and 5-25 µg/ml for linagliptin (Table 1, Figure 3 and Figure 4). The correlation coefficient of the calibration curves for the both drugs was 0.999 indicating good linearity. The representative linear equation was y = 0.0282x + 0.0077 and y = 0.0397x + 0.0014, respectively for vildagliptin and linagliptin. The limit of detection was found to be as

0.247µg/ml and 0.734 µg/ml while the limit of quantification was 0.748 µg/ml and 2.224 µg/ml for vildagliptin and linagliptin, respectively (Table 2).

Both of the drugs showed good regression values at their respective wavelengths and the results of recovery study reveal that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. The results of accuracy, inter-day and intra-day precision were listed in tables 3 and 4

respectively. Precision is validated by studying the repeatability and intermediate precision. Repeatability results indicate the precision under the same operating condition over a short interval of time and inter assay precision. Intermediate precision study expresses within laboratory variation in different days. In both inter-day and intra-day precision study, shows %RSD are not more than 2.0% which indicated good repeatability and intermediate precision (Table 4).

Table 1. Absorbance of vildagliptin and linagliptin solutions of varying concentrations at 197 nm and 294 nm, respectively.

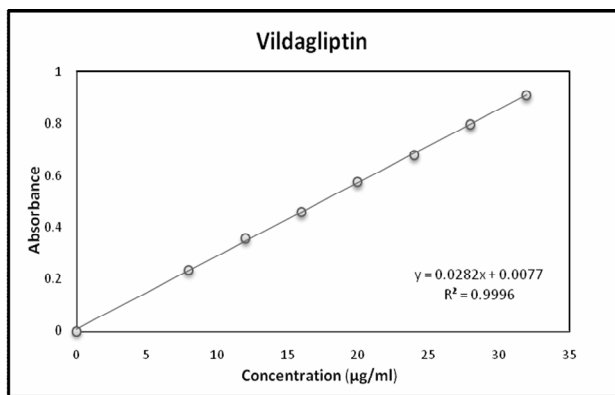| Drug	SN	Conc. (µg/ml)	Absorbance	Drug	SN	Conc. (µg/ml)	Absorbance
Vildagliptin	1	0	0	Linagliptin	1	0	0
	2	8	0.235		2	5	0.198
	3	12	0.358		3	10	0.402
	4	16	0.458		4	15	0.596
	5	20	0.575		5	20	0.804
	6	24	0.68		6	25	0.988
	7	28	0.797				
	8	32	0.909				

Figure 3. Calibration curve of vildagliptin

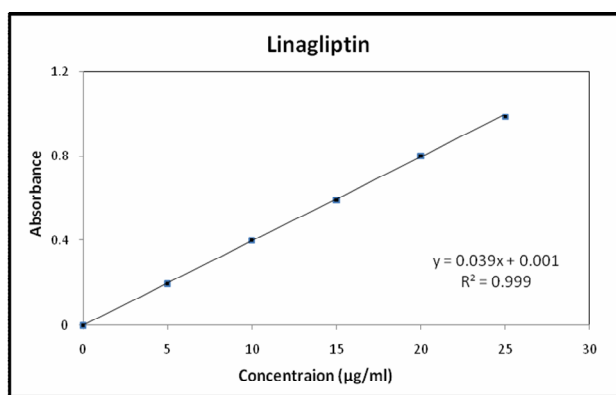

Figure 4. Calibration curve of linagliptin

Table 2. Linearity, LOD and LOQ data of vildagliptin and linagliptin.

SN	Parameter	Vildagliptin	Linagliptin
1	Wavelength detection	197	294
2	Slope	0.0282	0.0397
3	Intercept	0.0077	0.0014
4	Correlation coefficient	0.999	0.999
5	Beers law limit	8-32 µg/ml	5-25 µg/ml
6	Regression equation	y = 0.0282x + 0.0077	y = 0.0397x + 0.0014
7	Limit of detection	0.734 µg/ml	0.247 µg/ml
8	Limit of quantification	2.224 µg/ml	0.748 µg/ml

Table 3. Recovery study for vildagliptin and linagliptin (Accuracy).

Drug	Level of recovery	Sample ID	Theoretical value (mg)	Actual value (mg)	% recovery	Mean ± SD[*]	% RSD
		Spl_01	40.18	41.01	102.07		
	80%	Spl_02	40.18	40.23	100.12	100.69 ± 1.19	1.18
		Spl_03	40.18	40.13	99.88		
		Spl_01	50.38	49.18	97.62		
	100%	Spl_02	50.38	50.04	99.33	99.27 ± 1.62	1.63
Vildagliptin		Spl_03	50.38	50.81	100.85		
		Spl_01	61.54	62.09	100.89		
	120%	Spl_02	61.54	61.16	99.38	100.18 ± 0.75	0.74
		Spl_03	61.54	61.71	100.28		
		Spl_01	4.18	4.15	99.28		
	80%	Spl_02	4.18	4.20	100.48	99.76 ± 0.63	0.63
		Spl_03	4.18	4.16	99.52		
		Spl_01	5.10	5.05	99.02		
Linagliptin	100%	Spl_02	5.10	5.12	100.39	99.61 ± 0.70	0.79
		Spl_03	5.10	5.07	99.41		
		Spl_01	6.05	6.09	100.66		
	120%	Spl_02	6.05	6.04	99.83	100.22 ± 0.42	0.42
		Spl_03	6.05	6.06	100.17		

*mean of three determinations

Table 4. Result of precision (intraday and interday precision).

Component	Intraday precision		Interday precision	
	% Amount found ± SD[*]	% RSD	% Amount found ± SD[*]	% RSD
Vildagliptin	98.06 ± 0.780	0.795	97.62 ± 1.910	1.950
Linagliptin	100.06 ± 0.018	0.018	100.06 ± 0.032	0.032

*mean of six determinations

After the validation of the newly developed method, the method was applied successfully for the analysis of vildagliptin and linagliptin in pharmaceutical dosage forms (tablets). The potency of marketed formulation was determined by this validated method and the results are presented in Table 5. Percentage estimation of vildagliptin and linagliptin from tablet dosage forms by this method was 98.58% and 98.56%, respectively with standard deviation (SD) <2 (Table 5).

Table 5. Potency determination of the vildagliptin and linagliptin marketed dosage form.

Drug	Dosage form	SN	Sample code	Label claim (mg)	Amount found (mg)	Potency (%)	Mean ± SD
		1	VDA-1	50	49.51	99.01	
		2	VDA-2	50	48.98	97.96	
Vildagliptin	Tablet	3	VDA-3	50	49.37	98.74	49.29 ± 0.221
		4	VDA-4	50	49.29	98.56	
		5	LNA-1	5	4.950	99.01	
Linagliptin	Tablet	6	LNA-2	5	4.895	97.90	
		7	LNA-3	5	4.927	98.54	4.928 ± 0.024
		8	LNA-4	5	4.943	98.86	

Conclusion

Results of the present study suggested that the proposed method was found to be simple, accurate and rapid for the determination of vildagliptin and linagliptin in bulk drug and pharmaceutical dosage forms. The method showed high sensitivity, acceptable linearity and accuracy. Furthermore, the method required simple reagents with minimum steps and time for sample preparation, which allowed it to be useful for routine analyses and quality-control assays of vildagliptin and linagliptin in pharmaceutical preparations.

Competing Interest

The authors declared that they have no competing interest.

Acknowledgements

References

Balas, B., Baig, M.R., Watson, C., Dunning, B.E., Ligueros-Saylan, M. and Wang, Y. 2007. The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single-dose administration in type 2 diabetic patients. *J. Clin. Endocrinol. Metab.* **92**, 1249-55.

Banik, S., Kaisar, M.M. and Hossain, M.S. 2013. Development and validation of a simple and rapid uv spectrophotometer method for assay of linagliptin in bulk and marketed dosage form. *Indian. J. Nov. Drug. Delivery.* **5**, 221-224.

Four Phase III Trials Confirm Benefits of BI's Oral, Once-Daily Type 2 Diabetes Therapy. Genetic Engineering & Biotechnology News. 28 June 2010.

Gupta, R., Walunj, S.S., Tokala, R.K., Parsa, K.V., Singh, S.K. and Pal, M. 2009. Emerging drug candidates of dipeptidyl peptidase IV (DPP IV) inhibitor class for the treatment of Type 2 Diabetes. *Curr. Drug. Targets.* **10**, 71-87.

ICH: Harmonised Tripartite Guideline, Validation of analytical procedures: text and methodology, International Conference on Harmonization. Q2(R1), Geneva, Nov 2005.

ICH 2003. Harmonised Tripartitle Guideline, Stability Testing of New Drug Substances and Products. Q1A(R1), USA, pp. 1-18.

Kavitha, K.Y., Geetha, G., Hariprasad, R., Kaviarasu, M. and Venkatnarayanan, R. 2013. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form. *J. Chem. Pharm. Res.* **5**, 230-235.

Mayfield, J. 1998. Diagnosis and Classification of Diabetes Mellitus: New Criteria, American Family Physician. Indiana: Bowen Research Center, Indiana University. **58**, 1355-62, 1369-70.

Mentlein, R. 1999. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptidase. *Regul. Pept.* **85**, 9-24.

Spreitzer, H. 2008. NeueWirkstoffe - BI-1356.Österreichische Apothekerzeitung, 918.

Swamy, A.J. and Baba, A.K. 2013. Analytical method development and method validation for the simultaneous estimation of metformin HCL and linagliptin in bulk and tablet dosage form by RP-HPLC method. *Int. J. Pharm.* **3,** 594-600.

Verma, D., Laksmana, R.A. and Dinda, S.C. 2013. Simultaneous determination of metformin and vildagliptin is solid dosage form by stability indicating RP-HPLC Method. *Int. Res. J. Pharm.* **4**, 122-128.

World Health Organization. 2011. Fact Sheet No. 312: What is Diabetes?