RESEARCH ARTICLE

Development and Validation of New Stability Indicating Analytical RP-HPLC Method for Simultaneous Estimation of Metformin and Alogliptin

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Received: 20th December, 2023; Revised: 16th February, 2024; Accepted: 30th June, 2024; Available Online: 31st August, 2024

ABSTRACT

The current study focuses on developing and validating a reverse phase-high performance liquid chromatography (RP-HPLC) method for quantifying metformin HCL (MET) and alogliptin (ALO) in both dosage and bulk drug forms. ALO and MET in tablet form were determined simultaneously using an RP-HPLC technique. The Reverse Phase (Waters) C18 (4.6 mm x 150 mm; 5 µ) column, a 20-injection loop are the components of the gradient system for the RP manufactured by Agilent Technologies. In the procedure, a 50:50 volumetric combination of methanol and water (0.1% acetic acid) was used as the mobile phase at pH 4.5. The developed approach yielded retention times of 3.856 minutes for MET and 6.302 minutes for ALO. The reliability of the established technique was demonstrated in compliance with the International Council of Harmonization (ICH) standards. In general, all of the metrics including linearity, accuracy, range, and robustness, were found to be within the acceptable ranges. Consequently, the methodology was assessed to be simple, precise, cost-effective, and replicable. Hence, the analysis of MET and ALO in both bulk medication and formulated states can be regularly conducted utilising the procedures specified for the purpose of quality control.

Keywords: Metformin, Alogliptin, ICH guidelines, RP HPLC, Validation.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.06

How to cite this article: Jadhav PB, Mansuri T, Bairagi VA, Ahire SB. Development and Validation of New Stability Indicating Analytical RP-HPLC Method for Simultaneous Estimation of Metformin and Alogliptin. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1137-1143.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The growing population suffered from type 2 diabetes mellitus (T2DM) has made it necessary to create efficient pharmacological therapies to control this long-term illness.[1] Metformin (MET) and alogliptin (ALO) are two such drugs that have demonstrated significant efficacy in controlling blood glucose levels in T2DM patients. MET, a type of biguanide, primarily lowers blood sugar by enhancing insulin sensitivity.[2] On the other hand, ALO enhances the incretin system, increasing insulin secretion and decreasing glucagon release in a glucose-dependent manner.[3] The combination of these two drugs offers a synergistic approach to diabetes management, providing enhanced glycemic control with complementary mechanisms of action.

Given the widespread use of metformin and alogliptin in combination therapy, there is a critical need for robust and reliable analytical methods to ensure their stability and efficacy. Stability-indicating methods are essential for assessing the stability of pharmaceutical compounds under various conditions, including exposure to light, heat, and humidity, as well as during storage and handling. By detecting degradation products, these techniques assist in maintaining the safety and efficacy of the medications for the duration of their shelf life. The pharmaceutical business uses high-performance liquid chromatography (HPLC) because of its excellent resolution, sensitivity, and specificity.[4] Reverse-phase HPLC (RP-HPLC), in particular, is preferred for its ability to separate and quantify components in a mixture based on their hydrophobicity.[5]

A review of the literature indicated that several analytical techniques, such as UV spectroscopy and HPLC, have been described for the quantification of MET and ALO alone or in combination with other medications.[6] For the measurement of these two medications, the RP-HPLC technique yielded a stability indicator number. Stability indicator methods are now considered crucial from a regulatory and C-GMP perspective for evaluating the stability of pharmaceuticals.[7] For the simultaneous measurement of metformin and alogliptin, it is imperative to design and validate a novel stability-indicating analytical RP-HPLC technique for many reasons.

Firstly, a combined method reduces the time and resources required for analysis, enhancing efficiency in quality control processes.[8, 9] Secondly, it provides comprehensive data on the

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stability of both drugs when used together, which is essential for ensuring their combined therapeutic efficacy. Thirdly, such a method can identify potential interactions between metformin and alogliptin or their degradation products, thereby improving the safety profile of the combination therapy.

Standard samples of metformin and alogliptin, as well as their combinations in medication formulations, will be used to validate the suggested approach. Specificity will be assessed by ensuring that the method can accurately distinguish between the drugs and their degradation products. Linearity will be evaluated by preparing calibration curves over a range of concentrations. The limit of detection (LoD) and limit of quantitation (LoQ) will be established based on signal-to-noise ratios, and robustness will be tested by making deliberate variations to the chromatographic conditions and observing the effects on method performance.[9]

By offering this research work for the simultaneous measurement of MET and ALO, this research will make a substantial contribution to the area of pharmaceutical analysis. This approach will make it easier to monitor and guarantee the quality of these medications when used in combination therapy, guaranteeing their stability, safety, and efficacy. Moreover, the development of this method aligns with the growing trend towards more efficient and comprehensive analytical techniques in the pharmaceutical industry, ultimately benefiting patients by ensuring the availability of high-quality, stable medications for the management of T2DM.

MATERIALS AND METHODS

Chemicals and reagents

MET and ALO were received from Swapnroop drug and pharmaceutical. HPLC grade methanol, acetonitrile, was purchased from Merck Ltd., India. HPLC grade formic acid (FA) was purchased from Loba Chem Pvt. Ltd. Aloja-M Tablets were purchased from local pharmacy.

Instrumentation

Agilent Tech was utilized for the drug analysis. Gradient System with Gradient Detector and Auto Injector (DAD). outfitted with UV730D Absorbance detector, Reverse Phase (Agilent) C18 column, and Chemstation 10.1 software.

Chromatographic conditions

Chromatographic separation was tried using different mobile phases in different volume ratios at different flow rates. The sample analysis was performed at 240 nm using a UV730D Absorbance detector detector.

Methods:

Analytical method development

- *Preparation of buffer*

A solution containing 0.006M monobasic sodium phosphate and 0.032M anhydrous dibasic sodium phosphate was prepared in 1000 mL of water and thoroughly mixed while being constantly stirred. 250 mL of this buffer was diluted up to 1000 mL using water and pH was maintained at 7.6 using phosphoric acid. Trials of mobile phases are presented in Table 1. Various mobile phase trials are summarized in Table 1.

Preparation of metformin standard stock solution (SSS): (Stock I)

About 500 mg of precisely weighed MET was dissolved in 100 mL of volumetric flask volumetric flask and volume increased to 10.0 mL to yield a 5000 µg/mL solution.

Preparation Alogliptin standard stock solution (SSS): (Stock II)

A precisely measured 12.5 mg of ALO was dissolved in 100 mL of methanol in a volumetric flask, and the volume was increased to 100 mL to yield a 125 µg/mL solution.

Preparation of std. Metformin and Alogliptin solution: (Stock III)

A volume of 0.1 mL from SSS (5000 and 1.25 µg/mL) was pipetted into a 10 mL volumetric flask followed by dilution up to 10 mL with the mobile phase, resulting in final concentrations of 1.25 µg/mL of alogliptin and 50 µg/mL of metformin.

Method validation

The analytical technique was validated in accordance with ICH guidelines. Numerous factors, including limit of detection, accuracy, linearity, robustness, specificity, precision, and limit of quantification.[10-12]

Linearity and range

Plotting the calibration curve served as validation for the method's linearity and range.[13] Until a stable baseline was reached, the mobile phase and stationary phase were allowed to equilibrate. Concentrations ranging from 1.25 to 6.25 µg/mL for ALO and 50 to 250 µg/mL for MET were injected, and the peaks were measured at 240 nm.

System suitability

Data was gathered from five identical injections of the standard solution to conduct the test.[14]

Accuracy

MET and ALO (equal to 500 mg of MET and 12.5 mg of AlO, or 80, 100, and 120% of the label claimed, respectively) were used to calculate the accuracy of the dosage in relation to the average weight of marketed tablets. After being triturated, this powder mixture comprising 500 mg of metformin and 12.5 mg of alogliptin was analyzed chromatographically using

Table 1: Different trials of mobile phases

S. No.	*Mobile phase*	*Ratio*	*Flow (mL/min)*
1	Methanol: water (0.1% FA)	90:10% v/v	0.7
2		80:20% v/v	0.7
3		75:25% v/v	0.7
4		60:40% v/v	0.8
5		40:60% v/v	0.8
6	Methanol: Water 0.1% FA PH 4.5	40: 60% v/v	1

the previously mentioned technique. Over the course of three days, the resultant combinations were examined in triplicate.[12]

Repeatability

The RP-HPLC method sample was used to assess the system's precision. Peak areas were assessed and the percentage RSD was computed after six duplicates of the sample solution containing 500 mg of MET and 12.5 mg of ALO were injected.[12]

Precision

Intra-day precision

The percentage R.S.D. was calculated after three separate analyses of sample solutions containing 500 mg of MET and 12.5 mg of ALO at three different concentrations (50, 100, 150 µg/mL for MET and 1.25, 2.5, 3.75 µg/mL for ALO) on the same day.[12]

Inter-day precision

Sample solutions containing 500 mg of MET and 12.5 mg of ALO were prepared on separate days, with the percentage R.S.D. computed for each concentration (50, 100, 150 µg/mL for MET and 1.25, 2.5, 3.75 µg/mL for ALO). This variability is typically expressed using standard deviation or relative standard deviation.[12]

Robustness

The composition of the mobile phase, consisting of methanol and FA in water (40:60), was varied by ± 1 mL/min, along with the flow rate and the detection wavelength. The impact of these variations was investigated. Solutions containing 600 µg/mL of MET and 15 µg/mL of ALO were used in triplicate for the analysis.[12]

LoD and LoQ

The following formulae, 1 and 2, were used to derive LoD and LoQ.[12]

$$LoD = \frac{3.3 \times \text{SD of y-intercept}}{\text{Slope of a calibration curve}} \quad \text{----------(1)}$$

$$LoQ = \frac{10 \times \text{SD of y-intercept}}{\text{Slope of a calibration curve}} \quad \text{----------(2)}$$

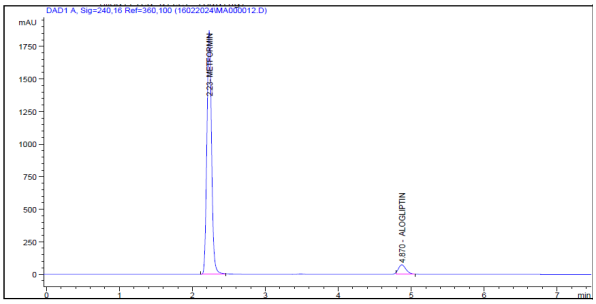

Figure 1: Representative chromatogram of metformin and alogliptin using trial 6

Analysis of marketed formulation

In order to ascertain the amount of metformin and alogliptin included in commercially available tablets (which on the label indicate 500 mg MET and 12.5 mg ALO). The tablets were ground into a powder and weighed out to be 845.3 mg. 100 milliliters of methanol were used to extract the medication from the powdered tablet. It was sonicated for fifteen minutes to guarantee full extraction. After that, 0.4 mL of supernatant was diluted with mobile phase to make 10 mL. After injecting the resultant solution into an HPLC, the drug peak area was observed. Peak regions of standard solutions were used to create the regression equation.

RESULT AND DISCUSSION

Analytical Method Development and Optimization

Several development trials were conducted using an isocratic method and chromatographic behavior was observed. Table 2 summarises the observations of various developmental trials.[15]

The aforementioned data indicates that the use of a mobile phase consisting of methanol and water (0.1% formic acid) (40+60% v/v), 240 nm, 1-mL, pH 4.5 (Trial 6), produced a peak shape and an adequate retention time of 2.231 and 4.870 minutes (Figure 1) for theoretical plates containing 5862 of metformin and 10730 of alogliptin.

The optimized chromatographic conditions of the analytical method development are summarised in Table 3.

Theoretical plates were discovered above 2000 in the typical combination of metformin and alogliptin, or for metformin and alogliptin 4777 and at least RT 2.221 and 4.911, respectively.

Table 2: Results of various mobile phase compositions

S. No.	*Mobile Phase*	*RT (min)*		*Remark*
		Metformin	*Alogliptin*	
1.	Methanol: water (0.1% FA pH-4.5) (90:10% v/v)	2.914	4..232	Splitting
2.	Methanol: Water 0.1% FA (80:20% v/v) 0.7 mL/min	3.020	4.048	No sharp peak
3.	Methanol: Water 0.1% FA (75:25% v/v) flow 0.7 mL/min	3.075	4.093	No sharp peak
4.	Methanol: Water 0.1% FA (60:40% v/v) pH 4.5 flow 1-mL/min	2.727	4.274	No sharp peak
5.	Methanol: Water 0.1% FA (40:60% v/v) pH 4.5 flow 0.8 mL/min.	3.124	5.405	No sharp peak
6	Methanol:Water 0.1% FA (40:60% v/v) pH 4.5 flow 1-mL/min.	2.231	4.870	Sharp peak obtain

Table 3: Optimised chromatographic conditions

S. No	*Parameters*	*Conditions*
1	HPLC	Agilent
2	Column	C18 column (250 mm x 4.6 mm, 5 µm)
3	Wavelength	240 nm
5	Mobile phase	Methanol: (0.1% Formic acid)
6	Run time	10 min
7	Injection volume	20 µL
8	Flow rate	1-mL/min

Table 4: Chromatograms of standard MET and ALO

No.	*RT [min]*	*Area[mV*s]*	*TP*	*TF*	*Resolution*
1	2.221	4660.8754	6710	0.80	-
2	4.911	208.700	10403	0.79	17.85

The details are summarised in Table 4.

Method Validation

Linearity and calibration curve

After undergoing linear regression analysis, the calibration data of MET and ALO revealed a linear connection between peak areas and concentrations between 50 to 250 µg/mL for MET and 1.25 to 6.25 µg/mL for the latter. The corresponding linear equations for MET and ALO were y = 33.56X + 3035 and y = 120.5 X + 55.44, respectively. There was a 0.999 correlation coefficient. Figures 2 and 3, respectively, show the metformin and alogliptin calibration curves.

Accuracy

To confirm the accuracy of the established approach, recovery tests were performed. Specific concentrations of the standard medication (80, 100, and 120%) were added to the pre-analyzed tablet solution, and their recovery was then examined (Table 5). The results of the recovery studies were statistically validated (Table 6). The accuracy of the RP-HPLC method was determined through recovery experiments conducted at three different concentration levels: 80, 100, and 120%. The recoveries were found to be between 98 and 102%.

Repeatability

Repeatability study results are presented in Table 7.

MET and ALO system suitability characteristics were investigated in order to evaluate the resolution and repeatability of the suggested chromatographic method for metformin measurement.[16,17] The outcome is displayed in Table 7. The percentage RSD was less than 2% in the repeatability testing conducted on RP-HPLC for metformin and alogliptin. This indicates a high percentage amount discovered in the range of 98 to 102%.

Precision

The examination of many duplicate standards of both Alogliptin and Metformin developed the methodology. To document any intra-day & inter-day variations in the final outcome, every solution underwent three analyses. The intraday outcome is displayed in (Table 8) correspondingly. The high accuracy percentage amount of 98 to 102% in the intraday and interday precision investigations using RP-HPLC for metformin and alogliptin demonstrates a finished analytical procedure.

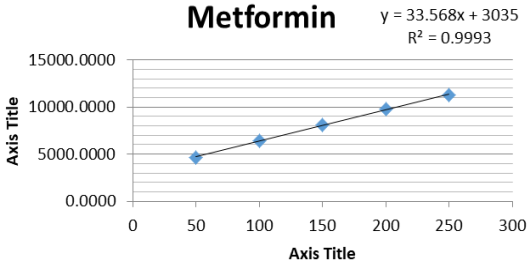

Figure 2: Calibration curve of Metformin

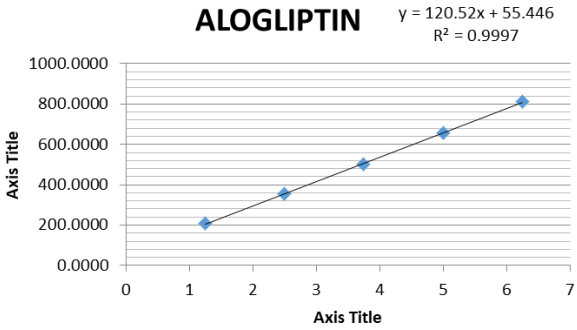

Figure 3: Calibration curve of Alogliptin

Robustness

A method is said to be robust if it can withstand slight, intentional changes in its parameters.[18] Modest but purposeful changes were made to the optimum technique parameters in order to assess the robustness of the suggested approach. Changes in the wavelength, content, and flow rate of the mobile phase were examined for their effects on the retention period and tailing factor of the drug peak. Tables 9 and 10 present the findings from the robustness studies. Since robustness parameters were also determined to be adequate, the analytical approach would be finished.

LoD and LoQ

The lowest detectable limit is known as the LoD.[19] The analytical approach revealed that the LoDs of alogliptin and metformin were 0.2145 (µg/mL) and 10.84 (µg/mL), respectively. The lowest concentration that is quantifiable is known as the limit of quantification, or LoQ.[19] Following an analytical approach, the LoQ of both metformin and alogliptin were determined to be 32.86 and 0.6500 µg/mL, respectively.[20,21]

Analysis of marketed tablet formulation

To make sure the marketed formulation would function as intended, the designed and validated analytical method was applied to analyze it. Table 11 displays the analysis's findings. A review of the commercial formulation revealed that 99 to 101% of the label claims were true.

Table 5: Result of Recovery data for Metformin and Alogliptin

Drug	*Level (%)*	*Amt. taken (µg/mL)*	*Amt. Added (µg/mL)*	*Area Mean* ± S.D.*	*Amt. recovered Mean * ± S.D.*	*%Recovery Mean * ± S.D.*
MET	80	50	40	90.75 ± 0.02	40.75 ± 0.02	101.88 ± 0.6
	100	50	50	101.1 ± 0.04	51.1 ± 0.044	102.2 ± 0.09
	120	50	60	109.8 ± 0.10	59.80 ± 0.10	99.67 ± 0.18
ALO	80	1.25	1	2.25 ± 0.005	1.00 ± 0.005	99.94 ± 0.55
	100	1.25	1.25	2.47 ± 0.117	1.22 ± 0.117	99.99 ± 1.43
	120	1.25	1.5	2.73 ± 0.004	1.48 ± 0.004	98.36 ± 0.27

*Mean of each 2 reading for RP-HPLC method

Table 6: Recovery Studies statistical validation data of MET and ALO

Method	*Level of Recovery (%)*	*Drug*	*Mean %Recovery*	*Standard Deviation**	*%RSD*
RP-HPLC Method	80	MET	101.88	0.06	0.06
		ALO	99.94	0.55	0.55
	100	MET	102.27	0.09	0.09
		ALO	99.99	1.43	1.43
	120	MET	99.67	0.18	0.18
		ALO	98.36	0.27	0.27

*Denotes average of three determinations for RP-HPLC

Table 7: Repeatability study results

Method	*Conc of Metformin and Alogliptin (mg/mL)*	*Peak area*	*Amount found (mg)*	*%amount found*
RP-HPLC method for metformin	150	8149.77	153.12	102.08
	150	8150.282	155.72	102.10
		Mean	154.50	102.9
		SD	0.73	0.73
		%RSD	0.01	0.01
RP-HPLC method for alogliptin	3.75	517.420	3.81	101.50
	3.75	510.770	3.76	101.48
		Mean	3.79	101.49
		SD	4.70	4.70
		%RSD	0.91	0.91

Table 8: Intraday and inter day precision study data

Method	*Drug*	*Conc^n (µg/mL)*	*Intraday precision*		*Interday precision*	
			Mean ± SD	*%Amt Found*	*Mean ± SD*	*%Amt found*
Rp- HPLC METHOD	Metformin	50	4692.29 ± 1.78	100.20	4698.74 ± 0.58	100.58
		100	6426.86 ± 25.15	101.78	6444.90 ± 6.72	102.32
		150	8161.11 ± 12.47	102.31	8150.74 ± 0.82	102.10
	Alogliptin	1.25	210.41 ± 1.53	102.88	210.26 ± 1.26	102.78
		2.5	354.88 ± 0.90	99.40	355.01 ± 0.91	99.44
		3.75	509.20 ± 0.93	100.42	509.81 ± 0.62	100.55

*Mean of each 3 reading for RP-HPLC

Table 9: Robustness study data of MET

Parameters	*Conc (µg/mL)*	*Amount detected)*	*%RSD*
Flow changes 0.9 mL	150	8105.75 ± 0.03	0.0
Flow changes 1.1 mL	150	8113.14 ± 2.86	0.04
Chromatogram of comp change 39 mL Meoh+61 mL formic acid Water	150	8161.637 ± 0.59	0.01
Chromatogram of comp change 41 mL methanol+59 mL formic acid Water	150	8137.41 ± 46.73	0.57
Chromatogram of comp change wavelength change 239 nm	150	8152.5 ± 4.43	0.05
Chromatogram of composition changes wavelength change 241 nm	150	8189.42 ± 1.12	0.01

Table 10: Robustness study data of ALO

Parameters	*Conc.(µg/mL)*	*Amount detected*	*%RSD*
Flow change 0.9 mL	3.75	618.88 ± 0.80	0.13
Flow change 1.1 mL	3.75	506.44 ± 6.82	1.35
Chromatogram of comp change 39 mL Meoh+61 mL formic acid water	3.75	591.672 ± 0.96	0.16
Chromatogram of comp change 41 mL Methanol+59 mL formic acid water	3.75	565.47 ± 1.62	0.29
Chromatogram of comp change wavelength change 239 nm	3.75	581.2 ± 1.40	0.24
Chromatogram of comp change wavelength change 241 nm	3.75	484.47 ± 0.93	0.19

Table 11: Marketed formulation study data

Drug	*Label claim*	*Amt. found*	*% Label claim*	*SD*	*%RSD*
Metformin	200	204.01	102.23	0.19	0.13
Alogliptin	5	20.24	101.24	0.42	0.14
Metformin	200	204.49	102.24	0.19	0.13
Alogliptin	5	20.15	100.79	0.42	0.414

CONCLUSION

An RP-HPLC method for quantitative detection of MET and ALO in both bulk and dosage forms was successfully developed and validated during the investigation. Utilizing a Waters C18 column, a methanol-water (0.1% acetic acid) mobile phase, and UV detection at 223 nm, the method demonstrated precise retention times of 3.856 minutes for metformin HCL and 6.302 minutes for alogliptin. Adhering to ICH guidelines, the method showed excellent linearity, accuracy, range, and robustness, making it reliable and reproducible. The approach's simplicity, cost-effectiveness, and improved peak resolution, coupled with the shorter retention period and isocratic mode, underscore its superiority over previous methods.

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