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RESEARCH

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A new gas chromatographic method for quantification of Metformin hydrochloride and Vildagliptin in bulk and pharmaceutical dosage form: development and validation

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Abstract

Background Metformin, an antidiabetic drug, assists in reducing the creation of glucose inside the liver. Vildagliptin, a DPP-4 inhibitor, enhances insulin release from the pancreas and reduces the hormones that elevate blood sugar levels. The combined medications work synergistically to lower blood sugar levels. This study was designed to develop and validate a reliable method of simultaneous assessment of Metformin and Vildagliptin in bulk and pharmaceutical dosage forms. For the chromatographic separation, a Gs-Tek INNOWAX column was utilized. This column has a length of 30 m, an internal diameter of 0.25 mm, and a 1.8 µm film thickness. For the detection, a Flame Ionization Detector was utilized. The ideal conditions included an injection volume of 1 µL with a split mode of 10 to 1 ratio, a flow rate of 1 mL/minute for the nitrogen carrier gas, an injector temperature of 300 °C, a detector temperature of 250 °C, an initial oven temperature of 100 °C that was maintained for seven minutes and then programmed to climb at a rate of 10 °C per minute up to a temperature of 300 °C.

Results A gas chromatographic method that is simple, precise, accurate, robust, and reliable has been developed and implemented for the simultaneous estimation of Metformin and Vildagliptin in the tablet dosage form. The retention time for Metformin and Vildagliptin was 10.203 and 22.021 min. respectively. Validation studies were performed on the method's Linearity, detection limit (LOD), and quantitation limit (LOQ), as well as its accuracy, precision, system suitability, and robustness, using the norms established by the International Conference on Harmonization (ICH). The mean recovery value for Metformin and Vildagliptin was 100.31% (% R.S.D. = 0.6743%) and 100.33% (% R.S.D. = 0.6900%). All the results are within the acceptable range.

Conclusion Validation of the developed method revealed that all the results were within an acceptable range, and techniques can be employed to analyze these two medications in combined dosage forms. It is the first method used for simultaneous estimation of these two drugs.

Keywords Metformin, Vildagliptin, Gas chromatography, Validation, ICH, System suitability

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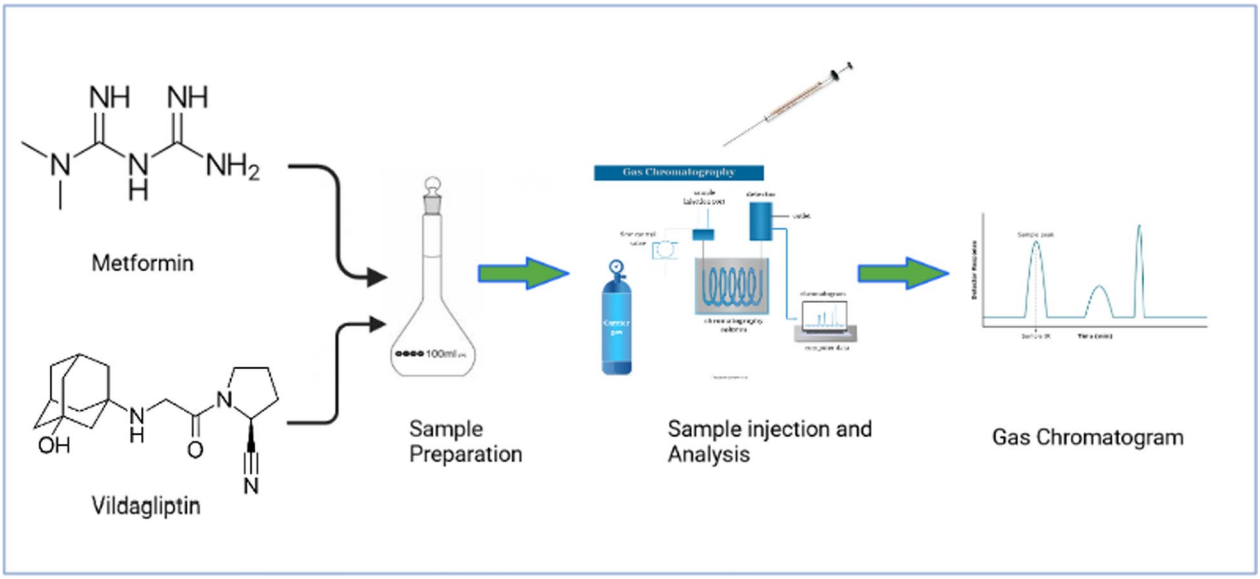
Graphical abstract



Background

Today's worldwide disease, Type 2 Diabetes Mellitus (T2DM), is treated by using drugs like Metformin (MET) and Vildagliptin (VID) [1]. Metformin, an antidiabetic drug, assists in reducing the creation of glucose inside the liver. Vildagliptin, a DPP-4 inhibitor, enhances insulin release from the pancreas and reduces the hormones that elevate blood sugar levels. In Fig. 1, the chemical structures of drugs are shown.

The combined medications work synergistically to reduce blood sugar levels. Patients with type 2 diabetes (T2DM) who take Vildagliptin, an orally active, potent, and selective inhibitor of dipeptidyl peptidase-IV (DPP-4), see improvements in their glycemic control, primarily because of improved pancreatic islet function [2]. In summary, Vildagliptin has been reported to enhance insulin levels and reduce the elevated glucagon release in type 2 diabetes people when paired with Metformin, a thiazolidinedione, a sulfonylurea, or insulin. Metformin has been used to treat diabetes for 50 years, and although it has a distinct mode of action and does not resolve ß-cell dysfunction, it is still the primary medication advised by all guidelines [3].

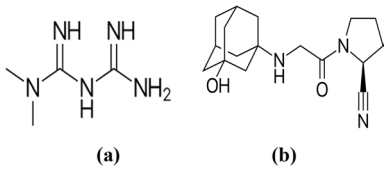


Fig. 1 Chemical structure of **a** Metformin and **b** Vildagliptin

The multiple pathophysiological anomalies and progressive nature of T2DM need various intensification techniques over time. As per recent guidelines, patients need therapeutic combinations much earlier to reach and maintain the ever-stricter glycemic goals. Adverse effects like hypoglycemia can be avoided with careful medicine selection. Metformin and Vildagliptin have merits over conventionally used combinations because they do not raise the probability of weight gain or induce hypoglycemia [4–6]. Compared to other diabetic drugs, the safety and tolerability profiles of the combination of Vildagliptin and Metformin are exceptional, and they appear to have positive effects on beta-cell activity [7, 8].

Various analytical methods were used to analyze Metformin when present alone or combined with other medications, including U.V. [9–12], HPLC [13–16], HPTLC [17] and GC techniques [18]. The analysis of the relevant literature showed that Vildagliptin, either on its own or in combination with other pharmaceuticals, has been evaluated by U.V. [18], HPLC [19], HPTLC [20, 21], and GC–MS techniques [22]. The literature study also suggests that only a few analytical methods are established for the determination of MET and VID in combined dosage forms, such as RP-HPLC [23], HPLC–MS/MS [24], and HPTLC [25]. This pharmacological combination has not yet been investigated using GC, and no attempts have been made to do so. This study proposes and validates a

new gas chromatographic approach for the simultaneous detection of MET and VID in both their bulk form and their pharmaceutical dosage form.

Methods

Instrument

The Star Chromatography Workstation version 6.41 software was used to record the data, and the HSGC—Shimadzu 2010 I GC system, which relates to a flame ionization detector (FID), was used for all experiments.

Gas chromatographic settings

The Gs-Tek INNOWAX column, which has a 30 m length, 0.25 mm I.D., and 1.8 µm df, was used for the separation. FID was used to carry out the detection. Injection volume 1 µL in split mode 10:1, nitrogen used as carrier gas at a constant flow of 1 mL/min, injector temperature 300 °C, detector temperature 250 °C, beginning oven temperature 100 °C kept for 7 min, then scheduled to climb at a rate of 10 °C/min up to 300 °C, were the optimal circumstances.

Preparation of standard stock solutions

The standard stock solution was prepared by dissolving accurately weighed 500 mg of MET and 50 mg of VID, in ethanol in a 100 mL volumetric flask. It is further diluted by taking 1 mL stock solution with 10 mL ethanol to get containing 500 µg/mL MET and 50 µg/mL VID respectively.

Assay procedure for tablet formulation

Twenty commercially available Galvus Met Novartis formulation tablets were ground adequately into powder using mortar and pestle. In a 100 mL volumetric flask, we weighed out powder equal to 500.0 mg of MET and 50.0 mg of VID, added roughly 50 mL ethanol, sonicated for 2 min to ensure appropriate dissolution, and then brought the volume up to the mark using the same solvent. Whatman No.41 filter paper was used to filter the sample solution and 5 mL of the filtrate was placed in a 50 mL volumetric flask, with the remainder of the volume filled with ethanol. The method is developed and validated with the help of this stock solution.

Validation of method

The results of the analysis, which included Linearity, precision, accuracy, detection limit (LOD), quantitation limit (LOQ), system suitability, and robustness, were statistically validated and performed following ICH guidelines Q2 (R1) [26].

System suitability

Three injections of a solution containing 500 µg/mL MET and 50 µg/mL VID were used to evaluate the system's suitability. The subsequent factors like Retention time values, peak areas, tailing factor, theoretical plates, and resolution are considered.

Linearity and range

The stock solution was diluted with ethanol to create standard working solutions with concentrations ranging from 125.0 to 750.0 µg/mL for MET and 12.5 to 75.0 µg/mL for VID. Five different concentrations of each chemical were selected using the previously established gas chromatographic conditions, and three injections were performed for each dilution. Regression equations and correlation coefficients were obtained using calibration curves to develop the Linearity of the suggested method.

Study of LOD and LOQ

The ICH guideline describes multiple methods for estimating detection limits and quantitation limits. The LOD and LOQ This study determined the proposed approach's values using Eqs. 1 and 2.

$$\mathrm{LOD} = 3.3x\sigma/s \tag{1}$$

$$\mathrm{LOQ} = 10x\sigma/s \tag{2}$$

where σ the standard deviation of the Y-intercept. s the slope of the calibration curves.

Precision

Six sample solutions ($n=6$) were produced and analyzed using gas chromatography, with concentrations of 500.0 µg/mL for MET and 50.0 µg/mL for VID, respectively, to ensure intraday accuracy. The same solution was analyzed over three days to evaluate the Interday precision.

Accuracy

Analysis of standard drug additions at three levels, i.e., multiple-level recovery experiments, were used to determine the reliability of the approach. A predetermined amount of the sample was mixed with a reference standard of varying concentrations (80%, 100%, and 120%) before being evaluated for the substance.

Robustness

The method robustness was determined by measuring the percentage relative standard deviation (R.S.D.) of retention time and peak area after the oven starting

temperature and flow rate were changed by 2.0 °C and 0.1 mL/sec, respectively.

Results

Chromatography

The INNOWAX column effectively separated drugs. As shown in Fig. 2, the retention times for MET and VID were 10.203 and 22.021 min. respectively.

System suitability

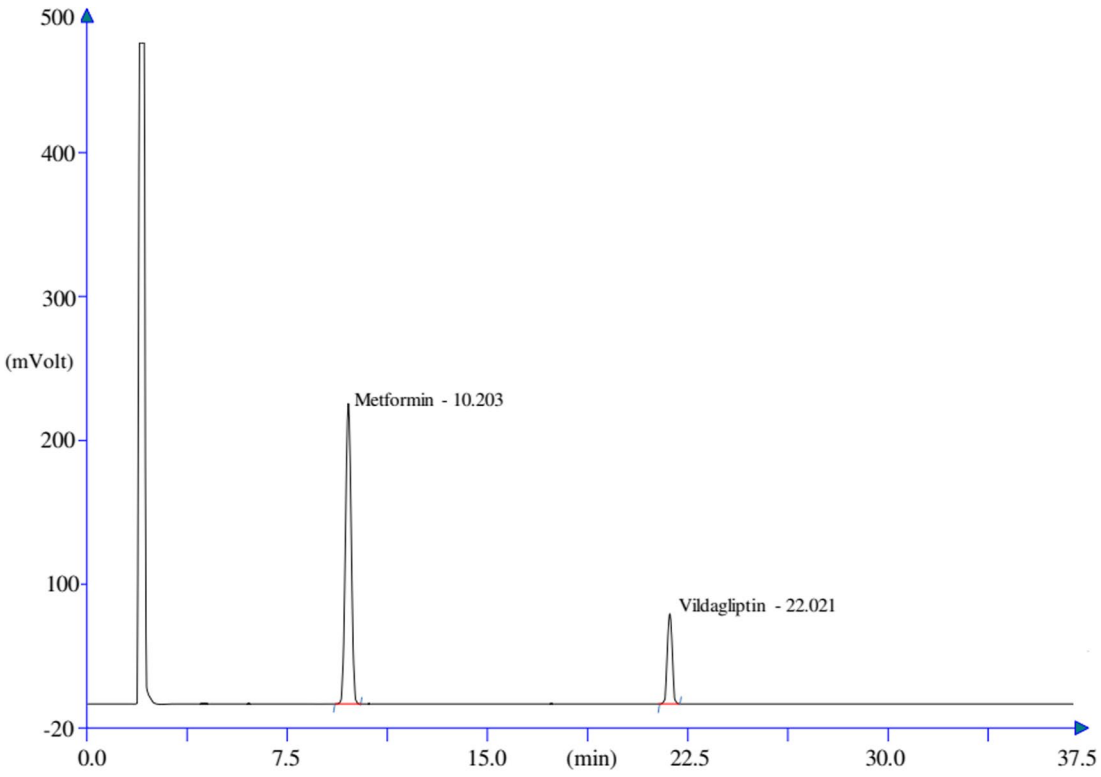
It was decided to inject five separate injections for the system suitability study. The acceptance limitations were satisfied for all the system suitability criteria, including the number of theoretical plates, the tailing factor, and the resolution. Also, the relative standard deviation (R.S.D.) of retention time values (Rt) and peak area (drug) for five separate injections does not exceed 2%, indicating that the GC system is suitable for the analysis of MET and VID combinations. Table 1 provides a concise summary of the findings.

Linearity and range

The parameters of the GC were discussed before they were used, and then the calibration curves for MET and VID were developed by plotting the detector's response against the concentration of the medications. The findings show a strong connection between the detector's response and the drug concentration. The concentration ranges and the slope, y-intercept, and correlation coefficient values for both drugs for MET and VID are provided in Figs. 3 and 4.

LOD and LOQ value

The Detection limit was found to be 12.14 µg/mL for MET and 2.44 µg/mL for VID, while the Quantitation limit was found to be 36.80 µg/mL for MET and 7.37 µg/mL for VID.



Peak Table

FID DET

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		

Fig. 2 Gas Chromatogram showing the presence of MET (500 µg/mL) and VID (50 µg/mL) in a standard solution

Table 1 System suitability parameters

Criterion	MET	VID
% R.S.D. of Rt	0.0247	0.0316
% R.S.D. of area	0.9673	0.8590
NTP	25,381	18,468
Tailing factor	1.03	1.09
Resolution	11.2	

Table 2 Study of precision

Drug	% R. S. D Intra-day	% R. S. D Inter-day
MET	0.9673	0.9663
VID	0.8590	0.8606

Precision

Table 2 presents the intraday and Interday precision % R.S.D. values, which all came in at less than 2.0%. These results indicate that the approach has an appropriate level of precision.

Study of accuracy

Multiple-level recovery experiments were conducted to conduct the three-level recovery test, consisting of conventional standard additions. The mean recovery value for MET and VID was 100.31% (% R.S.D. = 0.6743%) and 100.33% (% R.S.D. = 0.6900%), respectively. Table 3 shows the findings of the accuracy of the developed method.

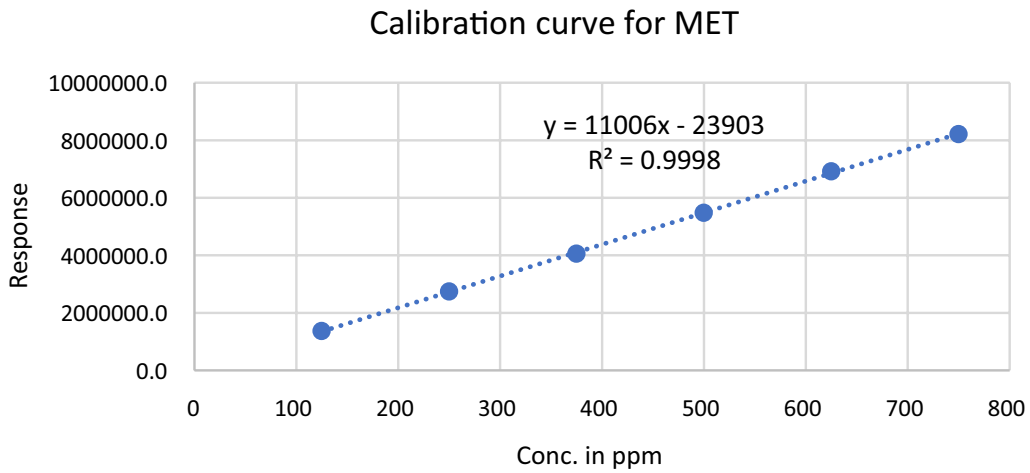


Fig. 3 Calibration curve for MET for 125, 250, 375, 500, 625 and 750 ppm

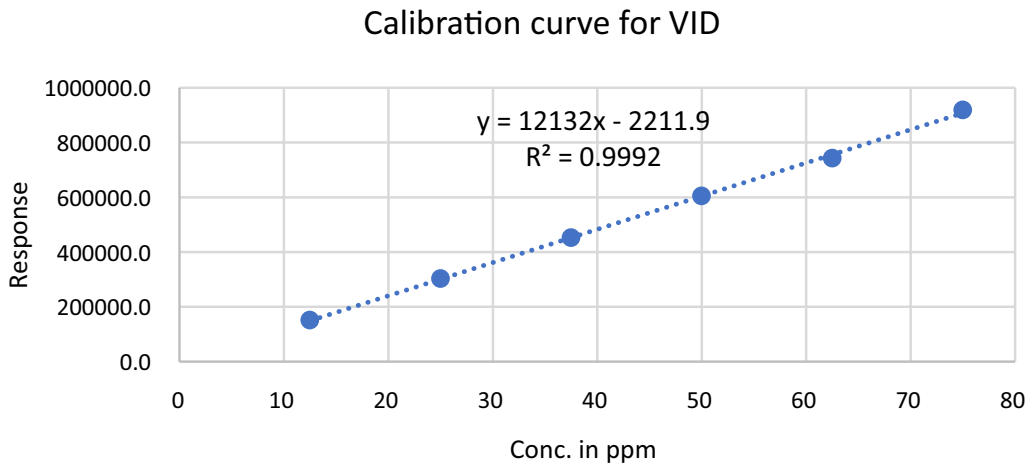


Fig. 4 Calibration curve for VID for 12.5, 25.0, 37.5, 50.0, 62.5 and 75.0 ppm

Table 3 Accuracy for the determination of M.E.T. and VID

Level of % Recovery	% Recovery*		% RSD*	
	MET	VID	MET	VID
80	100.77	99.98	0.6576	0.8466
100	99.94	100.37	0.7350	0.6625
120	100.20	100.62	0.6305	0.5610
Mean	100.30	100.32	0.6743	0.6900

* Mean of three estimations ($n = 3$)

Table 4 Robustness

Parameter changes		Retention time (Min)		Peak Area	
		MET	VID	MET	VID
The initial temperature of the oven	98 °C	10.225	22.045	5,486,025	605,845
	100 °C	10.204	22.021	5,475,845	603,654
	102 °C	10.189	21.995	5,474,554	602,965
%RSD		0.1772	0.1136	0.1147	0.2489
Flow rate (mL/min)	0.9	10.225	22.052	5,485,445	605,868
	1.0	10.205	22.018	5,475,841	603,674
	1.1	10.155	22.001	5,467,828	601,964
%RSD		0.3537	0.1179	0.1611	0.3241

Robustness

The procedure's reliability was analyzed by purposefully tinkering with the oven's beginning temperature (2.0 °C) and the flow rate (0.1 mL/min.). According to the data presented in Table 4, these adjustments did not substantially impact the retention times or peak areas.

Analysis of commercial tablet dosage form

The chromatographic approach assessed the two drugs using their combined tablet formulation (500 mg MET, 50 mg VID) as shown in Fig. 5. The analysis was carried out five times with the tablet formulation. The findings obtained using the suggested strategy are presented in Table 5. The assay results demonstrated good accuracy and precision, and the chromatograms of the tablets exhibited no interfering peaks at any point in the analysis.

Discussion

In this investigation, a novel gas chromatographic method employing the INNOWAX column was developed to simultaneously detect MET and VID in bulk and pharmaceutical dosage forms. In earlier experiments, the individual GC determination of MET and VID in a variety of samples was shown to be possible. How these two analytes are evaluated simultaneously

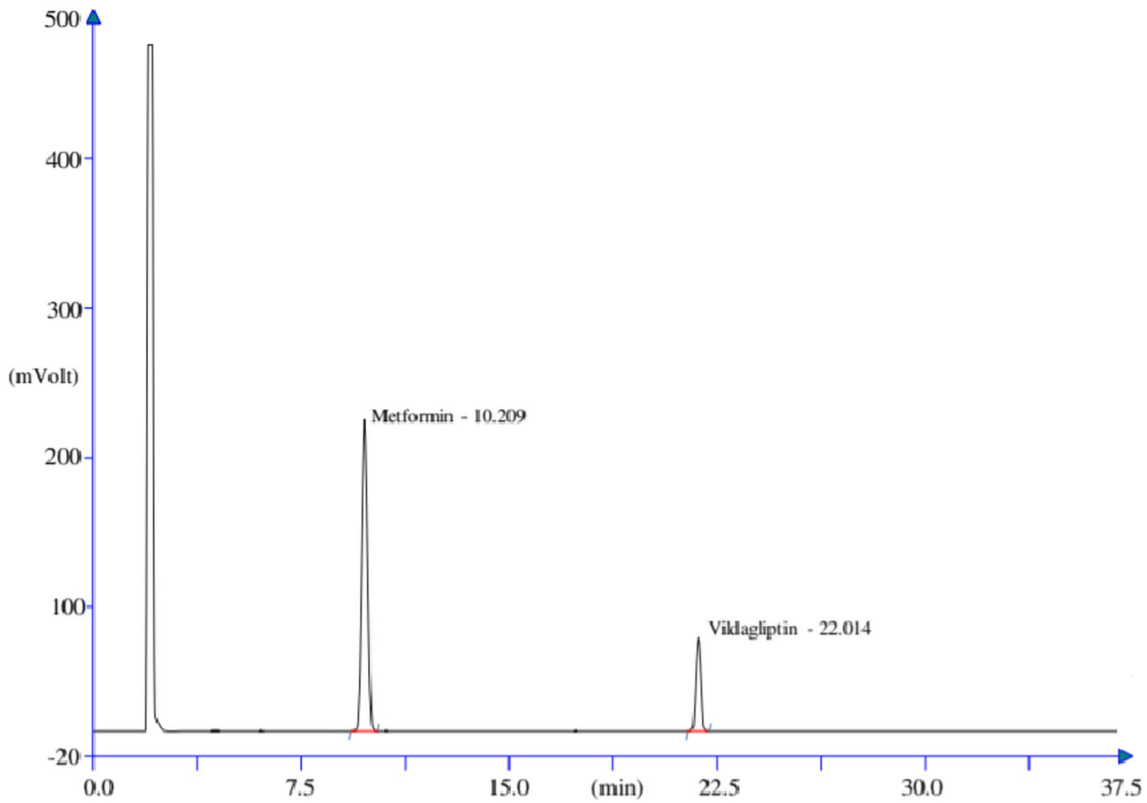


Fig. 5 Gas Chromatogram showing the presence of MET (500 µg/mL) and VID (50 µg/mL) in a sample solution

Table 5 Result of analysis of marketed formulation

Drug	Label claim (mg/tab)	The amount found (mg/tab)	Drug Found * (%)	R.S.D. (%)
MET	500	496.8	99.35	0.0964
VID	50	49.71	99.42	0.1685

* Mean of six estimations ($n = 6$)

employing GC in a pharmaceutical binary combination has not yet been documented. The established chromatographic approach mentioned here has greater specificity than methods like spectrophotometry, which does not segregate the chemicals being tested for. The HPLC techniques have a disadvantage. They require a significant amount of organic solvents, making them unfeasible for routine analysis in the pharmaceutical industry because of their high cost.

The primary purpose of this research was to establish a method that could make this determination in a quick, dependable, and direct manner with a minimum amount of sample preparation. This method does not involve any complexation or pretreatment of the medications that are the focus of the research, which contributes to its ease of use and cost-effectiveness. Also, this method supports the goal of the work that was done. The developed analytical technique has successfully passed validation regarding Linearity, range, LOD, LOQ, accuracy, and precision. The robustness of the developed analytical method was also verified.

Conclusion

The current study developed a simple, linear, precise, accurate, and robust gas chromatographic technique to evaluate MET and VID in bulk and pharmaceutical formulations. The resolution between the drugs was improved by the developed method. The procedure's performance was validated statistically per the ICH requirements, and the findings were deemed satisfactory. Also, this is the first instance that the gas chromatography technique has been invented and validated for estimating MET and VID in combination dosage form, to the greatest of our knowledge.

Abbreviations

MET	Metformin
VID	Vildagliptin
ICH	International conference of Harmonization
LoQ	Limit of detection
LoD	Limit of quantification
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
LC/MS	Liquid chromatography/Mass spectroscopy
LC–MS/MS	Liquid chromatography–Mass spectroscopy/Mass spectroscopy
R.S.D.	Relative standard deviation
U.S.P.	United Sate pharmacopoeia

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Competing interests
The authors declare that they have no competing interests.

Studies involving plants
Not applicable.

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