RESEARCH PAPER

Response Surface Methodology (Central Composite Design) Based Development and Validation of RP-HPLC Method for Simultaneous Estimation Of Evogliptin Tartrate and Metformin Hydrochloride in Combined Dosage Form

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ABSTRACT

A simple, specific, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Evogliptin Tartrate (EVO) and Metformin Hydrochloride (MET), two commonly co-prescribed anti-diabetic drugs, in combined tablet dosage form, using a Response Surface Methodology (RSM) based Design of Experiments (DoE) approach. Chromatographic separation was achieved on a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm) using a Shimadzu LC-2010 CHT HPLC system, with a mobile phase comprising 10 mM potassium dihydrogen phosphate buffer (pH 3.85, adjusted with ortho-phosphoric acid) and methanol in the ratio of 48:52 (%v/v), delivered at a flow rate of 1.1 mL/min, with UV detection at 262 nm. A Faced Central Composite Design (FCCD), comprising three independent variables - flow rate, buffer pH, and percentage of methanol - was employed over 15 experimental runs to optimize the method with respect to the retention time of Evogliptin and the tailing factor of Metformin. Under the optimized conditions, Metformin Hydrochloride and Evogliptin Tartrate eluted at 1.860 min and 5.221 min, respectively, with adequate resolution (6.590) and theoretical plate count. The method was found to be linear over the range of 1-6 µg/mL for Evogliptin Tartrate (r^2 = 0.9994) and 100-600 µg/mL for Metformin Hydrochloride (r^2 = 0.9999). Accuracy, expressed as percentage recovery, ranged from 99.13-100.77% for Evogliptin Tartrate and 98.31-98.88% for Metformin Hydrochloride. The method was precise, with %RSD values below 2% for repeatability, intraday, and interday precision, and remained robust to small deliberate changes in detection wavelength, mobile phase ratio, and flow rate. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.192 and 0.582 µg/mL for Evogliptin Tartrate, and 6.606 and 20.020 µg/mL for Metformin Hydrochloride, respectively. The method was validated as per ICH Q2(R1) guidelines and was found to be suitable for the routine, regulatory-compliant quality control analysis of Evogliptin Tartrate and Metformin Hydrochloride in combined tablet dosage forms.

Keywords: Evogliptin Tartrate (EVO), Metformin Hydrochloride (MET), RP-HPLC, Response Surface Methodology (RSM), Central Composite Design (CCD), Design of Experiments (DoE), Analytical Method Validation

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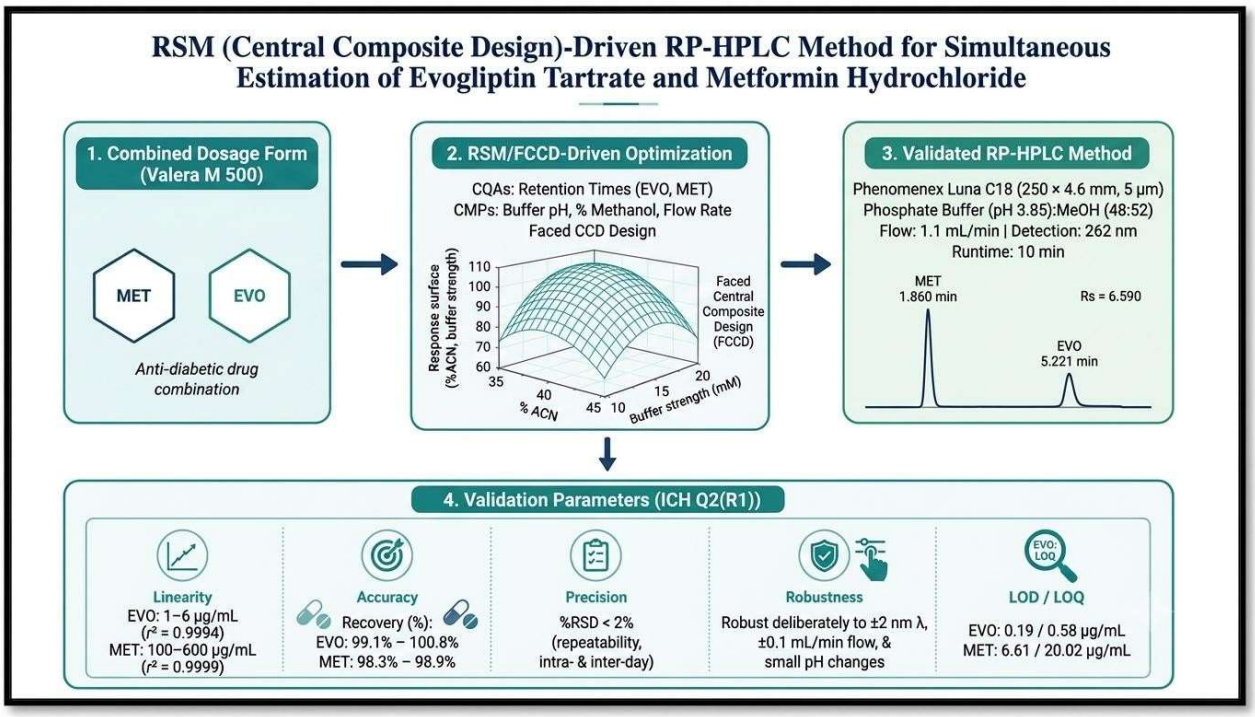
GRAPHICAL ABSTRACT



INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, which, if left inadequately controlled, leads to long-term complications affecting the kidneys, eyes, heart, and nervous system. Effective management of type 2 diabetes mellitus often requires combination therapy involving anti-diabetic drugs acting through complementary mechanisms, making reliable analytical methods essential for their simultaneous estimation in combined dosage forms [1,2].

Evogliptin Tartrate (EVO) is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances incretin activity, thereby increasing glucose-dependent insulin secretion and decreasing glucagon levels. Metformin Hydrochloride (MET) is a first-line biguanide anti-hyperglycaemic agent that reduces hepatic glucose production and improves peripheral insulin sensitivity. When combined, these agents offer complementary glucose-lowering action, and their simultaneous quantitative estimation in fixed-dose combination tablets is of considerable importance in pharmaceutical quality control [3,4].

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is a powerful and widely used analytical technique for the quantitative estimation, separation, and purity assessment of drugs, owing to its high sensitivity, specificity, and reproducibility [5]. Conventional (One-Factor-at-a-Time, OFAT) approaches to method development are often trial-and-error based, time-consuming, and may not guarantee a robust method across the intended operating range.

To overcome these limitations, Response Surface Methodology (RSM), employing a Faced Central Composite Design (FCCD), was applied in the present work as a systematic Design of Experiments (DoE) tool. RSM enables simultaneous evaluation of the effect of multiple independent variables - and their interactions - on selected chromatographic responses, and allows identification of an optimal design space within which the method consistently delivers the desired performance [6,7]. This systematic, statistically driven approach to method development results in a robust, reproducible, and regulatory-compliant RP-HPLC method, while also reducing the number of experimental trials required compared with conventional development.

Analytical method development and validation play a central role in pharmaceutical chemistry - they ensure drug quality, safety, and efficacy prior to formulation and market approval; assist in the identification and authentication of Active Pharmaceutical Ingredients (APIs); and support regulatory compliance with ICH guidelines [8,9]. The present study was therefore undertaken to develop and validate, using a Response Surface Methodology (Central Composite Design) based approach, a robust RP-HPLC method for the simultaneous estimation of Evogliptin Tartrate and Metformin Hydrochloride in combined tablet dosage form.

MATERIALS AND METHODS

Materials

Pure drug sample of Evogliptin Tartrate was supplied by Alkem Laboratories Ltd., Mumbai, and pure drug sample of Metformin Hydrochloride was supplied by Harman Finochem Ltd., Mumbai. Valera M 500 tablets (Alkem Laboratories Ltd.), labelled to contain Evogliptin Tartrate equivalent to 5 mg Evogliptin and Metformin Hydrochloride equivalent to 500 mg Metformin (sustained-release form), were procured from the local market. Methanol (HPLC grade) and ortho-phosphoric acid (AR grade) were procured from Merck, and HPLC-grade water was procured from Rankem. All reagents used were of analytical/HPLC grade.

Instruments and Apparatus

Instrument/Apparatus	Specification
HPLC system	Shimadzu LC-2010 CHT Liquid Chromatograph, with serial dual-plunger pump and UV-VIS detector
Column	Phenomenex Luna C18 (250 × 4.6 mm, 5 µm)
Data acquisition software	LabSolution
DoE / statistical software	Design-Expert version 13 (Stat-Ease Inc.)
UV-Visible spectrophotometer	Double-beam UV-VIS spectrophotometer
Analytical balance	Analytical weighing balance
pH meter	Digital pH meter
Ultrasonicator	Bath-type sonicator
Membrane filter	0.45 µm Nylon syringe/membrane filter
Glassware	Borosilicate glassware

Table 1: Instruments and apparatus used for method development

Preparation of Mobile Phase

10 mM phosphate buffer was prepared by accurately weighing 1.36 g of potassium dihydrogen phosphate, dissolving in 950 mL of water, and making up the volume to 1000 mL. The pH was adjusted to 3.85 using ortho-phosphoric acid, and the buffer was filtered through a 0.45 µm nylon membrane filter. The mobile phase consisted of this phosphate buffer and methanol, mixed in the ratio of 48:52 (%v/v), and was degassed by ultrasonication for 5 minutes prior to use. The mobile phase was also used as the diluent.

Preparation of Standard Stock and Working Solutions

A standard stock solution of Evogliptin Tartrate (100 µg/mL) was prepared by accurately weighing 13.74 mg (equivalent to 10 mg Evogliptin), dissolving in methanol with sonication, and making up to 100 mL with methanol. A standard stock solution of Metformin Hydrochloride (1000 µg/mL) was prepared by accurately weighing 25 mg, dissolving in methanol with sonication, and making up to 25 mL with methanol. Working solutions of 4 µg/mL (Evogliptin Tartrate) and 400 µg/mL (Metformin Hydrochloride), corresponding to the 100% assay level used for method development and validation, were prepared by appropriate dilution of the respective stock solutions with diluent.

Preparation of Sample Solution

Twenty tablets were accurately weighed and finely powdered. An accurately weighed quantity of powder equivalent to 1 mg Evogliptin and 100 mg Metformin was transferred to a 100 mL volumetric flask, dissolved in methanol with 15 minutes of sonication, made up to volume, and filtered through Whatman filter paper (No. 42). An aliquot of 4 mL of this stock sample solution was further diluted to 10 mL with diluent to obtain a working sample concentration of 4 µg/mL Evogliptin and 400 µg/mL Metformin, which was assayed as per the proposed method.

Identification of Standard Evogliptin Tartrate

Identification of standard Evogliptin Tartrate was carried out by IR spectroscopic and melting point studies. A pellet of Evogliptin Tartrate with KBr (spectroscopic grade) was prepared using a hydraulic pellet press at 7-10 tonnes pressure and scanned from 400-4000 cm^{-1} using an FT-IR instrument. Principal IR peaks were observed at 3500-3300 cm^{-1} (-NH_2), 3500-3200 cm^{-1} (-OH

stretching), and 1400-1000 cm^{-1} (C-F stretch) (Figure 1), consistent with the functional groups present in the molecule. The observed melting point of Evogliptin Tartrate was 208-212 °C.

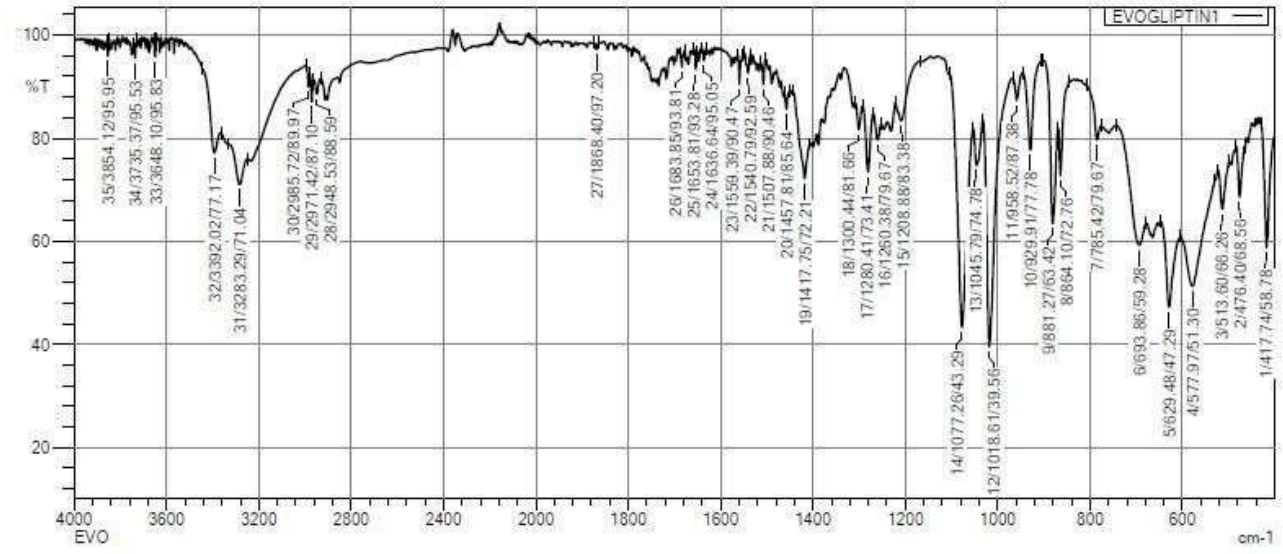


Figure 1: IR spectrum of Evogliptin Tartrate

Selection of Analytical Wavelength

Standard solutions of Evogliptin Tartrate (1 µg/mL) and Metformin Hydrochloride (10 µg/mL) were scanned in the range of 200-400 nm on a double-beam UV-Visible spectrophotometer against methanol as blank. A wavelength of 262 nm, at which both drugs showed considerable absorbance, was selected for detection using a PDA detector.

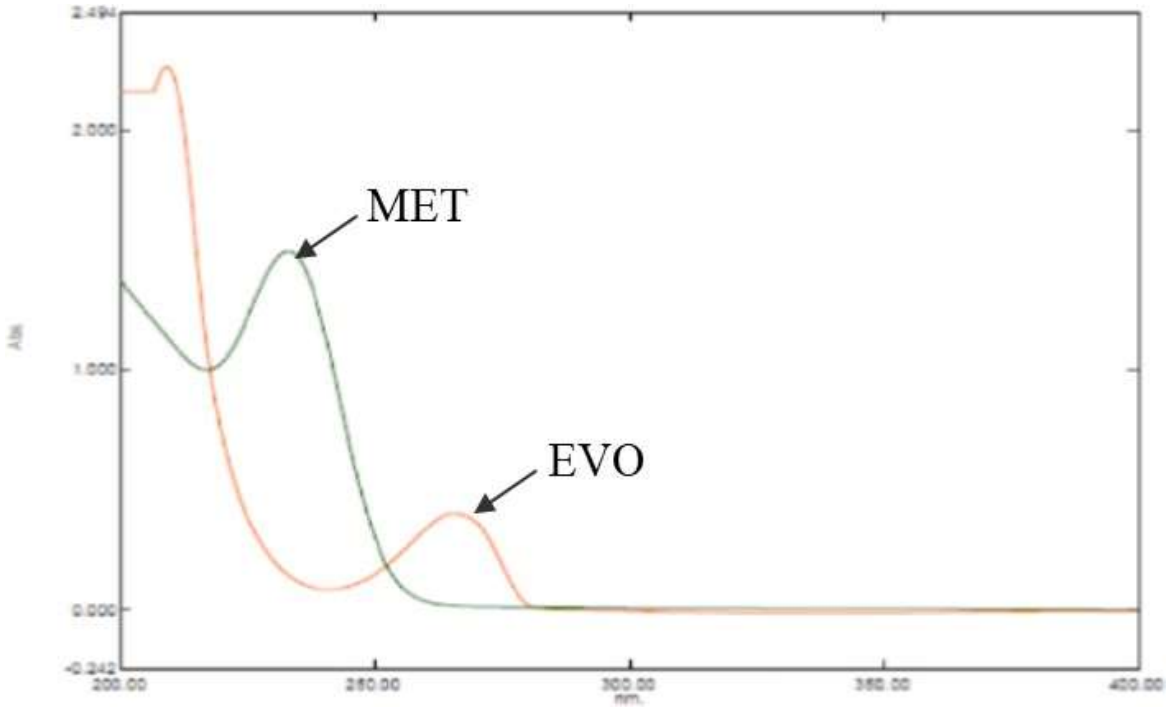


Figure 2: Overlain UV spectra of Evogliptin Tartrate (1 µg/mL) and Metformin Hydrochloride (10 µg/mL) used for selection of the detection wavelength

Method Development by Response Surface Methodology (Central Composite Design)

In the preliminary method development, a two-level factorial design with centre points was constructed, and the methanol concentration and flow rate of the mobile phase were screened over the range of 40-60% v/v and 0.8-1.2 mL/min, respectively, to achieve better peak symmetry and accurate quantification with a short run time. Based on the pKa values of the two drugs, the buffer pH range was optimized between 3.5 and 4.0, since peak tailing increased above pH 4.0 while column shelf life decreased below pH 3.5.

To determine the ideal pH of the mobile phase, flow rate, and percentage of organic modifier, a Faced Central Composite Design (FCCD) was constructed over these three independent variables, employing a partial factorial design coupled with five replicate centre points and five axial (face) points, comprising a total of 15 optimized experimental runs (Table 3). The retention time of Evogliptin (RT-EV) and the tailing factor of Metformin (Tailing-MET) were selected as the critical quality attribute responses. The quality of the fitted polynomial models was assessed using the adjusted coefficient of determination (adjusted R^2), model p-value, percentage coefficient of variation (%CV), and adequate precision, in order to identify the region of the true optimum within the experimental design space.

Factor	Name	Level (-)	Level (0)	Level (+)
A	Flow rate (mL/min)	0.8	1.0	1.2
B	Buffer pH	3.5	3.75	4.0
C	Methanol (%v/v)	40	50	60

Table 2: Independent variables (factors) and levels used in the Central Composite Design

Std	Run	Flow rate (mL/min)	Buffer pH	Methanol (%v/v)	RT of EVO (min)	Tailing of MET
9	1	0.7	3.75	50	9.60	1.52
1	2	0.8	3.5	40	12.35	1.31
14	3	1.0	3.75	66.82	5.01	1.66
12	4	1.0	4.17	50	8.62	1.75
15	5	1.0	3.75	50	8.92	1.49
6	6	1.2	3.5	60	6.02	1.27
10	7	1.3	3.75	50	5.68	1.63
13	8	1.0	3.75	33.18	14.09	1.50
4	9	1.2	4.0	40	9.74	1.56
5	10	0.8	3.5	60	6.35	1.42
3	11	0.8	4.0	40	12.35	1.62
8	12	1.2	4.0	60	5.98	1.64
2	13	1.2	3.5	40	11.45	1.28
7	14	0.8	4.0	60	7.36	1.75
11	15	1.0	3.33	50	9.12	1.22

Table 3: Faced Central Composite Design (FCCD) matrix and observed responses for Evogliptin Tartrate (EVO) and Metformin Hydrochloride (MET)

Model fitting and analysis of variance (ANOVA) were performed on the response data using Design-Expert software. Quadratic regression models were fitted for both responses - retention time of Evogliptin and tailing factor of Metformin - and were found to be statistically significant (model $p < 0.0001$ for both responses), with adjusted R^2 of 0.9140 and 0.8115, respectively (Table 4). Perturbation plots and three-dimensional/contour response surface plots were generated to visualize the effect of flow rate, buffer pH, and percentage methanol,

individually and through their interactions, on both responses.

Response	Regression model (coded)	Adjusted R^2	Model p-value	%CV	Adequate precision
Retention time of EVO	8.84267 - 0.888A - 0.115795B - 2.59569C - 0.345AB + 0.225AC + 0.335BC	0.9140	< 0.0001	9.29	15.5667
Tailing factor of MET	1.508 - 0.0148A + 0.159797B + 0.0424008C + 0.00125AB - 0.02125AC + 0.01375BC	0.8115	< 0.0001	5.03	10.3664

Table 4: ANOVA-based statistical parameters and regression models for the two response variables

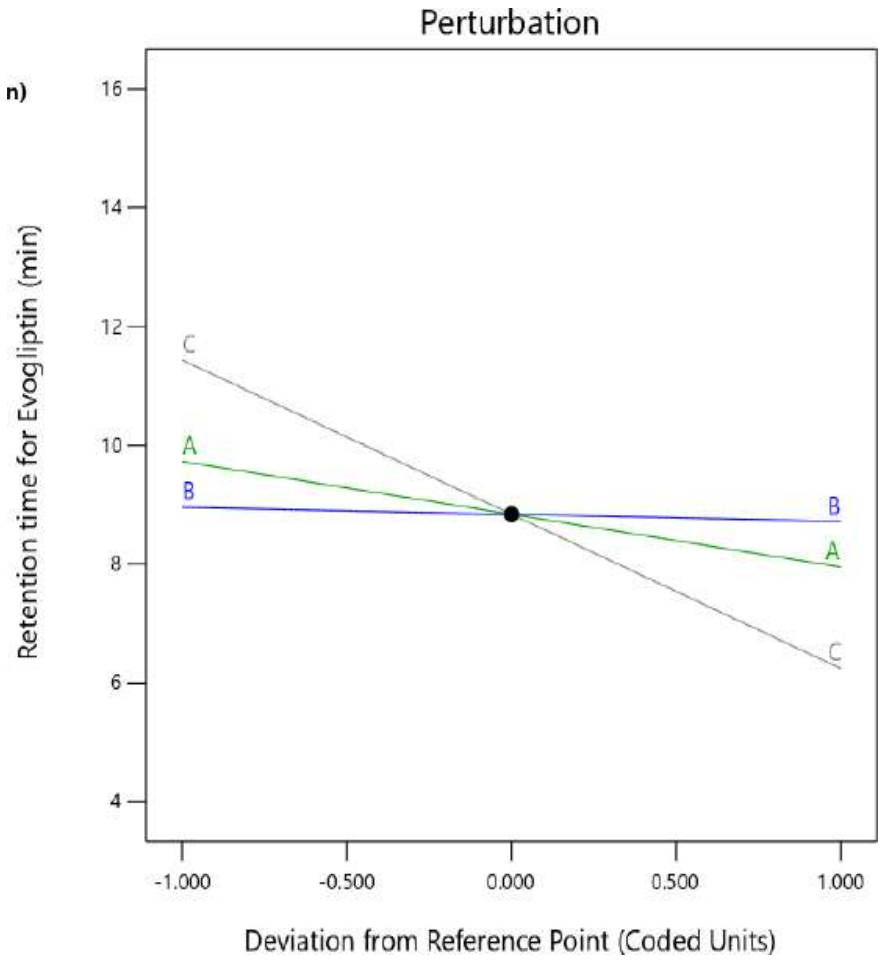


Figure 3: Perturbation plot showing the effect of each factor on the retention time of Evogliptin

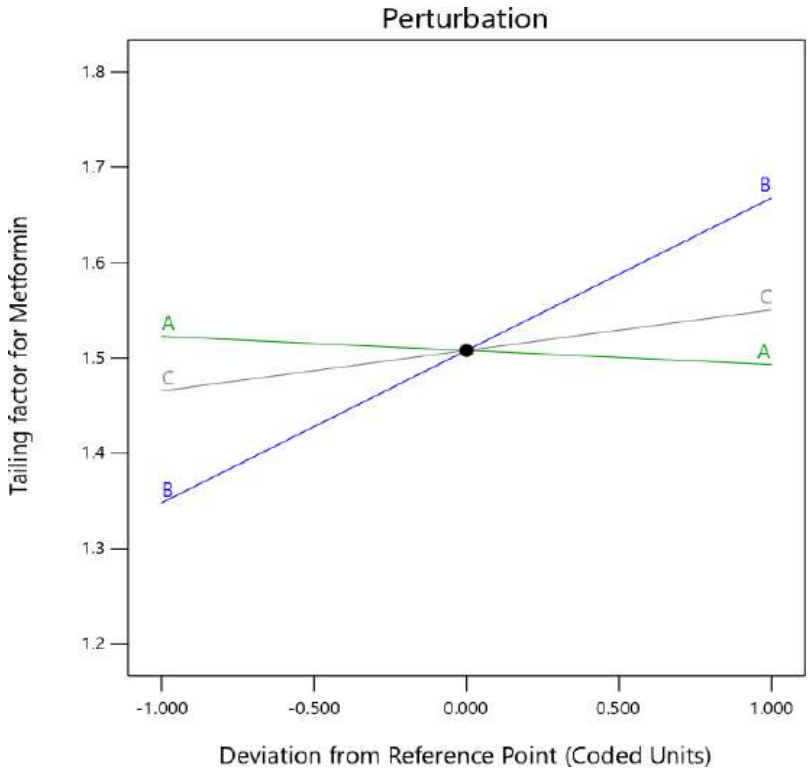


Figure 4: Perturbation plot showing the effect of each factor on the tailing factor of Metformin

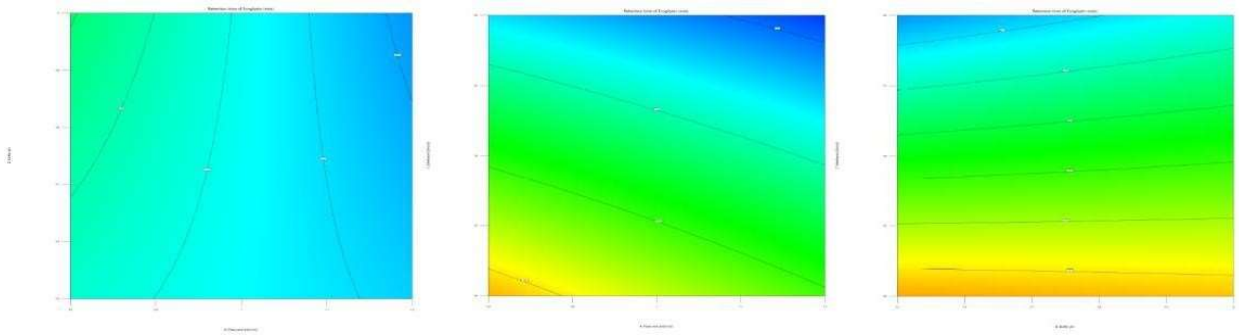


Figure 5: Contour plots for retention time of Evogliptin - (i) AB, (ii) AC, and (iii) BC interactions

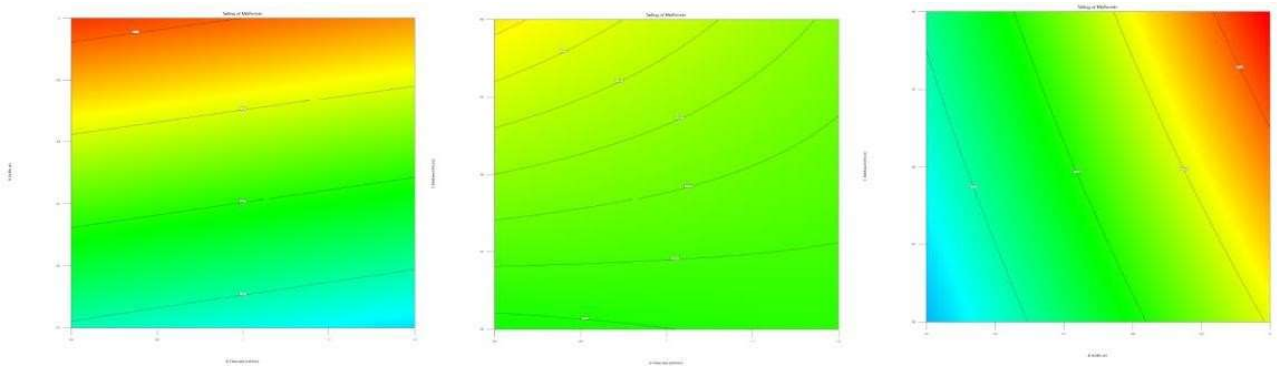


Figure 6: Contour plots for tailing factor of Metformin - (i) AB, (ii) AC, and (iii) BC interactions

Based on the desirability-based analysis of the fitted response surface models, the optimum region of the design space was identified at a buffer pH of 3.85, 52% v/v methanol, and a flow rate of 1.1 mL/min, giving the desired combination of quicker separation, good resolution, and optimal tailing of the Metformin peak. Confirmatory injections performed under these optimized conditions gave retention times of 1.860 min (Metformin) and 5.221 min (Evogliptin), confirming the robustness of the identified design space. The finalized chromatographic conditions used for method validation are summarized in Table 5.

Parameter	Optimized condition
Column	Phenomenex Luna C18 (250 × 4.6 mm, 5 µm)
Mobile phase	10 mM phosphate buffer (pH 3.85) : Methanol (48:52 %v/v)
Diluent	Mobile phase
Flow rate	1.1 mL/min
Injection volume	10 µL
Detection wavelength	262 nm
Retention time - Metformin Hydrochloride	1.860 min
Retention time - Evogliptin Tartrate	5.221 min

Table 5: Finalized (validated) chromatographic conditions

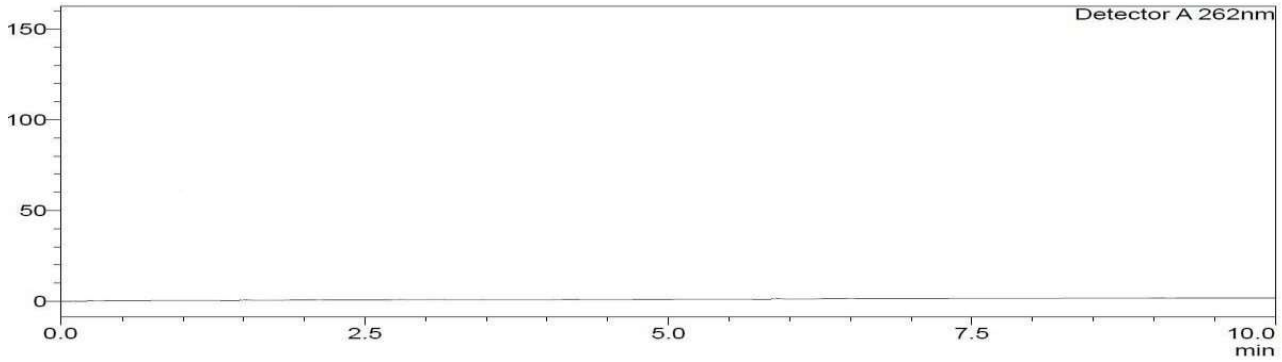


Figure 7: Chromatogram of blank

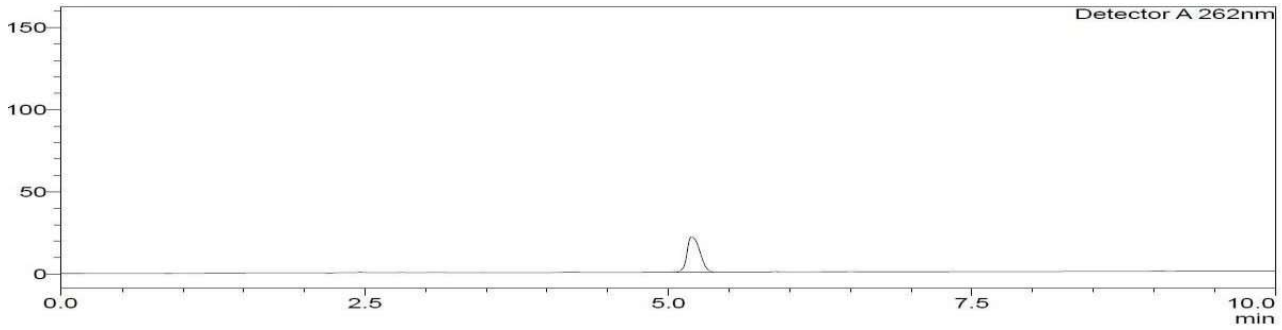


Figure 8: Chromatogram of Evogliptin Tartrate standard (4 µg/mL)

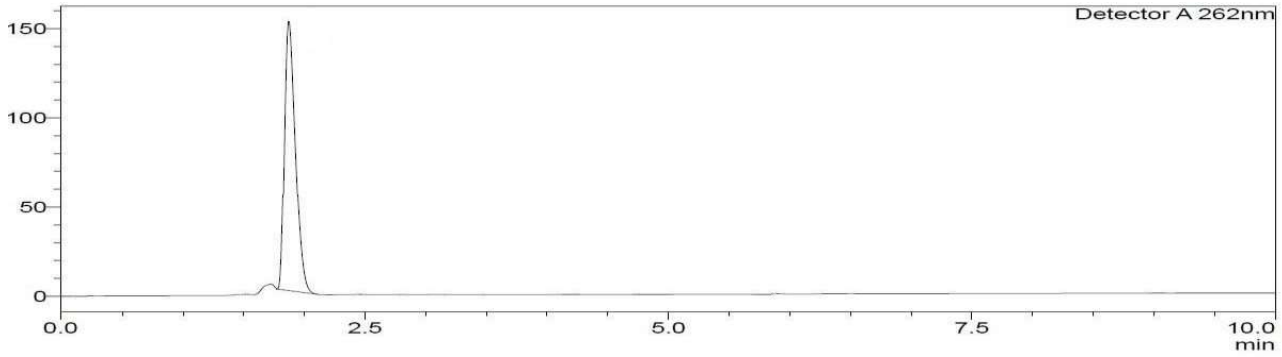


Figure 9: Chromatogram of Metformin Hydrochloride standard (400 µg/mL)

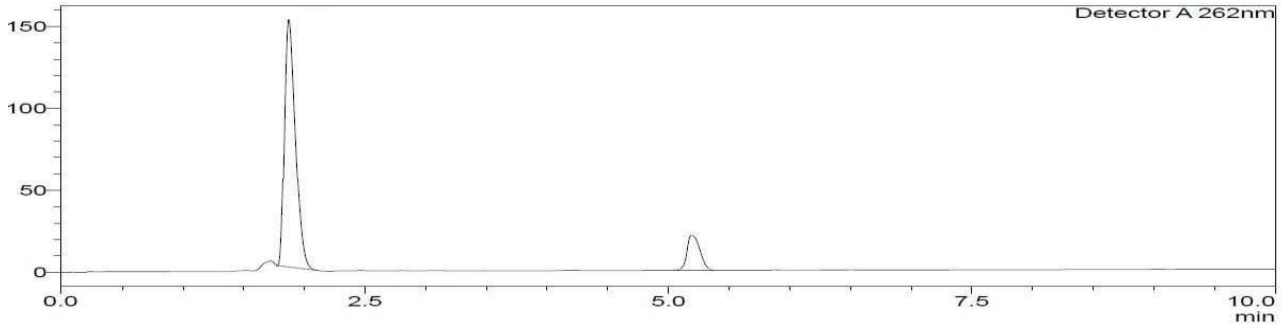


Figure 10: Optimized chromatogram of binary mixture - Evogliptin Tartrate (RT 5.221 min) and Metformin Hydrochloride (RT 1.860 min)

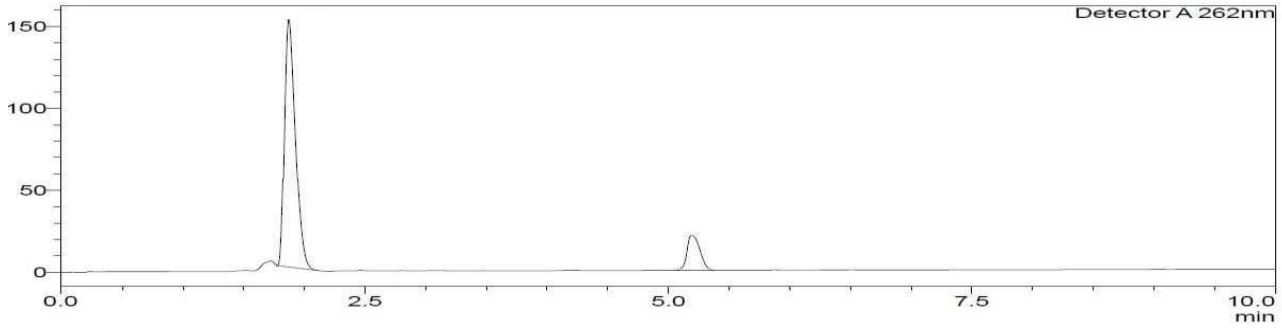


Figure 11: Chromatogram of sample (marketed formulation)

Method Validation

The finalized RP-HPLC method was validated as per ICH Q2(R1) guidelines [10] for system suitability, specificity, linearity and range, limit of detection/quantification (LOD/LOQ), accuracy, precision (repeatability, intraday, and interday), and robustness.

System Suitability

System suitability was evaluated by injecting the binary mixture solution of Evogliptin Tartrate (4 µg/mL) and Metformin Hydrochloride (400 µg/mL) six times (n = 6), and recording retention time, theoretical plates per meter, tailing factor, and resolution.

Specificity

Specificity was assessed by injecting blank (diluent), individual standard solutions, the mixed standard solution, and the sample solution, and comparing the resulting chromatograms for interference at the retention times of Evogliptin Tartrate and Metformin Hydrochloride.

Linearity and Range

Linearity was assessed by preparing a series of standard solutions of Evogliptin Tartrate (1-6 µg/mL) and Metformin Hydrochloride (100-600 µg/mL), n = 5. Each solution was injected under the finalized chromatographic conditions, and calibration curves of peak area versus concentration were constructed for both analytes.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the response and the slope of the calibration curve, using the standard formulae LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve [10].

Accuracy

Accuracy was determined by the standard addition method at three concentration levels (50%, 100%, and 150% of the target concentration) for both analytes. Known amounts of standard stock solutions were added to pre-analyzed sample solutions, each level was analyzed in triplicate (n = 3), and the mean percentage recovery, standard deviation, and %RSD were calculated.

Precision

Repeatability was assessed by injecting the working standard mixture solution six times (n = 6) on the same day. Intraday precision was determined by analyzing three concentrations of each analyte three times on the same day, and interday precision was determined by repeating the study on three different days over one week. The %RSD of peak area was calculated in each case.

Robustness

The robustness of the method was evaluated by introducing small, deliberate variations in detection wavelength (± 2 nm), mobile phase ratio (± 2 mL methanol), and flow rate (± 0.1 mL/min), and observing the effect on the peak area of both analytes.

RESULTS AND DISCUSSION

Method Development and RSM Optimization

A wavelength of 262 nm was selected as the optimum detection wavelength based on the overlapping UV spectra of both drugs, providing good detector sensitivity and responsiveness for simultaneous estimation. ANOVA of the FCCD design matrix (Table 3, Table 4) confirmed that flow rate, buffer pH, and percentage methanol had a statistically significant effect ($p < 0.0001$) on both the retention time of Evogliptin and the tailing factor of Metformin, with quadratic regression models providing the best fit for both responses. It was observed across multiple trials that the retention time, at any pH, decreased rapidly as the methanol content was raised, and that a slight increase in buffer pH caused a slight decrease in the retention time of Evogliptin; because of the resulting shorter analysis time, this interaction was found to be synergistic. All three factors were found to have a moderate effect on the tailing of Metformin.

Response surface and desirability-based analysis identified the optimum working point at a buffer pH of 3.85, 52% v/v methanol, and a flow rate of 1.1 mL/min, at which the method achieved rapid separation, good resolution, and optimal tailing of the Metformin peak; the finalized conditions (Table 5) gave retention times of 1.860 min for Metformin Hydrochloride and 5.221 min for Evogliptin Tartrate, with adequate resolution and peak symmetry, and were used for subsequent method validation.

System Suitability

The results of the system suitability parameters are summarized in Table 6. The %RSD for all system suitability parameters was found to be less than 2%, confirming that the chromatographic system was suitable for the intended analysis.

Parameter	EVO - value ± SD	EVO %RSD	MET - value ± SD	MET %RSD
Retention time (min)	5.221 ± 0.009	0.174	1.860 ± 0.011	0.602
Theoretical plates per meter	4092 ± 18	0.451	4509 ± 15	0.328
Tailing factor	1.590 ± 0.027	1.703	1.170 ± 0.006	0.470
Resolution	6.590 ± 0.021	0.317	-	-

Table 6: System suitability parameters for Evogliptin Tartrate (EVO) and Metformin Hydrochloride (MET) (n=6)

Specificity

No interfering peaks were observed at the retention times corresponding to Evogliptin Tartrate (5.221 min) or Metformin Hydrochloride (1.860 min) in the blank chromatogram, and the peaks were found to be free from co-eluting peaks when examined in PDA mode, confirming that the method is specific for the simultaneous estimation of both analytes in the presence of formulation excipients (Figures 7-11).

Linearity

The calibration curves of peak area versus concentration were linear over the range of 1-6 µg/mL for Evogliptin Tartrate and 100-600 µg/mL for Metformin Hydrochloride. The regression equation for Evogliptin Tartrate was found to be $y = 10154x + 24832$ (r^2 =

0.9994), and for Metformin Hydrochloride, y = 12418x - 9376.3 (r^2 = 0.9999), indicating an excellent linear relationship between peak area and concentration for both analytes over the studied range (Tables 7 and 8).

Sr. No.	EVO conc. (µg/mL)	EVO area ± SD	EVO %RSD	MET conc. (µg/mL)	MET area ± SD	MET %RSD
1	1	35339 ± 285	0.808	100	1226002 ± 17352	1.415
2	2	44954 ± 700	1.558	200	2459390 ± 25897	1.053
3	3	55252 ± 459	0.830	300	3760489 ± 24752	0.658
4	4	65347 ± 848	1.298	400	4948072 ± 11924	0.241
5	5	74903 ± 660	0.882	500	6177320 ± 44377	0.718
6	6	86427 ± 500	0.578	600	7450356 ± 23678	0.318

Table 7: Linearity data of Evogliptin Tartrate (EVO) and Metformin Hydrochloride (MET) (n=5)

Drug	Straight-line equation	r^2
Evogliptin Tartrate	y = 10154x + 24832	0.9994
Metformin Hydrochloride	y = 12418x - 9376.3	0.9999

Table 8: Regression analysis data of Evogliptin Tartrate and Metformin Hydrochloride

Limit of Detection and Limit of Quantification

The LOD and LOQ, calculated from the standard deviation of the response and the slope of the calibration curve, are shown in Table 9, indicating that the method is sufficiently sensitive for the estimation of both analytes.

Drug	LOD (µg/mL)	LOQ (µg/mL)
Evogliptin Tartrate	0.192	0.582
Metformin Hydrochloride	6.606	20.020

Table 9: LOD and LOQ data for Evogliptin Tartrate and Metformin Hydrochloride

Accuracy

Recovery for Evogliptin Tartrate and Metformin Hydrochloride was found in the range of 99.13-100.77% and 98.31-98.88%, respectively, by the standard addition method, confirming the accuracy of the method (Tables 10 and 11).

% Spiked	Theoretical amount (µg/mL)	Amount found (µg/mL)	% Recovery ± SD	%RSD
50%	1	1.01	100.77 ± 0.008	0.811
100%	2	1.98	99.13 ± 0.019	0.966
150%	3	2.99	99.67 ± 0.030	1.001

Table 10: Accuracy study data for Evogliptin Tartrate (n=3)

% Spiked	Theoretical amount (µg/mL)	Amount found (µg/mL)	% Recovery ± SD	%RSD
50%	100	99.13	98.88 ± 0.410	0.413
100%	200	196.63	98.60 ± 1.038	0.528
150%	300	295.98	98.31 ± 0.947	0.320

Table 11: Accuracy study data for Metformin Hydrochloride (n=3)

Precision

The %RSD for repeatability of Evogliptin Tartrate and Metformin Hydrochloride was found to be 0.973% and 0.391%, respectively. The %RSD for intraday precision was found to be in the range of 0.793-1.157% (mean 0.971%) for Evogliptin Tartrate and 0.207-1.013% (mean 0.671%) for Metformin Hydrochloride. The %RSD for interday precision was found to be in the range of 0.487-1.557% (mean 0.985%) for Evogliptin Tartrate and within 2% for Metformin Hydrochloride, confirming that the method is precise (Table 12).

Precision parameter	EVO %RSD	MET %RSD
Repeatability (n=6)	0.973	0.391
Intraday precision (n=3, mean of 3 concentrations)	0.971	0.671
Interday precision (n=3, mean of 3 concentrations)	0.985	0.575

Table 12: Summary of precision (repeatability, intraday, and interday) data for Evogliptin Tartrate (EVO) and Metformin Hydrochloride (MET)

Robustness

The method was found to be robust to small deliberate variations in detection wavelength, mobile phase ratio, and flow rate, with %RSD for peak area of both analytes found to be less than 2% in all cases (Table 13), except for a marginally higher %RSD (1.72%) observed for Metformin Hydrochloride across the mobile-phase-ratio variation, which remained within acceptable limits.

Factor varied	Levels studied	EVO %RSD	MET %RSD
Detection wavelength (± 2 nm)	260, 262 (optimum), 264 nm	0.14	0.28
Mobile phase ratio (± 2 mL methanol)	50:50, 48:52 (optimum), 46:54	0.69	1.72
Flow rate (± 0.1 mL/min)	1.0, 1.1 (optimum), 1.2 mL/min	1.64	1.37

Table 13: Robustness data - effect of variation in detection wavelength, mobile phase ratio, and flow rate on peak area of Evogliptin Tartrate (EVO) and Metformin Hydrochloride (MET)

Summary of Validation Parameters

Parameter	Evogliptin Tartrate	Metformin Hydrochloride
Specificity	Specific	Specific
Linearity range	1-6 µg/mL	100-600 µg/mL
Slope	10154	12418
Intercept	24832	-9376.3
Correlation coefficient (r^2)	0.9994	0.9999
Accuracy (% recovery, n=3)	99.13-100.77	98.31-98.88
Repeatability (%RSD, n=6)	0.973	0.391

Parameter	Evogliptin Tartrate	Metformin Hydrochloride
Intraday precision (%RSD)	0.971	0.671
Interday precision (%RSD)	0.985	0.575
LOD (µg/mL)	0.192	6.606
LOQ (µg/mL)	0.582	20.020
Robustness	Complies	Complies

Retention time (min) ± %RSD	5.221 ± 0.174	1.860 ± 0.602
Theoretical plates/meter ± %RSD	4092 ± 0.451	4509 ± 0.328
Tailing factor ± %RSD	1.590 ± 1.703	1.170 ± 0.470
Resolution ± %RSD	6.590 ± 0.317	-

Table 14: Summary of validation and system suitability parameters

SUMMARY AND CONCLUSION

A simple, specific, precise, accurate, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Evogliptin Tartrate and Metformin Hydrochloride in combined tablet dosage form, using a Response Surface Methodology (Central Composite Design) based approach [6,7]. The application of a Faced Central Composite Design allowed systematic, statistically driven identification and optimization of the critical method parameters - flow rate, buffer pH, and percentage of methanol - reducing the trial-and-error effort typically associated with conventional method development and establishing a design space within which the method consistently delivers the desired chromatographic performance. The optimized method achieved separation of both analytes within a short run time of 10 minutes. The method was validated as per ICH Q2(R1) guidelines [10] and was found to be linear, precise, accurate, and robust, with adequate sensitivity (LOD/LOQ) for both analytes. Owing to its simplicity, economy, and reproducibility, the developed method can be conveniently employed for the routine quality control analysis of Evogliptin Tartrate and Metformin Hydrochloride, either individually or in combination, in marketed tablet formulations.

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CONFLICT OF INTEREST

No conflicts of interest, either financial or otherwise, have been declared by the authors.

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