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A COMPREHENSIVE REVIEW ON ANALYTICAL PROFILE OF ANTI-DIABETIC DRUG

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ABSTRACT

Vildagliptin is an Oral Anti- Hyperglycaemic agent (antidiabetic drug) belonging to a new group of drugs called as dipeptidyl peptidase -4 inhibitor (DPP-4 inhibitor). Vildagliptin has been shown to reduce hyperglycaemia in type 2 Diabetes Mellitus. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the Islets of Langerhans in the pancreas & reduces the blood sugar level. The present review assesses the various approaches for analysis of Vildagliptin in bulk drug as well as various formulations. A concise review represents the compilation and discussion of about more than 35 analytical methods which includes HPLC, UHPLC, LC-MS and UV-Spectrophotometry methods implemented for investigation of Vildagliptin in biological matrices, bulk samples and in different dosage formulations. This detailed review will be of great help to the researcher who is working on Vildagliptin.

Keywords : Vildagliptin; Analytical Profile; HPLC; HPTLC; UPLC; Bio-analytical; Stability indicating

Abbreviations:

VLG: Vildagliptin

CAS: Chemical Abstracts Service pKa: Dissociation constant

BCS: Biopharmaceutical Classification System

API: Active pharmaceutical ingredient

HPLC: High performance liquid chromatography

RP-HPLC: Reverse phase-high performance liquid Chromatography

RP-UHPLC: Reverse phase- Ultra high performance liquid Chromatography

LC-MS: Liquid chromatography-mass spectrometry

PDA: Photodiode array

ICH: International conference on harmonization RH: Relative Humidity tR: Retention time

LOD: Limit of Detection

LOQ: Limit of Quantitation

FDA: Food and Drug Administration

SIM: Stability indicating method

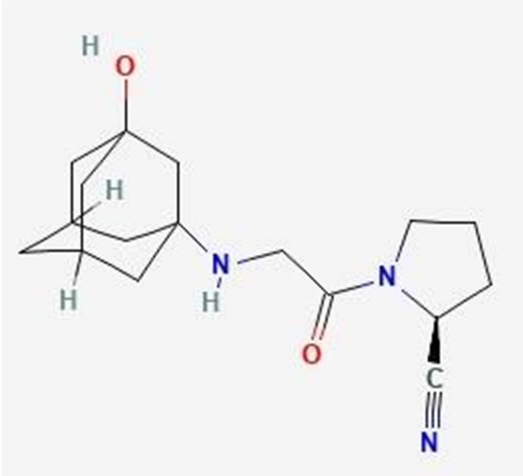
INTRODUCTION

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago **[1]**. In 1936, the distinction between type 1 and type 2 DM was clearly made **[2]**. Type 2 DM was first described as a component of metabolic syndrome in 1988 **[3]**. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycaemia, insulin resistance, and relative insulin deficiency **[4]**. Type 2 DM results from interaction between genetic, environmental and behavioural risk factors **[5]**. According to the International Diabetes Federation (IDF), approximately 415 million adults between the ages of 20 to 79 years had diabetes mellitus in 2015 **[6]**. In T2DM, the response to insulin is diminished, and this is defined as insulin resistance. During this state, insulin is ineffective and is initially countered by an increase in insulin production to maintain glucose homeostasis, but over time, insulin production decreases, resulting in T2DM. T2DM is most commonly seen in persons older than 45 years. Still, it is increasingly seen in children, adolescents, and younger adults due to rising levels of obesity, physical inactivity, and energy-dense diets **[7]**.

Vildagliptin is an oral antihyperglycemic agent (ant diabetic drug) of the dipeptidyl peptidase4(DPP-4) inhibitor class of drug. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP-4 allowing GLP-1 and GIP to potentiate the secretion of insulin in

the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas **[8]**. In investigative examination endeavor originate a novel reproducible and efficient HPLC technique with isocratic elution for assurance of vildagliptin in pharmaceutical formulation **[9]**.

Table 1: Drug profile of Vildagliptin [10]

Drug Name	Vildagliptin
Structure	
Category	Oral Hypoglycaemic agent
IUPAC name	(2S)-1-{2-[(3-hydroxyadamantan-1yl)amino]acetyl}pyrrolidine-2 carbonitrile
Chemical Formula	C17H25N3O2
CAS no.	274901-16-5
Molecular Mass	303.3993 g/mole
Physical State	White to off white solid powder
BCS class	I - High solubility and high bioavailability.
Solubility	Soluble in Water and Methanol
pKa	14.71 & 9.03 Strongest acidic and basic respectively
Absorption	Rapidly absorbed following oral administration with an oral bioavailability of greater than 90%.
T1/2	90 minutes
Therapeutic Use	Used to reduce hyperglycaemia in type 2 diabetes mellitus
Storage	At room temperature between 68ºF and 77ºF (20ºC and 25ºC).

Vildagliptin side effects:

Some of the common and major side effects of Vildagliptin are:

- Headache
- Cough
- Constipation
- Dizziness
- Hypoglycaemia
- Weakness
- Excessive sweating
- Heartburn
- Swelling of face, lips and eyelids
- Upper respiratory tract infection

Analytical techniques used for determination of Vildagliptin

A. **High-performance liquid chromatography (HPLC):-**

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product **[11]**.

Principle:-Solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase.

Depending upon the partition behaviour of different components, elution at different time takes

Place **[12]**.

Typical HPLC system consists of the following main components: [12]

1. **Solvent Reservoirs:-** Storage of sufficient amount of HPLC solvents for continuous operation of system.

   In normal phase chromatography: Mobile phase- Non polar

   Stationary phase-polar

   In reverse phase chromatography: Mobile phase- Polar

   Stationary phase- Non polar

   Types of solvent delivery system: a) Isocratic elution:-Mobile phase composition is fixed.

   b) Gradient elution:-Mobile phase composition is vary.

2. **Pump: -**This provides the constant and continuous flow of mobile phase through the system. Pumps can exert pressure up to 6000psi on mobile phase in HPLC. But for analytical work about 400-1500psi pressure is required. Pump can capable delivering 0.1-10ml/min mobile phase flow rate. There are three types of pump used in HPLC:
   I) Syringe/Displacement Pump
   II) Reciprocating /Hydraulic Pump
   III) Non-Reciprocating/Pneumatic Pump
3. **Injector:**-This allows an introduction of the analytes mixtures into the stream of the mobile phase before it enters the

column. This system can be divided into two types:

I) Syringe injection:-a) Direct Injection on Column
b) Stop and Flow Injection

II) Loop and Valve System:-a) Fixed Loop and Valve System
b) Variable Volume Loop and Valve System

4. **Column:** - It is heart of HPLC system. It actually produces a separation of the analytes in a mixture. A column is place where mobile phase is in contact with stationary phase, forming an interface with enormous surface.
5. **Detector: -** The function of detector in HPLC is to monitor the mobile phase with or without the solute as it emerges from the column. Apperance of the analyte in the detector flow cell causes change of absorbance. If the analyte absorbs greater than background (mobile phase) a positive signal is obtained e.g.: a) Refractive Index Detector
b) UV Detector-PDA c) Fluorescence Detector.

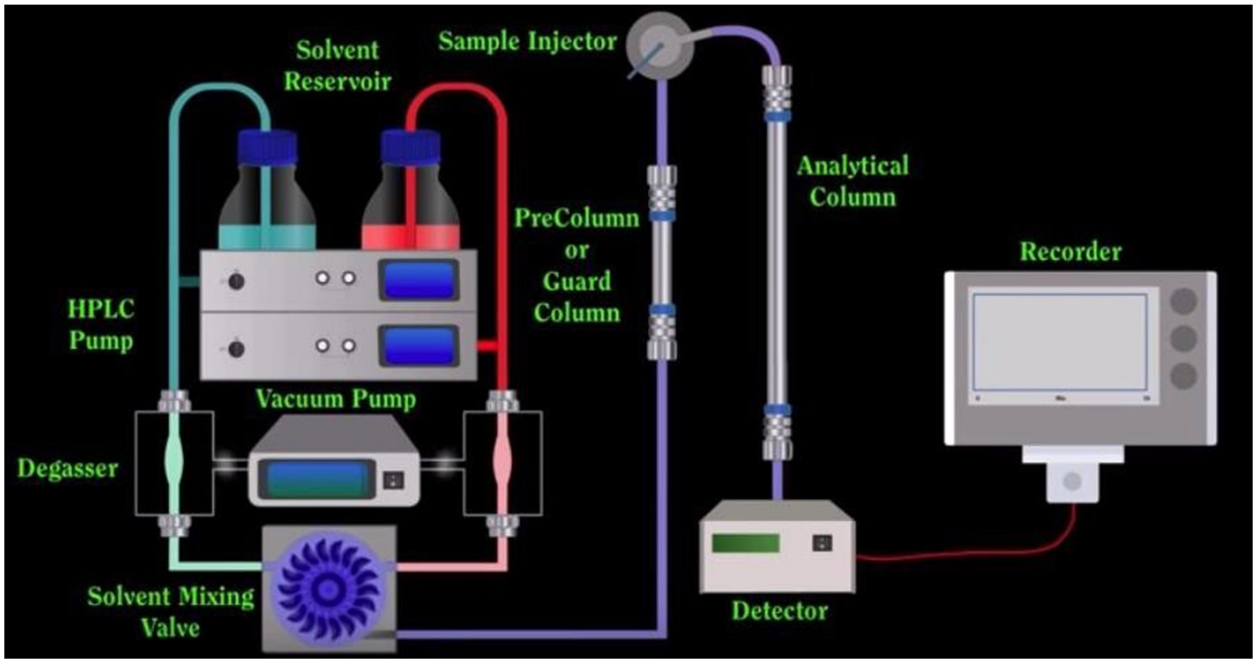


Figure 2: Instrumentation of HPLC Chromatography

Table 2:- HPLC method for vildagliptin

Sr. No.	Drug	Method	Stationary phase/ Column	Mobile phase	Detection	Linearity, LOD , LOQ	Retention Time (min) & Flow rate (ml/min)	Ref no.
1.	Vildagli ptin tablet	RP- HPLC	Xterra® waters C18 column (150mm× 4. 6mm ,5µm)	25%Ammonium hydroxide+50% phosphoric acid solution(pH9.5) : Methanol (60:40v/v)	210nm	Linearity - 5200µg/ml LOD - 1.47µg/ml LOQ - 4.90µg/ml	Flow rate1ml/min tR -6.3min	[13]
2.	Metform in HCl &	RPHPLC	Dionex C18 (250mm 4.6mm ,id-	0.01M DiPotassium hydrogen	215nm	MET : Linearity - 500-1500	Flow rate - 1.5ml/min	[14]
	Vildagli ptin tablet		5µm	phosphate buffer : Water (90:10v/v)		µg/ml LOD - 0.7057µg/ml LOQ - 2.3523µg/ml VLG : Linearity - 50-150µg/ml LOD - 0.6289µg/ml LOQ - 2.0964µg/ml	tR - 2.39min	
3.	Metform in HCl & Vildagli ptin tablet	RPHPLC	Phenomex kromosil250mm× 4.6 mm,5µm	0.1M phosphate buffer (pH6.8) : Acetonitrile (75:25v/v)	260nm	MET : Linearity- 25250µg/ml LOD - 0.219µg/ml LOQ - 0.669µg/ml VLG : Linearity - 2.5-25µg/ml LOD - 0.0053µg/ml LOQ - 0.0159µg/ml	Flow rate1ml/min MET : tR -2.4min VLG : tR -3.4min	[15]
4.	Vildagli ptin tablet	RPHPLC	Phenomex C18 column (5µm, 250mm × 4.6mm)	Methanol: water (60:40 v/v) pH 4.5 adjusted with OPA	207nm	Linearity- 1060µg/ml LOD - 0.98µg/ml LOQ - 2.98µg/ml	Flow rate- 0.8ml/min tR -3.58min	[16]

5.	**Metform in HCl &Vildag liptin tablet**	**RP-HPLC**	**Xterra C18 column (250mm×4. 6 mm I.D × 5µ)**	**Acetonitrile : Phosphate buffer (pH 6.0) : water (65:20:15v/v/v)**	**239nm**	**VLG : Linearity – 4-34 µg/ml LOD - 0.0040µg/ml MET : Linearity – 8-54 µg/ml LOD - 0.025µg/ml**	**Flow rate1ml/min VLG : tR -2.32min MET : tR -4.29min**	**[17]**
6.	**Metform in HCl &Vildag liptin**	**RP-HPLC**	**Chromosil ODS C18 column (250 x**	**0.1M DiPotassium hydrogen phosphate : Methanol**	**258nm**	**VLG : Linearity - 50150µg/ml LOD -**	**Flow rate-0.5ml/min MET :**	**[18]**
	tablet		**4.6mm 5µ)**	**(60:40%v/v) adjust pH-9.2 by using Ortho phosphoric acid**		**0.0015µg/ml LOQ - 0.0043µg/ml MET : Linearity - LOD - 0.005µg/ml LOQ - 0.014µg/ml**	**tR- 1.43min VLG : tR -5.32min**	
7.	**Vildagli ptin Tablet**	**RP-HPLC**	**Qualisil BDS C18 column (250 x 4.6mm, 5µ)**	**0.1M DiPotassium hydrogen phosphate buffer : Acetonitrile (70:30%v/v) adjust pH-7 by using O- phosphoric acid**	**263nm**	**Linearity - 1060µg/ml LOD - 0.45µg/ml LOQ - 0.98 µg/ml**	**Flow rate-0.5ml/min**	**[19]**
8.	**Metform in HCl &Vildag liptin tablet**	**RP- HPLC**	**ZODIAC C18 column (250 x 4.6mm, 5µ)**	**Disodium Hydrogen phosphate buffer(pH3.5) : Methanol (73.5:26.5v/v)**	**200nm**	**MET : Linearity - 75175µg/ml LOD - 0.09ppm LOQ - 0.28ppm VLG : Linearity - 7.5-17.5µg/ml LOD - 0.38ppm LOQ - 1.15ppm**	**Flow rate1ml/min MET : tR-2.490min VLG : tR - 4.243min**	**[20]**

9.	Metform in HCl &Vildag liptin tablet	RP- HPLC	Water's C18 column (150 x 4.6mm, 5µ)	0.1M DiPotassium phosphate buffer : Acetonitrile (70:30%v/v)	258nm	MET : Linearity - 1000-3000 µg/ml LOD - 1.1ng/ml LOQ - 3.6ng/ml VLG : Linearity - 100-300µg/ml LOD - 0.3ng/ml LOQ - 0.8ng/ml	Flow rate1ml/min MET : tR -1.4min VLG : tR - 5.3min	[21]
10.	Metform in HCl &Vildag liptin tablet	RP- HPLC	Lichrocart C18column (250 mm x 4.6mm, 5µ)	0.05M KH2PO4 : Acetonitrile (70:30%v/v)	215nm	MET : Linearity - 10-50µg/ml VLG : Linearity – 5-25µg/ml	Flow rate1ml/min MET : tR - 5.18min VLG : tR- 6.64min	[22]
11.	Vildagli ptin tablet	RP-HPLC	X-bridge C18 column	Phosphate buffer pH6.8 : Acetonitrile (67:33v/v)	239nm	-	Flow rate 1ml/min	[23]
12.	Metform in & Vildagli ptin tablet	RP-HPLC	Thermo Hypersil ODS C18 (250 mm x 4.6mm, 5µ)	0.1M Potassium Hydrogen phosphate buffer pH7 : Acetonitrile (60:40v/v)	263nm	-	Flow rate1ml/min MET : tR - 2.1min VLG : tR – 3.5min	[24]
13.	Vildagli ptin tablet	RP-HPLC	Agilent eclipse XDB C18 column (150 mm x 4.6mm, 5µ)	0.1M phosphate buffer (pH6.8) : Acetonitrile (85:15v/v)	210nm	Linearity - 10150µg/ml LOD - 0.0329µg/ml LOQ - 0.0998µg/ml	Flow rate 1ml/min tR-3.04min	[25]
14.	Vildagli ptin tablet	RP-HPLC	Thermosil C18 column (4.6 x 150mm, 5µm)	pH 8.2Buffer : Acetonitrile: Methanol (450: 480:70v/v)	254nm	Linearity 50–90 µg/ml LOD - 2.98 g/ml LOQ - 9.94 g/ml	Flow rate– 0.5ml / min tR - 3.906 min	[26]
15.	Vildagli ptin tablet	RP - HPLC	Altima C18 column (150	pH 2.6 buffer : Acetonitrile (72:28 v/v)	266 nm	Linearity - LOD - 0.06	Flow rate-1 ml/min	[27]

			mm x 4.6 mm, 5µm)			µg/ml LOQ - 0.21 µg/ml	tR- 3.25 min	
16.	Vildagli ptin tablet	RP - HPLC	ShimpackVP-ODScolumn(150 mm x 4.66 mm, 5µm)	0.02 M Phosphate buffer pH 4.6 : Acetonitrile (80:20 v/v)	PDA detector 210nm	Linearity - 20-70 µg/ml	Flow rate-1 ml/min	[28]
17.	Metform in & Vildagli ptin Bulk, Tablet	RP-UHPLC	Agilent Zorbax Eclipse Plus C18 column (150 mm x 4.66mm, 5µm)	Acetonitrile : Potassium dihydrogen phosphate buffer (pH4.2) : (80:20v/v)	DAD detection-207nm	MET : Linearity – 20-100 µg/ml LOD – 1.74µg/ml LOQ – 5.79µg/ml VLG : Linearity – 20-100µg/ml LOD – 2.20µg/ml LOQ – 7.33µg/ml	Flow rate0.6ml/min MET : tR-2.5min VLG: tR-3.67min	[29]
18.	Metform in HCL & Vildagli ptin Bulk, Tablet	RP - HPLC	BDS HYPERSIL C18 column (4.6mm ×250mm)	50 mM phosphate buffer (pH 6): Methanol : Acetonitrile (50:30:20v/v)	220nm	Linearity – 10-60µg/ml MET : LOD- 1.09 µg/ml LOQ - 3.32 µg/ml VLD : LOD- 1.70 µg/ml LOQ- 5.15 µg/ml	Flow rate 0.8ml/min. MET : tR-3.7min VLG: tR-4.8min	[30]
19.	Metform in & Vildagli ptin Bulk, Tablet	RP - HPLC	ODS column(4.6× 250mm, 5µm, Hypersil)	ACN: Methanol: Water (15:60:25v/v)	278nm	Linearity – 1-5µg/ml MET : LOD- 0.617µg/ml LOQ – 1.87 µg/ml VLD : LOD- 0.154µg/ml LOQ- 0.468 µg/ml	Flow rate 0.8ml/min.	[31]

B. UV- visible spectrophotometric method: [32]

UV – VIS spectrometry concerns with the consequences of electromagnetic radiation in the UV and/or Visible region with the absorbing species like atoms, molecules or ions. UV-VIS spectroscopy is type of absorption & molecular spectroscopy. UV radiations lie between 200 -400nm while visible radiations lies in between 400 800nm wavelength region.

Principle: - when UV-VIS radiations are incident on substance, then it interacted with molecule which absorbs these radiations & its electron goes from ground state to excited state by changing their electronic transition. In which wavelength & frequency of absorption is measured for qualitative & quantitative analysis respectively.

Absorption of UV-VIS radiation is directly proportional to conc^{n} of substance (Beers law) & thickness of medium (Lamberts law).

Typical UV-VIS spectrometry consists of the following main components:

1) Source: It is used to emit UV-VIS radiations. e.g.- Tungsten Source, Hydrogen discharge lamp, Deuterium lamp , Xenon discharge lamp , Mercury arc lamp.

2) Monochromators: These are used to select single wavelength radiations & block others. The essential elements are -

i) Entrance Slit– It allows UV-VIS radiations to incident on dispersing element.

ii) Dispersing element – Specific wavelength radiations can be selected. These are of 2 types:

a) Prism b) Grating

iii) Exit slit - It allows nominal wavelength together with a band of wavelength on either side selected by dispersing element.

3) Sample Holder/Cuvette:- Sample cells that are to be contains sample for analysis

4) Detectors:- These are used to detect intensity of UV radiations falls on it. There are three types of detectors:

a) Photo voltaic cell/Barrier layer cell

b) Photo cell / Photo tube

c) Photo multiplier tube

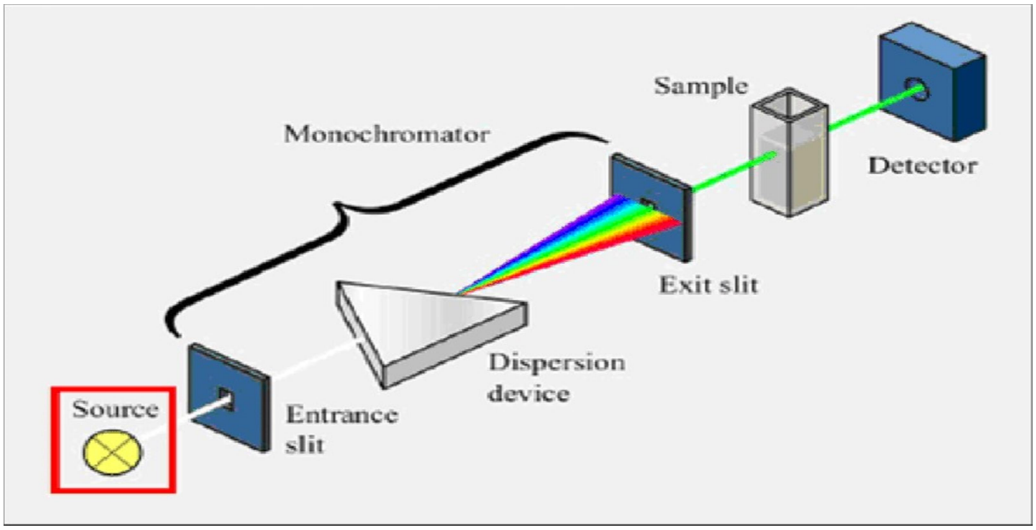


Figure 1: Single beam UV-Visible Spectrometer

Table 3: UV method for Vildagliptin

Sr. No.	Drug	Matrix	Method	Solvent	Detectio n	Linearity, LOD, LOQ	Ref. No.
1.	Vildagliptin tablet	Bulk	UV visible 1601 Shimadzu double beam spectrophotometer	Water	244 nm	Linearity - 12.5-200 µg/ml LOD – LOQ -	[33]
2.	Vildagliptin tablet	Bulk , Tablet	UV-VIS spectrophotometer (Shimadzu model 18001)	0.5M HCL	202.5 nm	Linearity - 10–35 µg/ml LOD – 0.055 µg/ml LOQ – 0.166 µg/ml	[34]
3.	Vildagliptin & Metformin HCL	Bulk, Tablet	LABINDIA spectrophotometer	Water	VLG 217 nm MET -234 nm	VLG : Linearity -0.7µg/ml LOD -0.074 µg / ml LOQ - 0.225 µg / ml MET : Linearity -7 µg/ml LOD -0.44 µg / ml LOQ -1.35 µg / ml	[35]
4.	Vildagliptin	Bulk, Tablet	UV-Visible double beam spectrophotometer	0.1 N HCL	210nm	Linearity –5-60 µg/ml LOD -0.951 µg/ml LOQ – 2.513 µg/ml	[36]
5.	Vildagliptin	Bulk, Tablet	Schimadzu 1800 version 1.12- Double Beam UV-Visible spectrophotometer	0.1N NaOH	216nm	Linearity - 10-100 µg/ml LOD – LOQ -	[37]
6.	Vildagliptin & Linagliptin	Bulk, Tablet	UV-Visible spectrophotometer (Shimadzu UV1800)	VLG – Distilled water LNG – Methanol	VLG – 197 nm LNG – 294 nm	VLG : Linearity - 8-32 µg/ml LOD -0.247µg/ml LOQ -0.748 µg/m LNG : Linearity - 5-25 µg/ml LOD –0.734 µg/ml LOQ – 2.224 µg/ml	[38]
7.	Vildagliptin & Metformin	Bulk, Tablet	Double beam spectrophotometer (Shimadzu UV– VIS 1700)	0.1N NaOH	VLG – 233nm MET 216nm	VLG : Linearity - 30-70 µg/ml MET : Linearity –5-25 µg/ml	[39]

C. Stability Indicating Method:

Singh and Bakshi discussed some conclusive points of developing SIM. Dolan suggests the comments on SIA. Smela discussed regulatory points about SIM is analytical method.

A Stability-indicating assay method can be defined as "Validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and drug products are specific so that the content of active ingredients and degradation products can be accurately measured without interference" **[40]**.

Generally forced degradation/stress testing is used to generate the samples for stability-indicating assay methods. Forced degradation/stress testing is defined as "the stability testing of drug substance and drug product under conditions exceeding those used for accelerated stability testing" **[41]**.

Degradation can be achieved by exposing the drug, for extended period of time, to extremes of pH (HCl or NaOH solutions of different strengths), at elevated temperature, to hydrogen peroxide at room temperature, to UV light, and to dry heat (in an oven) to achieve degradation to an extent of 5–20% **[42]**.

According to FDA guidelines (Guidance for Industry, Analytical Procedures and Methods Validation, FDA, 2000), a Stability Indicating Method (SIM) is defined as a validated analytical peptide testing procedure that accurately and precisely measure active ingredients (drug substance or drug product) free from process impurities, excipients and degradation products **[43]**.

SIM procedure is used to measure the diminution the quantity of API in drug substances prefer degradation Studies. SIM may also check stability of drug matter and Products changes in separate time intervals of study. These method accurately estimate the changes API concentrations .In the absence of impurities, excipients and other degradation products. Stress testing is done to demonstrate specificity of the created method to quantify the adjustments in grouping of substance when little data is accessible about prospective degradation product. The improvement of reasonable Stability indicating method provides a background for preformulation thinks about, stability examines and improve the proper storage condition **[44]**. These ICH guidelines are relevant to forced degradation Study:

- ICH Q1A: Stability testing of New Drug Substance and Products.

- ICH Q1B: Photo stability testing of New Drug Substance and Products.
- ICH Q1C : Stability testing of New Dosage Forms
- ICH Q1D : Bracketing and Matrixing Designs for stability testing of Drug Substances and Products
- ICH Q1E : Evaluation of stability data
- ICH Q1F : Stability data package for Registration Applications in Climatic Zones III and IV
- ICH Q2B: Validation of analytical procedure, methodology.
- ICH Q5C : Stability testing of Biotechnological/Biological Products

Solution state stability :

1. Acidic hydrolysis
2. Alkaline hydrolysis
3. Hydrolytic
4. Oxidative degradation **Solid state solubility:**

1. Thermal degradation
2. Photolytic degradation

Table 4: Types of Stability Studies

Study Type	Storage condition	Minimum time period covered by data at submission
Long Term	25°C±2°C and 60% RH±5% RH or 30°C±2°C and 65% RH±5% RH	12 months
Intermediate	30°C±2°C and 65% RH±5% RH	6 months
Accelerated	40°C±2°C and 75% RH±5% RH	6 months

Table 5: Stress Testing (forced degradation)

Degradation factor	Condition
Thermal	≥ 60°C
Humidity	≥ 75% RH
Acid	0.1 N HCL
Base	0.1N NaOH
Oxidative	Oxygen gas, 3% H2O2
Photolytic	Metal halide, Hg, Xe lamp, UV-B fluorescent

Types of Stability of drug substance:

Physical Stability:

The original physical properties, including appearance, palatability, uniformity, dissolution and suspend ability are retained. Physical stability affect to drug uniformity and release rate hence it is important from safety and efficiency point of view.

Chemical Stability:

Each active ingredient retains its chemical integrity and labelled potency within the specified limits. The chemical stability of drug is of great importance since it becomes

less effective as it undergoes degradation. Also drug decomposition may yield toxic by-products that are harmful to the patient.

Microbiological Stability:

Sterility or resistance to microbial growth is retained according to the Specified requirements. Antimicrobial agents retain effectiveness within specified limits. Microbiological instability of a sterile drug product could be hazardous.

Therapeutic Stability: The therapeutic effect remains unchanged.

Toxicological Stability: No significant increase in toxicity occurs **[45]**.

Table 6: Stability indicating method for Vildagliptin

Sr. No.	Drug	Method	Column/ Stationary Phase	Mobile Phase & Chamber saturation time	Flow Rate, R.T&R.T of Degradation Product/Develo pment Time Rf value of drug	Wavelength, Linearity, Coefficient Correlation	LOD & LOQ (µg/mL)	Ref no.
1.	Vildaglipti n Tablet, Bulk	RP-HPLC	Zorbax rapid resolution HT C18 column (150 mm x 4.6mm, 5µ)	Sodium dihydrogen phosphate buffer (pH 6.5) : Acetonitrile (50:50v/v)	Flow rate 1ml/min Run time – 10 min Retention time- 5.017min	UV detection -220 nm Linearity - 1060µg/ml R2 - 0. 9996	LOD – 0.025µg/ ml LOQ – 0.054µg/ ml	[46]
2.	Vildaglipti n Tablet	RP-LC	XBridge analytical column C8 (150 × 4.6 mm i.d., 5 µm	Acetonitrile : 0.3%Triethyl amine pH 7.0 adjust with phosphoric acid (15:85v/v)	Flow rate -1.0 ml/min Retention time– 6min	Photodiode array (PDA) detection -207 nm Linearity -20–80 µg/ml R2 - 0.9999	LOD – 0.63µg/ml LOQ - 2.82 µg/ml	[47]
3.	Metformin & Vildaglipti n tablet	RP-HPLC	Grace Cyano column (250 mm×4 .6 mm) 5 µm	25 mm Ammonium Bicarbonate buffer (pH7) : Acetonitrile (65:35 v/v)	Flow rate - 1.0 ml/min Retention Time – MET -7.5 min VLG - 5.3 min	UV detection- 207 nm MET: Linearity -25-125 µg/ml VLG : Linearity -50–250 µg/ml	MET : LOD – 0.36 µg/ml LOQ-1.22 µg/ml VLG : LOD – 0.75 µg/ml LOQ - 2.51 µg/ml	[48]
4.	Metformin & Vildaglipti n tablet	RP-HPLC	Thermo hypersil ODS C18 column (250 mm×4 .6 mm,5 µ m)	Methanol : Acetonitrile: Phosphate buffer pH 3.5(5:30:65v/ v)	Flow rate - 0.8ml/min Retention Time – MET -3.36 min VLG - 5.41min	UV detection- 212nm MET: Linearity -10-140 µg/ml R2-0.9917 VLG :	MET : LOD – 2.18 µg/ml LOQ-6.55 µg/ml VLG :	[49]
					Run time -7 min	Linearity -114µg/ml R^2 -0.9903	LOD – 0.13 µg/ml LOQ - 0.38µg/ml	
5.	Vildaglipti n tablet, Bulk.	RP-HPLC	Jasco crestpack RP C18 (250 mm x 4.6mm, 5µ)	0.01M Disodium Hydrogen phosphate buffer pH 6: Acetonitrile: Methanol (70:10:20)	Flow Rate-1ml/min Retention Time7.21	PDA Detection-210nm Linearity - 515µg/ml R^2-0.9999	LOD - 200ng/ml LOQ - 600ng/ml	[50]
6.	Vildaglipti n tablet	HPLC MS	RP C18 column (250 × 4.6 mm, 5-Hypersil Gold).	Acetonitrile Water (40:60) pH adjusted at 7.0 using triethylamine	Flow Rate-1ml/min Retention Time5.3min	Linearity - 2-12 ug/ml R^2 = 0.9999	LOD – 3.61 µg/ml LOQ - 10.96µg/ ml	[51]

D. Bio-Analytical Method :

These bioanalytical validation technique established by Karnes *et al*. in 1991which was intentional to give direction to bioanalytical chemists. After one year, Shah *et al*. established these report the convention on analytical technique validation of bioavailability, bioequivalence and pharmacokinetic studies organized in Washington in 1990 **[52]**.

Bio-analytical method promotes the quantitative analytical technique appropriate biochemical approach. HPLC, RP-HPLC, HPLC-MS/ESI, UPLC-MS, UPLC-TMS, LC and GC combined with mass spectroscopic procedure, LC-MS, LC-MS/MS. Bioanalysis is innovative technique for improve the accuracy, precision, efficiency, sensitivity, specificity, assays, data handling, processes, analysis cost, data quality **[53]**.

Table 7: Bio-analytical method for Vildagliptin

Sr. No.	Drug	Method	Bio-fluid	Column	Mobile phase	Flow rate & retention time	Detection /Detector	Linearity , LOD, LOQ	Ref. No.
1	Vildagliptin and Telmisartan	RP HPLC	Rabbit plasma	Kromasil C18 Column (100mm x 4.6mm, 5µ)	Acetonitrile: Methanol (75:25v/v)	Flow Rate1ml/min Retention Time : VLG- 2.5min TMS- 6.6min	PDA Detector at 225nm	VLG: Linearity - 24.979-5003.838µg /ml TMS: Linearity- 1.011-202.55938µ g/ml	[54]
2.	Metformin & Vildagliptin tablet	RP-HPLC	Human Plasma	Thermo hypersil ODS C18 column (250 mm ×4.6 mm ,5 µm)	Methanol : Acetonitrile: Phosphate buffer pH 3.5(5:30:65 v/v)	Flow rate - 0.8 ml/min Retention Time – MET - 3.36 min VLG - 5.41min Run time -7 min	UV detection-212nm	MET : Linearity - 10-140 µg/ml R^2- 0.9917 LOD –2.19 µg/ml LOQ-6.57 µg/ml VLG : Linearity-1 14µg/ml - R^2 -0.9903 LOD –0.14 µg/ml LOQ - 0.41µg/ml	[49]
3.	Vildagliptin tablet	LCMS/MS	Rat Plasma	Betasil C18 column (50 mm× 4.6 mm,	Acetonitrile: 2mM Ammonium acetate pH3.4	Flow rate - 0.35 ml/min Retention Time –	API 3200 Q trap triple quadrupole (TMS) via	VLG : Linearity-1.57501.21ng/ml R^2 ≥0.99	[55]

				5 µm	**(90:10v/v)**	**1.68min Run time -3 min**	**Electrospray Ionization Source**	**LOQ- 1.57ng/ml**	
4.	**Vildagliptin tablet**	**RP-HPLC**	**Human Plasma**	**XBridge Shield C18 column (150 mm ×4.6 mm ,3.5 µm)**	**50Mm Ammonium bicarbonate pH7.8 : Acetonitrile**	**Flow rate -1ml/min Retention Time – 11.2min**	**UV detection- 210nm**	**Linearity- 10-120 µg/ml**	**[56]**
5.	**Vildagliptin tablet**	**LCMS/MS**	**Rat Plasma**	**ACE3 C18 PEP Column (150 mm ×4.6 mm ,3 µm)**	**10 mM Ammonium acetate buffer :Acetonitrile (20:80v/v)**	**Flow rate - 0.7 ml/min Retention Time – 3-3.20 Min**	**Ionized in the positive Electrospray ionization ion source of mass spectrometer**	**Linearity- 7.063023.81 ng/ml R^2>0.99 LOQ - 7.06ng/ml**	**[57]**

CONCLUSION

The present review illustrates various analytical approaches exercised for the estimation of Vildagliptin. A numerous investigation had perform including, Bio-analytical, Stability Indicating, HPLC, UV-Visible Spectroscopy and LC-MS, etc. for estimation of Vildagliptin in bulk and in its combined pharmaceutical formulations and in plasma. Reverse Phase Liquid Chromatography with UV detection has been found to be most studied for estimation of Vildagliptin. In bulk as well as pharmaceutical dosage forms, while hyphenated such as LC-MS methods are reported for determination of Vildagliptin and its metabolite in plasma and other biological fluids. Few chromatography approaches like UHPLC and UV Spectrophotometry methods are also used for assay of Vildagliptin.

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