Research article

Development and validation of an RP-HPLC method for determination of Metformin and Evogliptin in bulk and pharmaceutical dosage forms

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ABSTRACT

Introduction and Aim: In this study, a new sensitive and rapid HPLC method was developed to determine Metformin hydrochloride and Evogliptin tartrate in pharmaceutical dosage forms.

Materials and Methods: Chromatographic separation of Metformin and Evogliptin was achieved on Waters Alliance-e2695 by using Waters XTerra RP-18 150X4.6 mm, 3.5µ column, and the mobile phase containing Acetonitrile: KH2PO4: Methanol in the ratio of 50:40:10% v/v.

Results: The flow rate was 1.0 ml/min; detection was carried out by absorption at 228 nm using a photodiode array detector at ambient temperature.

Conclusion: This method was a simple, economical, suitable, precise, accurate and robust method for quantitative analysis of Metformin and Evogliptin study of its stability.

Keywords: RP-HPLC; Metformin; Evogliptin; Chromatographic conditions.

INTRODUCTION

Globally, about 422 million people (8.8% of the adult population) are suffering from diabetes mellitus (DM) and the high prevalence is observed in middle and low-income countries (1). The most important reason for polytherapy in the treatment of DM is to give a rationale for drug regulatory mechanisms and improve the drug's therapeutic effectiveness (2). Recently the Food and Drug Administration approved the drug combination of Metformin and Evogliptin for treating patients with DM. Metformin is used to treat patients with Type-2 DM (T2DM), followed by Evogliptin, a DPP-4 inhibitor (3). Metformin is an oral antidiabetic that belongs to the group of biguanides, with proven efficacy in the treatment of T2DM and considered the drug of first choice in monotherapy for this entity. Among its beneficial effects, increased insulin sensitivity, weight loss, favorable modification of the lipid profile and improvement of glycemic control and vascular function were observed (4). It stimulates adenosine monophosphate-activated protein kinase, a liver enzyme that plays a key role in insulin signaling, whole balance, and metabolism of glucose and fats (5-7).

Evogliptin is a hypoglycemic drug of the dipeptidyl peptidase 4 (DPP-4) inhibitor class prescribed for patients with T2DM (8). The increased glycated hemoglobin (HbA1c) was observed at the start of therapy, and the decreased HbA1c was observed after therapy (9, 10), but DPP-4 have a moderate hypoglycemic effect (9), and therefore the response to therapy in terms of HbA1c parameter <7.0% is expected in patients with a moderate increase and the probability of reaching the target HbA1c value decreases by 36% with a 1% increase in HbA1c (11). In addition, DPP-4 in combination with metformin increases the effectiveness of therapy by 2.6 times compared with monotherapy with DPP-4, while the combined use of DPP-4 and the combination Metformin and a sulfonylurea are only 42% more effective than DPP-4 monotherapy in terms of the likelihood of achieving target HbA1c values (<7.0%) (11).

In this study, we aimed to describe the method appropriate for expected results which is based on International Council for Harmonization (ICH) guidelines. In this study, the HPLC method was developed to determine Metformin hydrochloride and Evogliptin tartrate in pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation

For the process of method development and validation, HPLC LC Waters 2695-Empower software (Waters, United States), 2700 pH Meter (Thermo Fisher, United States), Analytical Balance (Cole-Parmer, United States), UV-Vis Spectrophotometer

UV-1700 (Shimadzu, Japan), and Ultrasonicator UCA 701 Unichrome were used. The HPLC instrument was equipped with a pump with the isocratic model.

Preparation of buffer solution

KH_2PO_4 Buffer Preparation: 1.36 g of KH_2PO_4 was dissolved in 1-liter HPLC grade water, pH-3.4 was adjusted using TFA and filtered through a 0.45µ nylon filter.

Determination of wavelength (λ_{max})

PDA Detector was used to scan the wavelength range of 200–400 nm, using Acetonitrile, KH2PO4, and Methanol (50:40:10) as the reference material, to determine the wavelength of maximum absorption of the drug solution in these three solvents. An isosbestic point at 228 nm is visible on the absorption curve. So, the detector wavelength for the HPLC chromatographic process was decided upon as 228 nm.

Chemicals and reagents

The chemicals used in the experiment are acetonitrile, water, methanol, and KH_2PO_4 (Rankem, United States). These chemicals used are of HPLC grade.

Chromatographic conditions

The optimized chromatographic conditions at which the peaks were eluted (Table 1).

Table 1: Optimized chromatographic condition

Column	Waters XTerra RP-18 (150, 4.6mm, 3.5µ)
Mobile phase ratio	Acetonitrile: KH_2PO_4: Methanol (50:40:10)
Detection wavelength	228 nm
Flow rate	1 ml/min
Injection volume	10µl
Run time	7min
Observation	This method is suitable for validation
Temperature	Ambient (25° C)
Mode of separation	Isocratic mode

The Metformin peak (2.730 min), peak area (2152885), tailing factor (1.07), Evogliptin peak (4.468 min), peak area (314237), tailing factor (1.00), and resolution (8.27) were observed respectively.

Preparation of standard stock solution

Metformin (50 mg) and Evogliptin (5 mg) were carefully weighed and then added to a 10 ml volumetric flask. Later, diluent was added, and the sonification of mixture to wholly dissolve and increase the volume to the appropriate level using the same solvent. Evogliptin solution (1 ml) was pipetted into a 10 ml volumetric flask and diluted to the appropriate concentration with diluents (Stock solution).

A further 1 ml of the above mentioned stock solutions was pipetted into a 10 ml volumetric flask and diluted with diluent to the appropriate concentration (Metformin [500 ppm], Evogliptin [5 ppm]).

Sample solution preparation

A sample weighing 62.4 mg was deposited into a 10 mL clean, dry volumetric flask, diluent was added, sonicated for 30 min to dissolve, and then centrifuged for 30 min to thoroughly dissolve it and get the volume up to the target with the same solvent. After that, it is filtered via a 0.45-micron Injection filter (Stock solution). A volumetric flask was filled with an additional 1 ml of the above-mentioned stock solution, which was then diluted with diluents to the appropriate concentration (Metformin [500 ppm], Evogliptin [5 ppm]).

Method validation

Following ICH requirements, the evolved method was validated (Q2) and the parameters "specificity, accuracy, precision, linearity, robustness, the limit of detection (LOD) and limit of quantification (LOQ)" were evaluated (5,6,9).

Evaluation of system suitability

The tailing factor, plate count, and column efficiency were all recorded as system suitability metrics.

Forced degradation studies

Induced degradation studies like acid, hydrolysis (1N HCl), alkali (1N NaOH), peroxide hydrolysis (3% H_2O_2), and reduction ($NaHSO_4$) were performed based on ICH guidelines.

RESULTS

Method development and optimization

The mobile phase, which was optimized as the best chromatographic conditions for this study, consisted of Acetonitrile:KH_2PO_4: Methanol (50:40:10) mobile phase with a flow rate of 1 mL/min, injection volume of 10 µl, run time of 7 min, column temperature of 25°C (ambient), at the wavelength (λ) 228. Metformin and Evogliptin were eluted, forming symmetrical peak shapes, resolution. The optimized chromatogram is displayed in Fig. 1, and the Metformin and Evogliptin retention durations, USP plate counts, USP tailing, resolution, and area are displayed in Table 2.

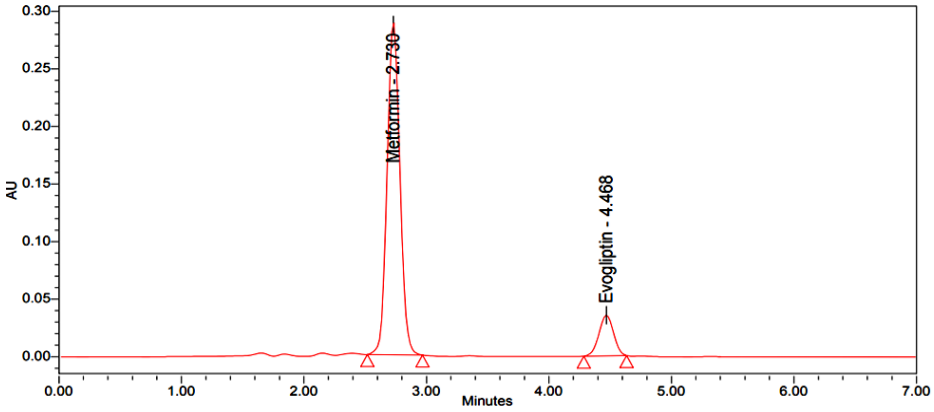


Fig. 1. Optimised chromatogram

Table 2: Optimised chromatographic conditions

S.No	Name	RT	Area	USP Plate Count	USP Tailing	USP Resolution
1	Metformin	2.730	2152885	3173	1.07	-
2	Evogliptin	4.468	314237	6509	1.00	8.27

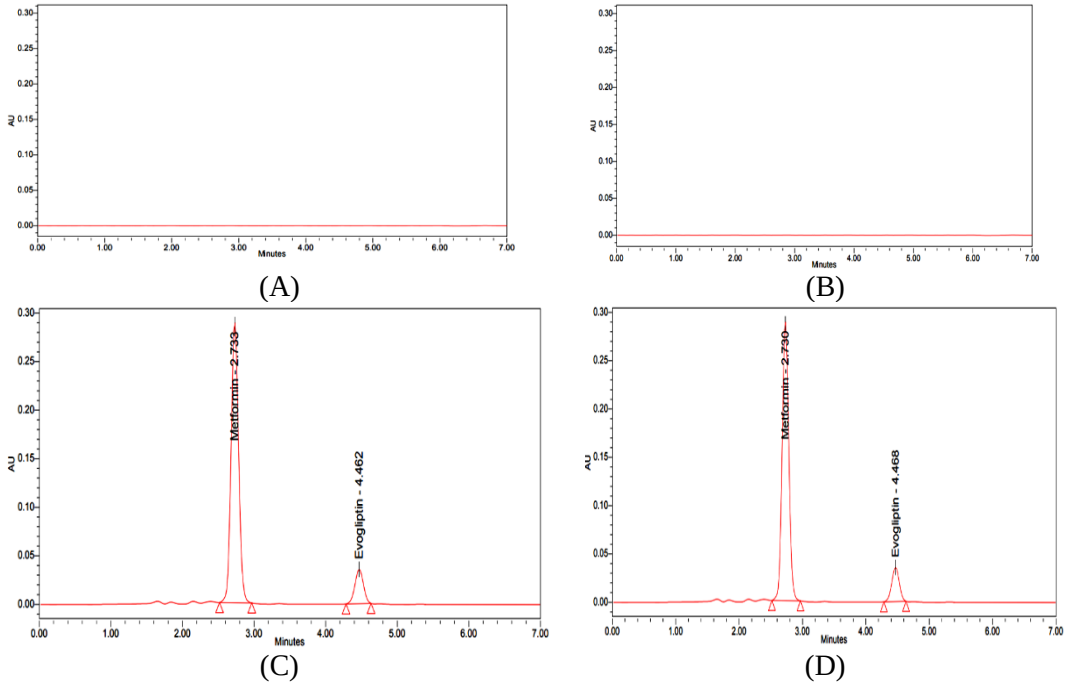


Fig. 2: (A) Chromatogram of blank, (B) Chromatogram of placebo, (C) Chromatogram of standard, (D) Chromatogram of sample

Method validation

Specificity

Evogliptin and metformin had retention durations of 4.468 and 2.730 minutes, respectively. This approach does not detect any interference peaks in the placebo or blank groups at the medication retention times. This procedure was therefore described as being precise. Fig. 2a, 2b, 2c, and 2d show the chromatograms of the blank, standard sample samples.

Accuracy

By using the conventional addition approach, three degrees of accuracy samples were created. For each level of precision, triplicate injections were performed, and the mean % recovery for the two drugs, Metformin and Evogliptin, was determined to be 100.1% and 99.6%, respectively (Table 3)

Precision

Six standard replication sets were used to test the repeatability of the system, the method, and the intermediate precision; the results demonstrated that the method is accurate within the permitted ranges. After calculating the RSD, tailing factor, and number of theoretical plates, the results were all within bounds (Tables 4 and 5).

Table 3: Accuracy results of Metformin and Evogliptin by RP HPLC method

Drug name	% concentration (At specification level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
Metformin	50%	1071560	25	24.96	99.8	100.1
	100%	2169284	50	50.54	101.1	
	150%	3195881	75	74.45	99.3	
Evogliptin	50%	157634	0.25	0.25	100.0	99.6
	100%	314326	0.50	0.5	100.0	
	150%	465443	0.75	0.74	98.7	

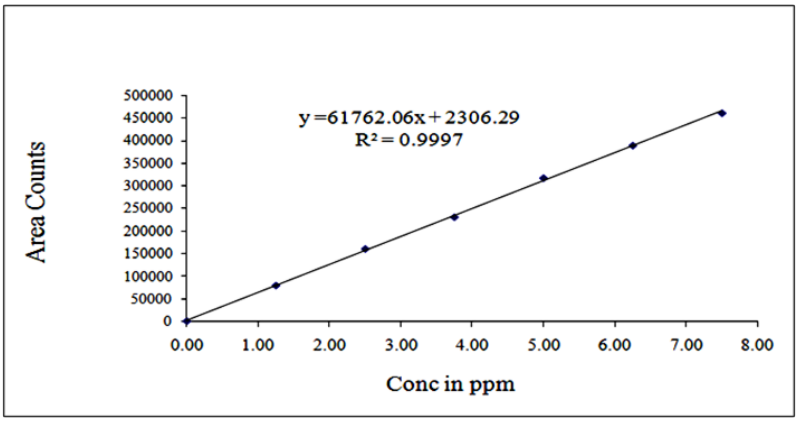
Table 4: System and method precision table of Metformin and Evogliptin

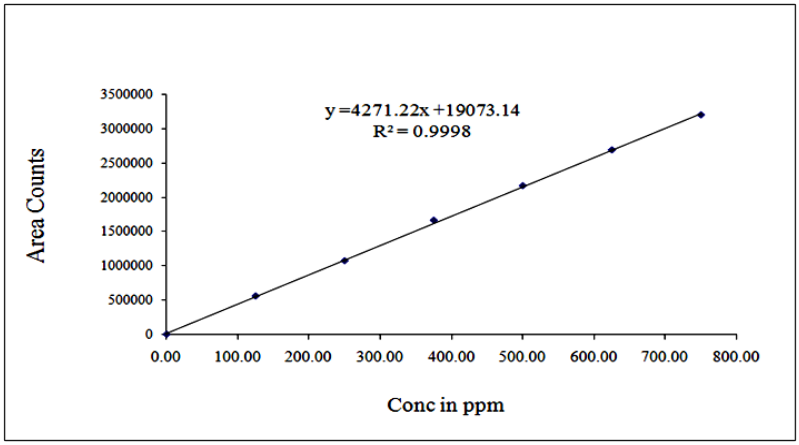
S.No	System Precision		Method Precision	
	Area of metformin concentration (500µg/ml)	Area of evogliptin concentration (5µg/ml)	Area of metformin concentration(µg/ml)	Area of evogliptin concentration(µg/ml)
1.	2152885	314237	2162891	314314
2.	2153793	310356	2153789	310341
3.	2135800	312747	2135810	315939
4.	2154609	311340	2154608	311852
5.	2139390	312332	2149396	313343
6.	2141308	313594	2141312	317161
Mean	2146298	312434	2149634	313825
S.D	8384.09	1429.66	9779.244	2533.785
%RSD	0.39	0.46	0.45	0.81

Table 5: Intermediate precision (day variation) for Metformin and Evogliptin

S. No.	Area for Metformin		Area for Evogliptin	
	Day-1	Day-2	Day-1	Day-2
1	2152889	2143218	314320	315124
2	2113794	2126547	311346	311935
3	2135815	2144705	312514	315861
4	2154611	2148624	315359	314329
5	2139397	2125106	316540	317246
6	2181324	2147981	313156	316463
Average	2146305	2139364	313873	315160
Standard Deviation	22598.207	10685.934	1911.418	1877.929
% RSD	1.05	0.50	0.61	0.60

(A)





(B)

Fig. 3: (A) Calibration curve for Metformin, (B) Calibration curve for Evogliptin

Linearity and range

According to the findings of the linearity investigation, Metformin and Evogliptin have linear relationships spanning the concentration ranges of 125–750 g/ml and 1.27–7.50 g/ml, respectively. Regression study results showed the linear equations for the drugs metformin and Evogliptin are: y = 4271.22 X + 19073 and y = 61762.06 X + 2306.29, respectively. The R^2 values were 0.9998 and 0.9997, respectively (Fig. 3).

Robustness

In order to assess the method's response to deliberate changes in flow rate, the makeup of the mobile phase, and temperature fluctuation (Table 6).

LOD (limit of detection) and LOQ (limit of quantification)

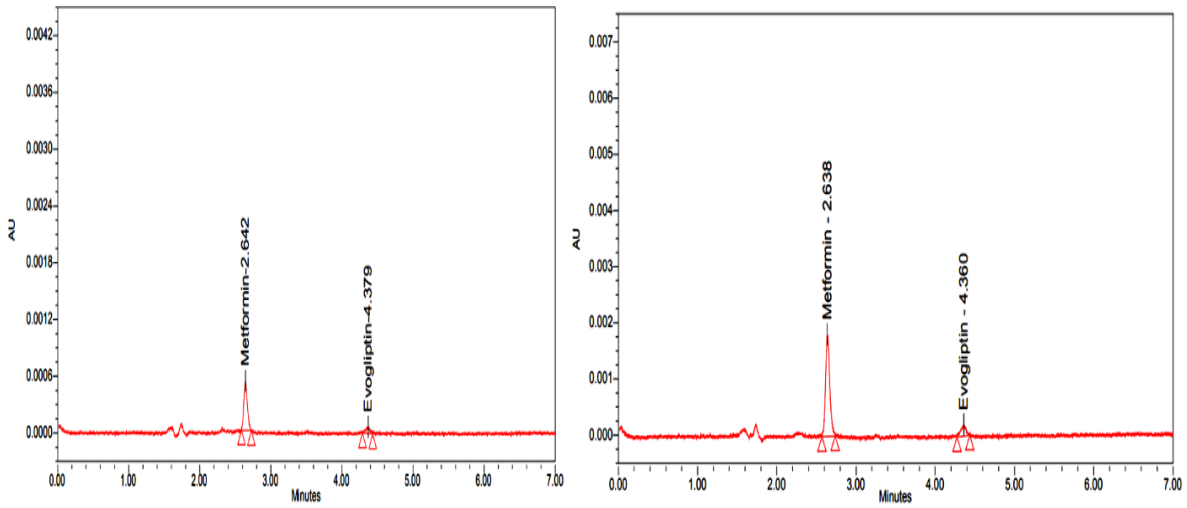
LOD and LOQ for Metformin and Evogliptin, respectively, were determined to be 1 µg/mL and 0.01 µg/ml, and 3.3 µg/mL and 0.03 µg/ml, respectively (Fig. 4A and 4B).

System suitability

According to ICH criteria, every system suitability metric fell within the acceptable range (Table 7).

Table 6: Robustness results of Metformin and Evogliptin

Drug name	Parameter	Condition	Retention time	Peak area	Resolution	Tailing	Plate count
Metformin	**Flow rate** Change (mL/min)	Less flow (0.8ml)	2.999	2367452		1.15	3245
		Actual (1ml)	2.730	2152885		1.07	3173
		More flow (1.2ml)	2.471	1906947		1.03	3102
	Organic Phase change	Less Org (47.5:43:9.5)	3.202	2557416		1.11	3269
		Actual (50:40:10)	2.733	2153793		1.05	3168
		More Org (52.5:37:10.5)	2.410	1861411		0.99	3084
Evogliptin	**Flow rate** Change (mL/min)	Less Flow (0.8ml)	4.938	332323	9.31	1.08	6586
		Actual (1ml)	4.469	314237	8.27	1.00	6509
		More Flow (1.2m)	4.049	289861	8.17	0.96	6471
	Organic Phase change	Less Org (47.5:43:9.5)	6.403	342478	14.10	1.11	6634
		Actual (50:40:10)	4.462	310356	8.31	1.01	6518
		More Org (52.5:37:10.5)	3.389	263370	5.28	0.98	6425



(A) (B)

Fig. 4: (A) Chromatogram of LOD, (B) Chromatogram of LOQ

Table 7: System suitability parameters for Metformin and Evogliptin

S.no	Parameter	Metformin	Evogliptin
1	Retention time	2.733	4.462
2	Plate count	3159	6513
3	Tailing factor	1.09	0.98
4	Resolution	----	8.30
5	%RSD	0.39	0.46

Table 8: Forced degradation results of Metformin and Evogliptin

% Degradation results	Metformin		Evogliptin	
	Area	% Degradation	Area	% Degradation
Control	2147823	0	312118	0
Acid	1843017	14.2	275512	11.8
Alkali	1865075	13.1	273849	12.3
Peroxide	1803507	16.0	267125	14.4
Reduction	1904461	11.3	279976	10.3
Hydrolysis	2120156	1.3	310154	0.7
Thermal	1853792	13.7	271405	13.1
Photolytic	2110408	1.7	311289	0.3

Degradation studies

Forced degradation studies were performed under the stress conditions like acid, base, peroxide, alkali, reduction, hydrolysis, thermal, and photolytic. The results showed that the highest and lowest percentage of degradation was seen in peroxide and hydrolysis respectively (Table 8).

DISCUSSION

Only liquid chromatography with tandem mass spectrometry (3), Orbitrap mass spectrometry methods were showed for the description of Evogliptin in plasma, urine, and liver of humans (13), Ultraviolet-visible spectrophotometer (5), and a single RP-HPLC method for method development and validation for Evogliptin. Even though there are many methods present for the development and validation of Metformin and Evogliptin individually, but no method was performed for the antidiabetic drug combination of Metformin and Evogliptin. Hence, a combination formulation of Metformin and Evogliptin was selected for the study.

The newly created HPLC process is quick, straightforward, linear, accurate, precise, and reliable. As a result, it can be used for standard quality control analyses. Metformin and Evogliptin had good resolution thanks to the mobile phase solvents and analytical technique conditions.

CONCLUSION

The key characteristics of the new approach also include a short run time of 7 min and Metformin and Evogliptin retention times of 2.730 min and 4.468 min, respectively. The technique was validated based on ICH criteria. Under these chromatographic circumstances, the procedure is reliable enough to replicate exact and precise results.

CONFLICT OF INTEREST

None.

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