ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 11, pp.22878-22881, November, 2022
DOI: https://doi.org/10.24941/ijcr.44281.11.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN AND EVOGLITPIN IN DOSAGE FORM

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ARTICLE INFO

Article History:
Received 18th August, 2022
Received in revised form
24th September, 2022
Accepted 15th October, 2022
Published online 30th November, 2022

Key words:

Metformin, Evogliptin, RP-HPLC, Simultaneous Estimation, Validation, ICH Guidelines.

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ABSTRACT

A new RP-HPLC method was developed and validated for the simultaneous estimation of metformin and evogliptin in dosage form. The chromatogram of the mixed standard was found to be satisfactory on C-18 (4.6×150mm, 5µ Hypersil column) using isocratic mixture of methanol 700 ml and 300 ml water (pH adjusted to 3.0 with orthophosphoric acid) at a flow rate of 0.8 ml/min and detection wavelength of 254 nm. The retention time of metformin and evogliptin were found to be 2.548 min and 2.107 min respectively. The system suitability parameters justify that the proposed method is suitable for simultaneous estimation of metformin and evogliptin with the theoretical plates for separation being 5633 and 4154 respectively. The method was found to be linear in the range of 10-50µg/ml for metformin and 1 to 5µg/ml for evogliption. The precision of the method was good and the recovery of drugs is well within the acceptance limits of 80-120%. The LOD was found to be 0.058 µg/ml for metformin and 0.015 µg/ml for evogliptin while the LOQ ws found to be 0.178 µg/ml for metformin and 0.047 µg/ml for evogliptin.

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Citation: ***Rupali Bagde, S Karole, Anup K Chakraborty and Kavita R Loksh.*** **2022.** "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin and Evoglitpin in Dosage Form*". International Journal of Current Research*, 14, (11), 22878-22881.

INTRODUCTION

Evogliptin (Figure 1) is a novel Dipeptidyl peptidase-4 (DPP-4) inhibitor used in treatement of type 2 diabetes via stimulation of glucose-mediated incretin secretion.[1] It has a chemical formula of $C_{19}H_{26}F_3N_3O_3$. Metformin (Figure 2) is a biguanide drug used in conjunction with diet and exercise for glycemic control in type 2 diabetes mellitus with molecular formula $C_4H_{11}N_5$.[2] Metformin is known to reduce the blood glucose by reducing gluconeogenesis, reducing intestinal absorption of glucose, and enhancing insulin sensitivity by increasing peripheral glucose uptake and utilization. Several HPLC based methods for estimation of metformin either alone or in combination with other drugs have been reported[3-10] in literature while only two HPLC methods were found for evogliptin.[11-12] The fixed combination of metformin and evogliptin has been approved very recently as an adjunct to diet and exercise to improve glycaemic control in adults patients with type 2 diabetes mellitus who are appropriate for co-administration of Evogliptin and Metformin. Hence no method related to the estimation of this combination has been reported till date. The objective was the present work was to develop a simple high performance liquid chromatography (HPLC) method for simultaneous estimation of metformin and evogliptin in tablets and validate the method as per ICH guidelines.

MATERIAL AND METHODS

Metformin and evogliptin pure drugs were purchased from Yarrow Pharmaceuticals, Mumbai. All solvents used were of HPLC grade while the other reagents were of analytical grade. HPLC Shimadzu, LC-10 HPLC system equipped with UV detector was used for development of the method. Electronic balance (Wensar), pH meter (Labtronics, LT-53), sonicator (Labman) and double beam UV Visible spectrophotometer (Labtronics, LT-2201) were used in the study.

***Determination of Working Wavelength*[13]:** The spectra of evogliptin and metformin were obtained in methanol using UV spectrophotometer. The isosbestic point was determined and used as the detection wavelength for the two drugs. This wavelength was found to be 254 nm.

Instrument Used for method development: Shimadzu HPLC system (LC-10AT) equipped with Rheodyne injector (20 µL loop), SPD 10 UV detector, hypersil C18 column (4.6 x 150 mm) with 5 µ particle size and Surwit N 2000 data acquisition software was used for the development and validation of the method.

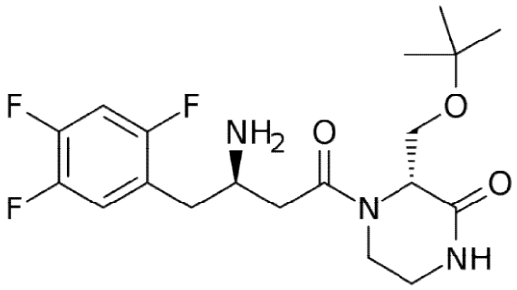

Figure 1. Chemical structure of evogliptin

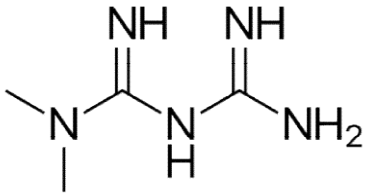

Figure 2. Chemical structure of metformin

Preparation of standard solution: Evogliptin (1 mg) and metformin (10 mg) were accurately weighed and transferred into a 10 ml clean, dry volumetric flask and about 5 ml of diluent was added and sonicated to dissolve the drugs completely and the volume was made up to the mark with the same solvent to obtain the standard stock solution. 1 ml of the stock solution was pippetted out from the above stock solution into a 10 ml volumetric flask and the volume was made up to the mark with the diluent to obtain the working standard solution. A series of dilutions of the working standard were prepared to obtain concentrations of 1 to 5 µg/ml of evogliptin and 10 to 50 µg/ml of metformin. These solutions were analyzed using the optimized chromatographic conditions to create the calibration curve.

Tablet sample for analysis: Tablet powder equivalent to 1 mg evogliptin and 10 mg metformin was accurately weighed and transferred into a 100 ml clean dry volumetric flask and about 35 ml of diluent was added and sonicated to dissolve the drug completely and the volume was made up to the mark with the same solvent. 5 ml of this solution was pipetted out in to a 50 ml volumetric flask and diluted up to the mark with mobile phase. An aliquot of this solution was injected into HPLC system.

Optimized conditions: Mobile phase consisted of isocratic mixture of methanol 700 ml and 300 ml water (pH adjusted to 3.0 with orthophosphoric acid) and chromatographic conditions include:

Column: Octadecylsilane (ODS) (4.6 x 150mm, 5µm, Hypersil).

Flow rate: 0.8 ml per min.

Wavelength: 254 nm.

Injection volume: 20 µl.

Run time: 5 min

Validation of method[14]

The developed method was validated for various parameters like linearity, specificity, precision, accuracy, LOD & LOQ in compliance with ICH guidelines.

System suitability: The working standard solution was injected six times into HPLC system as and the chromatographic study was performed as per the developed and optimized conditions. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention times, theoretical plates and peak areas from six replicate injections.

Specificity: Standard and sample solution were prepared and injected into the HPLC system to obtain the chromatograms. Mobile phase was injected to establish the interference of blank.

Linearity: Dilutions of the working standard were prepared at five different levels (1 -5 µg/ml for evoglitin and 10-50µg/ml for metformin) by appropriate dilution with the mobile phase.

Accuracy: Accuracy of the method was determined by performing recovery studies using regular addition method at three different levels (50%, 100%, 150%). The sample solution with the added (50%, 100% and 150%) solutions were injected into the HPLC system. The amounts added, amounts estimated and the individual recovery and mean recovery values were calculated.

Precision

Repeatability: The working standard solution was analyzed by the optimized conditions in six replicates and % RSD was calculated.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision of the method, precision was evaluated on different days using the same conditions.

Limit of Detection and limit of quantification: LOD and LOQ was calculated using the signal to noise ratio and the slope of the calibration curve by the equation 1 and 2 respectivley

$$LOD = \frac{3.3\,\sigma}{S} \text{----------} \quad \text{Eq. 1}$$

$$LOD = \frac{10\,\sigma}{S} \text{----------} \quad \text{Eq. 2}$$

Where σ is the standard deviation of the calibration curve; S is the slope of the calibration curve.

Robustness: In order to evaluate the robustness of the method, deliberate changes in the flow rate were made and the drug was assayed using the proposed conditions.

RESULTS AND DISCUSSION

The overlay spectrum of metformin and evogliptin was obtained using the software attached to the UV spectrophotometer and 254 nm was found to be the isosbestic point and was selected as the working wavelength for the simultaneous estimation of both the drugs. The chromatogram was obtained using the optimized conditions (Figure 3-5). The retention time were found to be 2.107 min for evogliptin and 2.548 min for metformin during the simultaneous estimation.

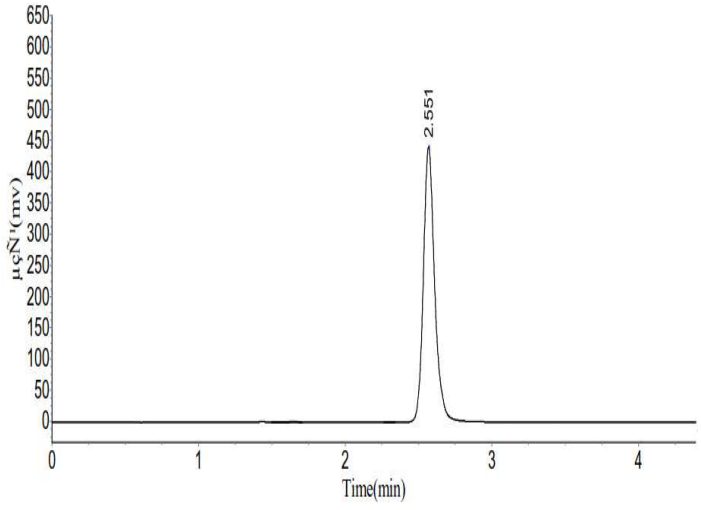

Figure 3. Chromatogram of metformin

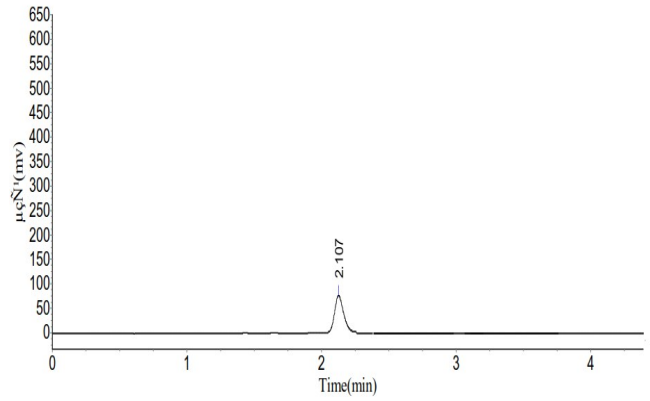

Figure 4. Chromatogram of evoglitpin

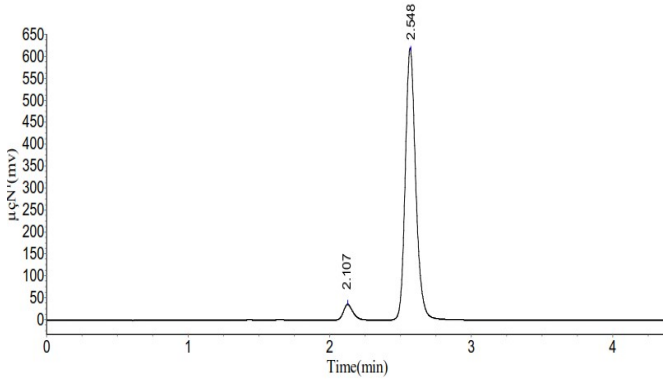

Figure 5. Chromatogram of metformin and evogliptin combination

Validation of the method

System Suitability: The % RSD of retention time was less than 2% and tailing factor and number of theoretical plates were found to be satisfactorily within the limits for both Metformin and Evogliptin (Table 1). Hence the selected system parameters were found to be suitable for the simultaneous estimation.

Table 1. system suitability parameters

System suitability parameters	Metformin	RSD	Evogliptin	RSD
Retention Time	2.551	0.002	2.104	0.001
No.of Theoretical plates	5633		4154	

Linearity: The linearity range was found to be from 10 to 50 µg/mL for metformin and 1 to 5µg/ml for evogliptin (Table 2).

Table 2. Linearity

Concentration (µg/ml)	Mean AUC* (Metformin)	Concentration (µg/ml)	Mean AUC* (Evogliptin)
10	593932	1	41534
20	1096789	2	87107
30	1779806	3	129894
40	2264621	4	173498
50	2872078	5	217769
Correl Coeff (r^2)	0.998	Correl Coeff (r^2)	0.999
Slope (m)	57301	Slope (m)	43644
Intercept (c)	2003	Intercept (c)	-808.5

*Average of six replicates

Accuracy: Accuracy of the method was determined by recovery analysis of spiked samples. Pre-analyzed sample was spiked with 50%, 100% and 150% of standard drug solution. The spiked sample was recovered within a range of 99.4-100.17% for metformin and 98.5 to 100.4% for evogliptin suggesting that the method was accurate in estimating the concentration of the drugs of mixture (Table 3).

Table 3. Accuracy

Conc. of metformin in tablet sample µg/ml)	Conc. of metformin added to final (µg/ml)	% Recovered (mean)*	Conc. of evogliptin in tablet sample µg/ml)	Conc. of evogliptin added to final (µg/ml)	% Recovered (mean)*
10	5	100.17	1	0.5	100.22
10	10	99.55	1	1	98.50
10	15	99.40	1	1.5	100.40

*Average of six replicates

Precision: Precision depicts the ability of the method produce the same results irrespective of the instrument used, or day of analysis or even the analyst performing the analysis (Table 4 and 5). The % RSD in both the repeatability and intermediate precision studies was less than 2%, confirming the method to be precise in estimation.

Table 4. Repeatability of the developed method

Concentration (mg/ml)	Metformin (10 µg/mL)	Evoglitpin (1 µg/mL)
	Retention time (min)	Retention time (min)
Mean*	2.549	2.105
SD	0.0014	0.0017
%RSD	0.055	0.0832

*Average of three replicates

Table 5. Intermediate precision

Concentration (mg/ml)	Metformin (10 µg/mL)	Evoglitpin (1 µg/mL)
	Retention time (min)	Retention time (min)
Mean*	2.545	2.103
SD	0.0151	0.0049
%RSD	0.594	0.234

*Average of six replicates

Robustness: A few deliberate changes in flow rate were made for studying its effect on the results obtained by the method. The method was able to adjust to the changes with no significant change in the retention time of the eluted components (Table 6).

Table 6. Effect of flow rate on retention time of metformin and evogliptin

Flow rate (ml/min)	Drug	Retention time (min)*	Standard deviation	% RSD
0.7 (-0.1)	Metformin	2.564	0.001	0.0587
0.9 (+0.1)		2.54	0.002	0.0787
0.7 (-0.1)	Evoglitpin	2.115	0.001	0.0716
0.9 (+0.1)		2.094	0.001	0.0875

*Average of six replicates

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ were calculated using the slope of the calibration curve obtained in the linearity study.

The lowest amount that could be detected (LOD) for metformin was found to be 0.0580µg/mL while the LOD for evogliptin was found to be 0.015µg/mL. On the other hand the lowest concentration of drug that could be quantified with accuracy (LOQ) for metformin was calculated to be 0.178µg/mL whereas for evogliptin the LOQ was calculated to be 0.047µg/mL.

Assay of marketed formulation: The validated method was applied to estimate the amount of evogliptin and metformin present in the marketed formulation (Valera M) and the result (Table 7) revealed that the percentage assay of metformin and evogliptin were 99.71 % and 99.2 % respectively. This suggests the suitability of the method for routine analysis of formulations.

Conclusion

A new RP – HPLC method for the simultaneous estimation of metformin and evogliptin was developed and validated and successfully applied for estimation of these drugs in tablet formulation. The method was simple, specific, accurate and reproducible and can be applied for routine pharmaceutical analysis.

Acknowledgements

The authors are thankful to the management of Oriental College of Pharmacy for providing the facilities to carry out the work. The authors are also thankful to Dr. Richa Mishra, Director, RB Science Research Lab, Bhopal for permitting to perform the intermediate precision studies using their HPLC system.

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