Biological Forum – An International Journal **15(3): 471-474(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

RP-HPLC Method Development and Validation for Determination of Metformin and Vildagliptin in Tablet Dosage Form

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(Received: 21 January 2023; Revised: 22 February 2023; Accepted: 24 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: Analytical method development involves screening various column and eluent conditions, method optimization includes iterative testing of various separation conditions of the HPLC method and is performed to achieve the best possible resolution, speed, and reproducibility, robustness testing and method validation.

This study aimed to develop fast, accurate, linear, sensitive and efficient high performance liquid chromatography [RP-HPLC] Methods for determining metformin and vildagliptin in pharmaceutical dosage forms. Chromatographic separation on a chromasil-C18 column [4.5 × 250 mm; 5 Î¼m] with a mobile phase consisting of methanol: 0.1% orthophosphoric acid (80:20) adjusted to pH 4 with orthophosphoric acid. The flow is 0.7ml/min, detection wavelength is 206nm. The peak retention times of the chromatographic conditions, metformin and vildagliptin were 1.87 min and 2.54 min, respectively. The method has been validated according to ICH Q2 R1 guidelines. Calibration curves for metformin and vildagliptin were found to be linear over the concentration ranges of 2-30µg/ml and 1-15µg/ml/ml. The detection and assay limits for Metformin and vildagliptin were established at 0.21 µg/ml/ml and 0.65 µg/ml, 0.09 µg/ml/ml and 65 µg/ml respectively. A new sensitive and simple method of reversed-phase high performance liquid chromatography [RP-HPLC] has been developed and validated for the determination of metformin and vildagliptin. This method can be used for routine dosing of vildagliptin and metformin.

Keywords: Metformin, Method validation, Reverse phase high performance liquid chromatography, vildagliptin.

INTRODUCTION

The chemical name of metformin (MTF) is [1-carbamimidamido-N,N-dimethylmethanimidamide] , is similar to oral ant diabetic biguanides. Used as a first-line drug in the treatment of non-insulin dependent diabetes. It improves glycemic control factors by reducing glucose production in the liver, reducing glucose uptake and increasing insulin-mediated glucose uptake. The therapeutic indication for metformin is second-line therapy in adults with type 2 diabetes, particularly overweight patients who fail to achieve adequate glycemic control at the maximum tolerated dose of oral metformin alone. The decrease in glucose and lipid concentrations is regulated by AMPK via activation of AMP-activated protein kinase (AMK) and the Peutz-Jeghers protein LKB1 (Inzucchi and Bergenstal 2012). Vildagliptin (VGT) [(S)-1-[N-(3-hydroxy-1-ada-mantyl) glycyl] pyrrolidine-2-carbonitrile], is a new type of oral antidiabetic belonging to the dipeptide Peptidase -4 (glucose-induced decreased secretion of glucagon-like peptide 1 and gastric inhibitory polypeptide)3 inhibitors are used as monotherapy in adults with type 2 diabetes, especially if insufficient controlled by patients' diet and exercise alone. Vildagliptin can be used as dual oral therapy in combination with metformin in patients with poor glycemic control despite metformin monotherapy at the maximum tolerated dose. Compared to sulfonylureas, vildagliptin has similar efficacy when combined with metformin and may reduce the risk of hypoglycemia without weight gain control and reduce hypoglycemia. Various methods have been developed to analyze the combination of vildagliptin and metformin and also sinle dosage form or with other combination by using HPLC and LCMS/MS methods (Abu Dayyih *et al.,* 2018; Mastan Ali and Ponnuri 2021; Jayaprakash and Senthil Kumar 2017; Patel and Patel2022; Attimarad *et al.,* 2022; Napate and Napate 2020).

Transient evaluation of these compounds by the RP-HPLC method indicated long analysis times and complex procedures; therefore, this study focused on the chromatographic analysis of vildagliptin and metformin to simultaneously analyze the inactive components of these compounds in a faster way (API) and drug dosage forms in the pharmaceutical market.

MATERIALS AND METHODS

The LC system consisted of the following components: Chromatography was performed using Spinchrom software on a Kromasil C18 measuring (250 mm × 4.6mm id) 5 micron. A Shimadzu electronic balance (AX 200) was used for weighing purposes. Analytical pure MET and VIL were obtained as gift samples from M/s Blue Cross Labs.ltd. (Mumbai, India) and M/s Mercury Laboratories Ltd. (Vadodara, India). Acetonitrile, methanol, water (E. Merck, Mumbai, India) were HPLC grade, while orthophosphoric acid (S.D. Fine Chemicals, Mumbai, India) was of analytical grade for mobile phase preparation. The Galvus Met tablet formulation containing the indicated amounts of vildagliptin 50 mg and metformin 500 mg was purchased from a local market.

Preparation of stock solution: Weigh 10 mg of metformin hydrochloride and vildagliptin, transfer to a clean and dry 20 ml volumetric flask, add 15 ml of water, sonicate until completely dissolved and dilute to volume with 500 ppm of water. Further dilute the stock solution from 2 ml to 10 ml with the mobile phase to obtain a concentration of 100 PPM. It was prepared during the flux phase of each trial and injected into the development trial.

Standard Mixture: Pipette 2 mL of each stock drug solution, transfer to a 10 mL volumetric flask and bring to volume with mobile phase to obtain 100 PPM.

Selection of analytical wavelength for HPLC method development: The analytical wavelength used for research is selected from the Q point of the spectrophotometric analysis, which is 206 nm.

Determination of metformin and vildagliptin in combined dosage forms: Weighed 20 tablets placed in a mortar and grind them into a fine powder. Mix the contents well. Weigh the powdered material (420.8 mg) equivalent to 200 mg metformin hydrochloride and 20 mg vildagliptin and transfer to a clean, dry 100 ml volumetric flask. Add 70 ml of water and leave to act for 15 minutes, shaking occasionally. After 15 minutes, allow the solution to cool to room temperature and make up to the mark with water. Filter the solution through a suitable O filter.45 Âµ syringe filter Discard 3 to 5 mL of initial filtrate. Further dilute 2.5 ml of the filtered stock solution to 50 ml with the mobile phase. (100 ppm metformin hydrochloride and 10 ppm vildagliptin), inject the resulting solution, record the chromatogram and record the results.

Accuracy: Take nine clean and dry 100 ml volumetric flasks. Weigh and transfer 200.8 mg of placebo into each 100 ml volumetric flask. The metformin hydrochloride (MTF) and vildagliptin (VGL) APIs were accurately weighed and transferred to the same 100 mL volumetric flask. Add 70 mL of water and sonicate for 15 min with intermittent shaking. Add water up to the mark. Filter the solution through a 0.45 µ PVDF syringe filter and discard 3-5 ml of the filtrate. Dilute another 2.5 ml of the filtrate to 50 ml with the mobile phase.

The accuracy of the is achieved over a range of 50% to 150% of working concentration. The solutions for each level of precision were prepared in triplicate. Calculate percent recovery for each sample, average percent recovery for each level, and total recovery, and also calculate %RSD for each level and %RSD for total recovery.

Acceptance Criteria

1. The percent recovery for each sample as well as the average and overall recoveries must be between 98 and 102%.
2. The relative standard deviation must not exceed 2.0%.

Precision: Intra-day and between-day precision studies of metformin hydrochloride and vildagliptin were performed on the same day and 3 days (day 1, day 2 and day 5) for 3 different concentrations of MTF (5) It is estimated that the corresponding response is performed 3 times. (5, 10, 15 µg/ml) and VGL (0.5, 1, 1.5 µg/ml) and report the results as relative standard deviation (RSD, Table 2). Reproducibility studies were performed by evaluating the responses of 3 different concentrations of MTF (5, 10, 15 µg/ml) and VGL (0.5, 1, 1.5 µg/ml) in triplicate, and the results are expressed in relative standard deviation (SD).

Specificity. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) are administered in pre-weighed drug amounts. Chromatograms were obtained by appropriate dilution and the amount of drug was determined.

Limits of detection and limits of quantification. Standard curves were prepared using MTF in the concentration range of 2 to 30 µg/ml and VGL in the range of 1 to 15 µg/ml (limit range of expected detection). The standard deviation of the intercept of the regression line was determined and recorded in the following equation, which was used to determine the limit of detection and the limit of quantification. Detection limit= 3.3σ /s; quantitation limit= 10σ/s; where σ is the standard deviation of y intercepts of regression lines and s is the slope of the calibration curve.

Robustness: The robustness of the method was studied by varying the composition of the organic phase by ±% and by varying the pH by ±0.2. It is also possible to observe the stability of the drug in the mobile phase at a temperature of 35o for 24 hours.

RESULTS AND DISCUSSION

Mobile phase optimization was performed based on the resolution, asymmetric factor and peak area obtained for MTF and VGL. Adjustment of methanol: 0.1% orthophosphoric acid (80:20) mobile phase to pH 4 proved satisfactory and provided two symmetrical and well-separated peaks for MET and VIL. MTF and VGL have a retention time of 1.87 minutes and 2.54 minutes, respectively (Fig. 1). The asymmetry factors

for MTF and VGL are 1.30 and 1.48, respectively. The overlaid UV spectra of MTF and VGL showed significant absorption at 206 nm for both drugs, therefore 206 nm was chosen as the detection wavelength in LC.

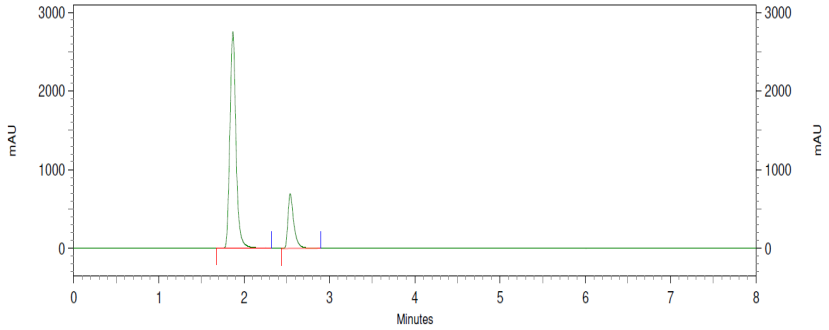

Fig. 1. Typical chromatogram of Metformin and Vildagliptin.

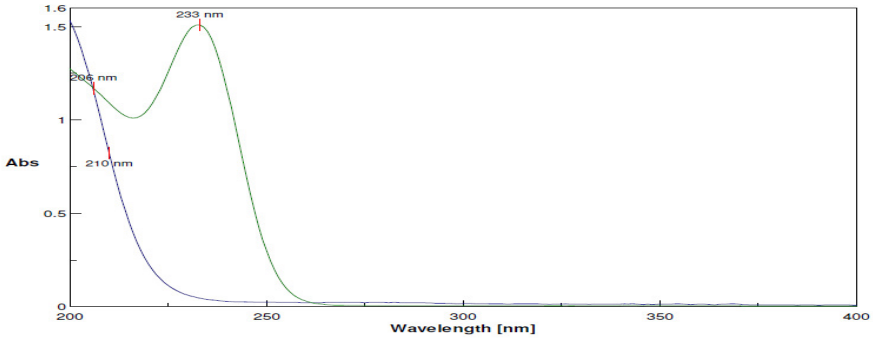

Fig. 2. Overlay UV spectrum of Metformin hydrochloride & Vildagliptin.

The calibration curve for MTF was obtained by plotting the MTF peak area against the MTF concentration in the range of 2-30 µg/ml and was found to be linear with r=0.9982. Similarly, a calibration curve for VGL was obtained over the range of 1-15µg/ml and was found to be linear with r = 0. 9993.The detection limits for MTF and VGL were 0.09 µg/ml and 0.21 µg/ml respectively. The quantification limits for MTF and VGL were 0.28µg/ml and 0.65 µg/ml, respectively, indicating that nanogram quantities of these two compounds can be accurately estimated. Table 1 summarizes the validation parameters.

The recoveries of MTF and VGL are between 98.02 to100.68% and 98%.38 to 101.42%, respectively. The system suitability test parameters are shown in Table 2. The content of MTF and VGL in the dosage form of the compound was determined by liquid chromatography. The results for MTF and VGL are comparable to the corresponding labeled amounts (Table 3).

Table 1: Summary of validation parameters.

Parameters	MTF	VGL
Detection limit (µg/ ml)	0.09	0.21
Quantitation limit (µg/ ml)	0.28	0.65
Accuracy (%)	98.02-100.68	98.38-101.42
Precision (RSDa, %)		
Intraday (n=3)	0.97-1.42	0.84-0.65
Interday (n=3)	1.01-1.82	0.71-1.21
Repeatability (RSDa,n=3)	0.59-0.95	0.26-0.34

Table 2: System suitability test parameters for MET and VIL by the proposed method.

System suitability Parameters	MTF	VGL
Retention time (min)	1.87	2.54
Resolution	5.89	-
Theoretical plates	3371	8778
Tailing factor (asymmetric factor)	1.22	1.44

Table 3: Assay results of combined dosage form using proposed method.

Trial No.	Labelled amount (mg)		Amount obtained (mg)b		% Recovery b	
	MTF	VGL	MTF	VGL	MTF	VGL
1	100	10	100.15	10.18	100.15	101.8
2	100	10	100.33	10.09	100.33	100.9
3	100	10	101.62	10.2	101.62	102

DISCUSSION

In most of literature analytical method development for metformin and vildagliptin was carried out by using acetonitrile: phosphate buffer (pH 6.0): water (65: 20:15v/v/v) as a mobile phase at a flow rate of 1.0 ml/min. Acetonitrile is expensive solvent and flow rate is also higher i.e. 1ml/min. whereas we had developed method by using methanol: 0.1% orthophosphoric acid (80:20) adjusted to pH 4 with orthophosphoric acid. The flow is 0.7ml/min, detection wavelength is 206nm comparatively methanol is less expensive. The method developed was simple, robust, accurate, and resolution of peak was good and which can be used commercially.

CONCLUSIONS

The study presented in describes a new RP-HPLC method for estimating MTF and VGL combinations in mixtures using single mobile phases with low buffer concentrations compared to reported methods. The method provided good resolution between the two compounds with short analysis times (<10 minutes). The method has been verified to be simple, sensitive, accurate and precise. The recoveries indicated that the method was not interfered with by the excipients used in the formulation. Therefore, this method can be used for routine analysis of MTF and VGL in compound dosage forms.

FUTURE SCOPE

The method which is developed for metformin and vildagliptin is a simple, rapid with low cost and can be commercially used in industry for the combination.

Acknowledgements. The author thanks to Dr. S.B. Bhawar, Principal Pravara Rural college of Pharmacy Pravaranagar, for his encouragement in carrying out this work.

Conflict of Interest. None.

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How to cite this article: Godge R.K., Dhanvate G.S., Shinde G.S. and Mankar S.D. (2023). RP-HPLC Method Development and Validation for Determination of Metformin and Vildagliptin in Tablet Dosage Form. *Biological Forum – An International Journal, 15*(3): 471-474.