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An Analytical Review-Based Study of Vildagliptin and Metformin

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder and a major public health concern worldwide. Among the available therapies, metformin, a biguanide, remains the first-line drug due to its ability to reduce hepatic glucose production and improve insulin sensitivity. Vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, complements metformin by enhancing incretin activity, thereby promoting insulin secretion and suppressing glucagon release in a glucose-dependent manner. Their combination offers improved glycemic control with low hypoglycemia risk and good tolerability. To ensure quality, efficacy, and safety, several analytical methods have been applied for their evaluation, including spectroscopy, HPLC, HPTLC, UPLC, LC-MS/MS, GC-MS, and capillary electrophoresis. These validated techniques enable accurate quantification in formulations and biological samples. Fixed-dose combinations (FDCs) of vildagliptin and metformin further enhance treatment adherence and therapeutic outcomes.

Keywords*:* T2DM, Dipeptidyl peptidase inhibitor, Vildagliptin, Metformin, Analytical Methodology.

1. INTRODUCTION

Diabetes mellitus (DM) is a complex chronic disease caused by hyperglycemia, impaired secretion, impaired function, or both. The chronic metabolic imbalance associated with this disease puts patients at risk of long-term macrovascular and microvascular complications, including frequent hospitalizations and cardiovascular disease, if not treated appropriately.[1]In the world. The International Diabetes Federation estimates that approximately 387 million people worldwide have diabetes.[2]According to the Centers for Disease Control and Prevention, 29.1 million adults in the United States, or 9.3% of the population, were diagnosed with diabetes in 2012. In that year, 1 million people had diabetes, and 15-30% of them developed full-blown diabetes.[3]When it comes to treating type 2 diabetes, especially in individuals who are overweight or obese and have normal renal function, MET is the recommended first line medication. It is used to treat polycystic ovarian syndrome and has been studied for additional conditions where a possible contributing factor is insulin resistance. A combination of Vildagliptin and Metformin is used to improve glycemic control whose diabetes is not controlled by them when administered alone. Numerous analytical techniques, including UV-Visible Spectroscopy, HPLC, GAS, LC-MS, and HPTLC.[4]

2. Objectives

1. Metformin:

Metformin is a biguanide and is the main oral drug for the management of T2 diabetes in all age groups. Metformin activates hepatic adenosine monophosphate-activated protein kinase, causing hepatic glucose uptake and inhibition of gluconeogenesis through complex effects on mitochondrial enzymes.[5] Metformin is well tolerated with mild side effects, low risk of hypoglycemia, and low potential for weight gain. Metformin has been shown to reduce the progression of T2DM by reducing hepatic glucose synthesis (gluconeogenesis) and increasing the sensitivity of peripheral tissues to insulin, reducing the risk of complications and reducing patient mortality. In addition, it improves insulin sensitivity by activating insulin receptor expression and increasing tyrosine kinase activity. Recent evidence also suggests that metformin lowers plasma lipid levels and prevents CVD via the peroxisome proliferator-activated receptor (PPAR)-α pathway.[1,5]

IUPAC name: 3-(diaminomethylidene)-1,1dimethylguanidine

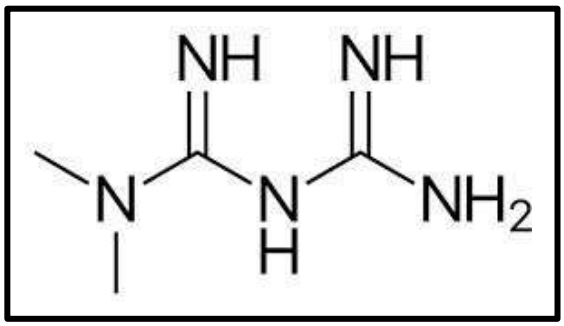

Figure 1: Chemical structure of Metformin (C4H11N5)

2. Vildagliptin: A drug Galvus (vildagliptin) was registered in Russia in 2008. This drug is effective as monotherapy along with blood sugar lowering agents and insulin. DPP-4

inhibitors are a new treatment for type 2 diabetes. Literature review showed that there is only one spectrophotometric method for measuring vildagliptin by the same author in this study. 3- Amino-1-adamantanol (AAD), reported in the synthesis of vildagliptin, is expected to be an impurity of vildagliptin. Since there is no published liquid chromatography method for vildagliptin, the aim of this study was to develop a reversed-phase liquid chromatography (RP- LC) method for the measurement of vildagliptin alone or in the presence of AAD.[6]

IUPAC name: S-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile

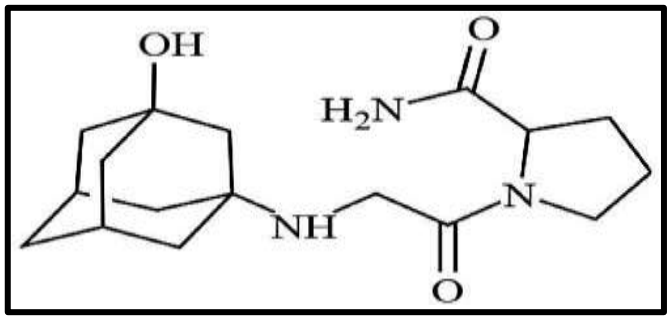

Figure 2: Chemical structure of Vildagliptin (C17H25N3O2)

3. Analytical Methods

Analytical methods are an interesting part of chemical analysis and can be defined as tools that interact with all branches of chemistry and disciplines of pure and applied sciences. Analytical tools play an important role in the development and evaluation of new products. These devices provide the detection limits needed to ensure the safety of food, medicine, water and air. This analytical method is based on four main principles: electrochemistry, spectroscopy, chromatography and linear methods.[7] Spectroscopy, liquid chromatography-mass spectrometry (LC-MS), high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), ultra-high performance liquid chromatography (UPLC) and capillary electrophoresis (CE), GC MS, LC-ESI/MS and diagnostic tests are used to analyze antidiabetic drugs.[8]

1. **Spectrophotometry:** Vildagliptin lacks a conjugated double bond system, and therefore does not exhibit a strong absorption peak in the UV region. However, it shows detectable absorbance around 260 nm. Reported studies have proposed valid spectrophotometric methods for its estimation, including analysis at 266 nm using water as a solvent at a concentration of 200 µg/mL.[9] Alternative methods have reported absorption at 244 nm, and at 202.5 nm when prepared in 0.5 M HCl with a concentration of 25 µg/mL. Derivative spectroscopic approaches, such as first and second derivative spectrometry, have also been applied for simultaneous measurement of vildagliptin with metformin hydrochloride. A simple and sensitive spectrophotometric method for the estimation of metformin hydrochloride in bulk and tablet formulations has been developed and validated. The primary amino group of metformin hydrochloride reacts with ninhydrin in an alkaline environment to form a purple chromogen that is measured spectrophotometrically at 200-400 nm. In the range of 18-8 µg/ml, beer from the laws of The recovery of the drug by the proposed method is 97-100%, which indicates that there is no interference from side substances in the tablets.[6,7,8]

Table 1: Optical parameters of Vildagliptin and metformin

Sr. No	Parameters	Vildagliptin	Metformin
1	Wavelength of maximum	244-266 nm	200-400 nm
2	Beer's law limit	30-70 µg/ml	8-18 µg/ml
3	Regression Equation	Y=0.011x – 0.029	Y=0.080x + 0.088
4	Slope	0.011	0.080
5	Corel. Coeff. (r^2)	0.990	0.990

6	Molar absorptivity (L mol^{-1} cm^{-1})	0.0462 X 10-4	1.334 X X 10-4
7	Sandell's Sensitivity	0.00358 X 10-4	0.440X 10-4

2. High-performance liquid chromatography MS/MS (HPLC):

High-performance liquid chromatography (HPLC) is an ideal technique for routine analysis because its sensitivity, reproducibility, and ability to separate compounds from a variety of matrices result in high resolution and short analysis times. Despite these advantages, existing HPLC methods can only monitor vildagliptin and metformin. In addition, the same dose may not contain compounds related to metformin as recommended by European and US pharmacopoeias.[10] Longer wavelengths (263 and 293 nm) have been used to improve selectivity in some HPLC methods, but are not used as chromophore molecules of vildagliptin and metformin, which causes a decrease in sensitivity. In other cases, the sensitivity is increased by using shorter wavelengths (210-220 nm), but the selectivity of the method is not sufficient. This validation is an important step to prove that the method is in accordance with the intended purpose.[11]

HPLC tandem mass spectrometry (MS/MS) has been shown to offer several advantages over other methods, including the ability to analyze drugs in complex matrices with high sensitivity and selectivity. HPLC-MS/MS is a promising method for simultaneous quantification of vildagliptin and metformin.[12]

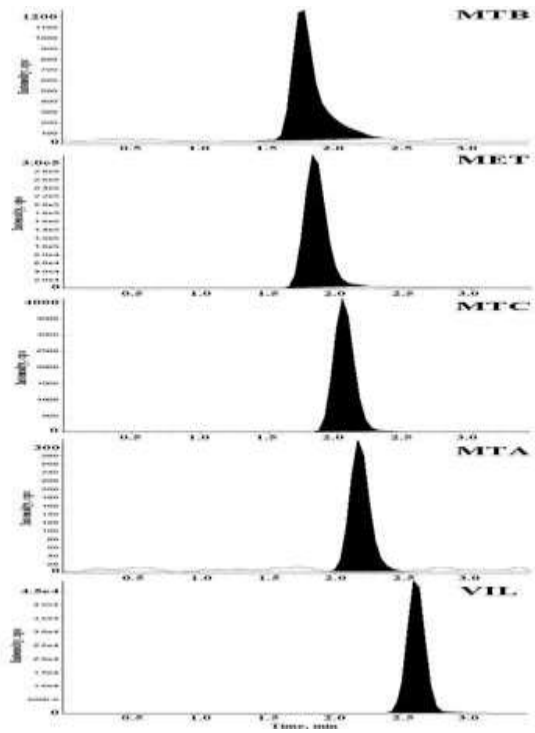

Figure 3: High-performance liquid chromatography-tandem mass spectrometry chromatograms of metformin (MET), MET-related compound A (MTA), MET-related compound B (MTB), MET- related compound C (MTC), and vildagliptin (VIL)Method validation

3. High-performance thin-layer chromatography (HPTLC):

A high-performance thin layer chromatography (HPTLC) method was developed for the simultaneous evaluation of major and commercial combination dosage forms of metformin hydrochloride (MET) and vildagliptin (VLD). The HPTLC method was developed using the Camag HPTLC system. A TLC plate coated with silica gel 60GF254 is used as the stationary phase. Mobile phase ammonium acetate (1% w/v) in methanol: toluene; This is (10:0.5). Spot detection is performed by densitometry at absorbance at 214 nm. Rf values of MET and VLD were found to be 0.44 and 0.55, respectively. The performance characteristics of the HPTLC method for the simultaneous estimation of MET and VLD and the commercially available combination doses were statistically validated according to the recommendations of the ICH guidelines for the validation of the analytical method.[13] The HPTLC method was linear between 1000 and 5000 ng/spot and 2000 and 500 ng/spot for MET and VLD, respectively. For HPTLC, the LOD values for MET and VLD were 17.22 ng/dot and 34.60 ng/dot, respectively, and the LOQ values for MET and VLD were 52.20 ng/dot and 104.85 ng/dot, respectively. The HPTLC method is simple, accurate, linear, accurate, precise and reliable, so this method can be used for the routine analysis required and simultaneous evaluation of formulations containing MET and VLD.[13,14]

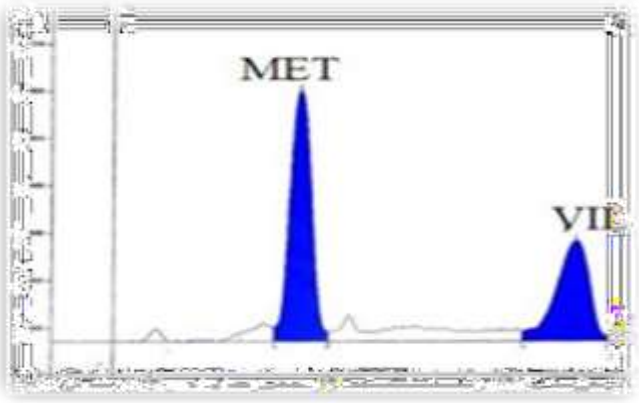

Figure 4: HPTLC chromatograms of metformin (MET) and vildagliptin (VIL)

4. Ultra-performance liquid chromatography (UPLC):

Ultra-performance liquid chromatography (UPLC), a new category of separation technology, is the most promising development in high-speed chromatographic separation with high chromatographic resolution, speed, and analytical sensitivity. A new, sensitive and rapid screening method using ultra-performance liquid chromatography (UPLC) was developed and validated according to ICH guidelines for the simultaneous determination of two binary mixtures. Vildagliptin and Metformin Hydrochloride and Ciprofloxacin Hydrochloride and Dexamethasone Sodium Phosphate.[15,16]

Method Overview: UPLC Assay Development & Validation Chromatographic Setup

Stationary Phase (Column): The separation is carried out using a Phenomenex C18 column with dimensions of 100 mm length × 2.1 mm internal diameter and packed with 1.8 µm particles, optimized for UPLC performance.

Mobile Phases: For **Vildagliptin + Metformin HCl**, a phosphate buffer (potassium dihydrogen phosphate)

and acetonitrile are mixed in a **70:30 (v/v)** ratio.

For **Ciprofloxacin HCl + Dexamethasone Sodium Phosphate**, the buffer-to-acetonitrile proportion is **80:20 (v/v).**

Flow Rate & Temperature: Flow is maintained at **1 mL/min**. The column temperature remains at **ambient** conditions.[17]

Detection: The mixture is monitored at **220 nm or 254 nm**, enabling simultaneous observation of both compounds.

Validation According to ICH Guidelines

The method was thoroughly validated following the International Council for Harmonisation (ICH) guidelines, with key parameters including:

Linearity: Confirmed across specific concentration ranges:

Vildagliptin: 0.5–5 µg/mL Metformin HCl: 5–50 µg/mL

Ciprofloxacin HCl and Dexamethasone Sodium Phosphate: 2–20 µg/mL These ranges reflect a clear proportional relationship between concentration and detector response. **Accuracy & Precision**: Results demonstrated both accuracy and repeatability, underscoring the method's reliability for pharmaceutical analysis.[17]

Robustness: The method showed resilience against minor variations (e.g., changes in pH, temperature, flow rate), affirming its suitability for routine laboratory applications.

Selectivity & Applicability: No interference from common formulation excipients was observed, implying the method is specifically reliable for the target analytes in both bulk and dosage forms.[17]

Table 2: Optimized UPLC Conditions and Validation Parameters for Simultaneous Determination of Binary Mixtures.

Feature	Details
Column	Phenomenex C18, 100 mm × 2.1 mm, 1.8 µm
Mobile Phase	V–M: 70:30 (buffer:ACN); C–D: 80:20 (buffer:ACN)
Flow Rate	1 mL/min
Temperature	Ambient
Detection Wavelengths	220 nm and 254 nm
Linearity Ranges	Vildagliptin: 0.5–5 µg/mL Metformin HCl: 5–50 µg/mL Ciprofloxacin & Dexamethasone: 2–20 µg/mL
Validated Parameters	Accuracy, precision, robustness, selectivity
Benefits	Rapid, sensitive, high-resolution, low-solvent use, suitable for regular QC

5. Capillary electrophoresis (CE):

Capillary electrophoresis (CE) is now a safe and reliable method for drug analysis and is recommended by

several pharmacopoeias, including the British Pharmacopoeia and the US Pharmacopoeia (USP 34 2011; BP 2012). Capillary zone electrophoresis (CZE) and micellar electro kinetic chromatography (MEKC) are two widely used techniques for the separation of pharmaceuticals (whether pharmaceutical formulations or body fluids) and anti-drugs. Various substances such as pollutants.[18,19] CE is important for drug quality control in quantitative and qualitative analysis and is now as important as HPLC in drug analysis. Quantitative analysis is mainly determined by comparing the migration time and standard deviation of the target compound. Quantitative analysis of abnormal nature calculated according to the standard calibration curve. CE separation relies on the interaction of solutes in an electric field, and electrophoresis is performed in a narrow-angle capillary filled with background electrolyte (BGE).[20,21]

6. Gas chromatography-mass spectrometry (GC-MS):

The new analytical technique of gas chromatography-mass spectrometry (GC-MS) is mainly used to analyze volatile drugs and waste solutions, some weak compounds and the absence of chromophores. GC-MS offers several advantages for the analysis of metformin and vildagliptin compared to HPLC, including higher throughput, sensitivity, specificity, shorter analysis time, and lower sample volume.[22,23]

GC-MS Analysis of Vildagliptin (VIL)

A sensitive and reliable method for analyzing vildagliptin in pharmaceutical formulations via GC-MS involves:

Derivatization: Vildagliptin is converted to its O-TMS derivative using MSTFA, NH_4I, and β-mercaptoethanol at 60 °C for 30 minutes.

Detection: Performed in selected ion monitoring (SIM) mode using characteristic ions at **m/z 223** and **252**, with nandrolone serving as the internal standard.

Validation Metrics:

LOD: 1.5 ng/mL

LOQ: 3.5 ng/mL

Linearity: 3.5–300 ng/mL

Precision: Intraday/interday RSD ≤ 3.62%

Accuracy: Ranged between –0.26% and 2.06%

Successfully applied to quantify VIL in tablets, yielding reliable and precise results (e.g., 50.12 ± 0.35 mg; RSD 1.71%).[24]

GC Method for Simultaneous Determination of Metformin and Vildagliptin

Though direct GC-MS methods for both metformin and vildagliptin simultaneously are rare, there is a well-validated **GC (not GC-MS)** method:

Column Used: INNOWAX column (30 m × 0.25 mm, 1.8 µm film thickness)

Operational Conditions:

Injection: Split 10:1, 1 µL

Carrier Gas (Nitrogen): 1 mL/min Injector Temp: 300 °C

Detector Temp: 250 °C

Oven: Start at 100 °C (held 7 min), ramp 10 °C/min to 300 °C.[25]

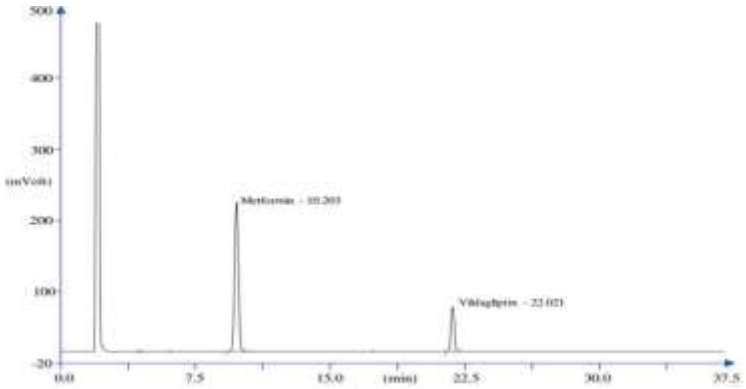

Figure 5: GC chromatogram (single-detection trace) of Metformin (MET) and Vildagliptin (VID) from a validated gas chromatographic method.

Table 3: Gas Chromatogram showing the presence of MET (500 µg/mL) and VID (50 µg/mL) in a standard solution

Peak	Name	Ret. Time	Area	Area %	USP plates	USP tailing
1	Metformin	10.203	5475875	90.071	25347	1.030
2	Vildagliptin	22.021	603652	9.929	18450	1.093
Total			6079527	100.000		

7. Liquid Chromatography with tandem mass spectrometry (LC-MS-MS):

Liquid chromatography and tandem mass spectrometry (LC-MS-MS) is a powerful analytical technique that combines the separation power of liquid chromatography with the sensitive and selective mass spectrometry capabilities of a quadrupole mass spectrometer. The sample solution containing the desired analyte is transferred through the mobile phase to the stationary phase (LC column) under high pressure. The chemical interaction between the sample components, the stationary phase, and the mobile phase affects the different migration rates through the LC column and affects the separation. A variety of combinations of stationary and mobile phases allows the separation to be customized for many complex solutions.[26,27] After washing through the LC column, the liquid is sent to the mass spectrometer. The mass spectrometer of the LC/MS/MS system contains an ionization source that atomizes, dissolves, and ionizes the LC column flow to produce charged

particles.

Conclusion:

The combined use of vildagliptin and metformin has emerged as a promising therapeutic strategy for the management of type 2 diabetes mellitus, offering enhanced glycemic control, low hypoglycemic risk, and favorable tolerability. As the clinical relevance of this fixed-dose combination continues to expand, the need for accurate, reliable, and validated analytical methods becomes essential for ensuring product quality, safety, and efficacy. A wide range of techniques—including spectrophotometry, HPLC, HPTLC, UPLC, GC-MS, LC-MS/MS, and capillary electrophoresis—have been developed and optimized for their simultaneous estimation in bulk drug substances, formulations, and biological matrices. Each method presents unique advantages in terms of sensitivity, selectivity, reproducibility, and application scope. Collectively, these advancements not only support routine quality control and pharmacokinetic investigations but also contribute to regulatory compliance and the assurance of therapeutic effectiveness.

Future research should focus on further refining multi-analyte approaches and integrating high- throughput, cost-effective platforms to strengthen the analytical framework for vildagliptin– metformin combination therapy.

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