RESEARCH ARTICLE

Development and Analytical Method Validation for Simultaneous Estimation of Evoglptin Tartrate and Metformin Hydrochloride in Combine Dosage Form

Aesha A. Patel, Manisha C. Kotadiya*, Bhakti Shah

Department of Pharmaceutical Chemistry, Shree Swaminarayan Sanskar Pharmacy College, Gujarat technological university, Zundal, Gandhinagar, , Gujarat, India.

Received: 18th October, 2022; Revised: 10th November, 2022; Accepted: 03rd December, 2022; Available Online: 25th December, 2022

ABSTRACT

Metformin hydrochloride is a diabetes medication that is used orally. Evogliptin tartrate is a dipeptidyl peptidase-4 (DPP-4) inhibitor which has been authorized as a new and powerful treatment in the process of reducing glucagon-like peptide 1 (GLP-1) deficiency while increasing insulin production and decreasing glucagon levels. The current study created a simple, highly exact, and rapid reverse phase high-performance liquid chromatography (RP-HPLC) approach for the simultaneous measurement of metformin hydrochloride and evogliptin tartrate in their combined dose form. High-performance liquid chromatography (HPLC), was performed on a phenomenex Luna C18 column with a mobile phase of water 25: acetonitrile 75 (25:75) at a 1.2 mL/min flow rate, with detection at 210 and 230 nm for metformin hydrochloride.and evogliptin tartrate. The validation method comprised of several variables like linearity, accuracy, selectivity, precision, robustness and specificity. The retention time of the metformin hydrochloride and evogliptin tartrate was 2.009 and 2.956 minutes, respectively. The method procedure linear responses of metformin hydrochloride and evogliptin tartrate was found to be 1 and 0.997, respectively. The percent relative standard deviation was less than 2% for intraday variation/precision, interday variation/precision, intermediate ruggedness, and robustness. The projected technique produced excellent results for many dependent variables. The projected method showed outstanding results with respect to different dependent and independent variables like limit of detection and limit of quantitation, suitability of system, linearity range etc., linearity, accuracy, precision, and specificity. The results were within the acceptance criteria given in ICH guideline.

Keywords: Antidiabetic medication, Dipeptidyl peptidase-4, Metformin hydrochloride, Reverse phase high-performance liquid chromatography Method, Validation.

International Journal of Pharmaceutical Quality Assurance (2022); DOI: 10.25258/ijpqa.13.4.25

How to cite this article: Patel AA, Kotadiya MC, Shah B. Development and Analytical Method Validation for Simultaneous Estimation of Evoglptin Tartrate and Metformin Hydrochloride in Combine Dosage Form. International Journal of Pharmaceutical Quality Assurance. 2022;13(4):504-509.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Diabetes mellitus (DM), usually referred to as diabetes, is a collection of metabolic illnesses characterized as persistently high blood sugar levels (hyperglycemia). Diabetes can lead to a variety of healthcare complications.[1,2] Metabolic acidosis,[3,4] hyperosmolar hyperglycemia, and mortality are all possible acute consequences. Heart disease, stroke, chronic renal disease, foot ulcers, nerve damage, vision problems, and cognitive impairment are all serious long-term complications.[5,6] Metformin hydrochloride is an oral antidiabetic drug[7,8] that has been used in the treatment of non-insulin-dependent diabetes, which improves glycemic control by primarily inhibiting hepatic gluconeogenesis and glycogenolysis. Metformin hydrochloride is classified as a biguanide, a group of antidiabetic drugs that lower blood sugar.[9,10]

Evogliptin tartrate is dipeptidyl peptidase-4 (DPP-4) inhibitors[11,12] are novel, potent oral antihyperglycemic agents that reduce degradation of endogenous glucagon-like peptide 1 (GLP-1) to increase insulin secretion and decrease glucagon. DPP-4 inhibitors enhance insulin secretion in a glucose-dependent manner, which potentially reduces hypoglycemia[13,14] risks during monotherapy or combination therapy with other antidiabetic agents. Evogliptin tartrate (Suganon is a new oral DPP-4 inhibitor developed for the treatment of patients with type 2 diabetes[15,16] inadequately controlled by diet and exercise.

This research aims to develop a sophisticated, highly precise, sensitive, and selective High-performance liquid

*Author for Correspondence: manishakotadiya3@gmail.com

chromatography (HPLC)[17,18] approach in quantifying evogliptin tartrate and metformin hydrocloride in solid formulation. The validation of the established analytical procedure in line with intracerebral brain hemorrhage (ICH)[19,20] recommendations (Q2) R1 using metrics such as accuracy, precision, Limit of Detection (LoD), Limit of Quantification (LoQ), linearity, and range[21,22] among others. Estimating evogliptin tartrate and metformin hydrocloride in combination dosage form using a newly built and verified analytical technique.[23,24]

METHODS AND MATERIALS

Metformin Hydrocloride and Evogliptin Tartrate Preliminary Analysis

The color and texture of the evogliptin tartrate sample were examine. Metformin hydrocloride and evogliptin tartrate samples were collected in capillaries to check its melting point. The melting point was measured and verified with the standard. Metformin hydrocloride and evogliptin tartrate were scanned in the 4000–400cm^{-1} area of fourier-transform infrared spectroscopy (FTIR). The resulting IR spectra were verified with the reference spectrum of metformin hydrocloride and evogliptin tartrate. For solubility, metformin hydrocloride and Evogliptin tartrate samples were placed in test tubes. They measured their solubility in suitable mobile phases such as water, methanol, 0.1 N HCl, DMSO, 0.1 M sodium hydroxide etc.

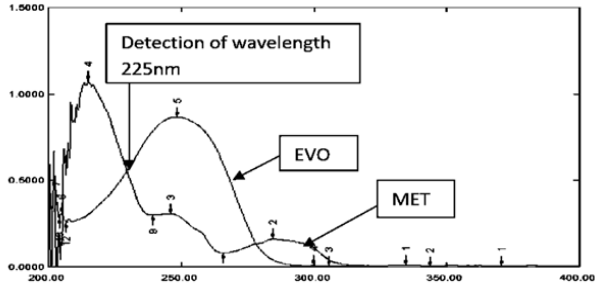

Figure 1: UV Spectra of Metformin Hydrochloride & Evogliptin (50 ppm) (maximum absorbance 225 nm).

Reagents and Materials

Methanol HPLC grade and water HPLC grade (Finar Limited), acetonitrile HPLC grade (Finar Limited), potassium di-hydrogen phosphate AR grade and phosphoric acid, AR grade (Ranchem, New Delhi) were brought. metformin hydrocloride and evogliptin tartrate were obtained from DC Chemical Limited.

Table 1: Mobile Phase trials for Metformin Hydrochloride and Evogliptin Tartrate.

S. NO.	*MOBILE PHASE COMPOSITION*	*RATIO (V/V)*	*pH*	*RT(MIN)*		*REMARKS*
				EVO	*MET*	
1.	Water :Methanol	50 : 50	-	-	-	No Peak observed
2.	Buffer :Methanol	50 : 50	7.5	-	-	
3.		60 : 40		-	-	
4.		70 : 30		-	-	
5.	Buffer : Methanol (pH adjusted with 0.5%OPA)	50 : 50	4.5	@ around 3-4 min		No Separation observed
6.	Buffer :Acetonitrile (pH adjusted with 0.5%OPA)	50 :50	4.0	7.5	3.20	Ill Separated but Peak tailingand long retention was observed for both
7.	Water: Acetonitrile (25:75)	25:75	4.0	3.0	2.0	Effective separation with proper peak shape & resolution and less retention time

Table 2: System suitability Variables.

Variables	*Metformin Hydrochloride*	*Evogliptin*
Retention Time (Min)	2.009	2.956
Theoretical Plates	6743	9243
Asymmetry	1.2	1.1
Resolution	1.7	

Variables	*Chromatographic Condition*
Elution Mode	Isocratic Mode
Solvent proportion	Water:Acetonitrile (25:75)
Column	C18 @ 25°C
Flow rate	1.2mL/min
Injected volume	20µL
Isobestic point	225 nm

Analytical Procedure for Method Validation of an RP-HPLC Method

Metformin hydrocloride and Evogliptin tartrate standard solution preparation

- Evogliptin tartrate (50 g/mL) standard stock solution I weighed 0.5 mg of evogliptin tartrate and added it to a 10 mL flask. To compensate for the volume difference, mobile phase was employed.
- Metformin hydrocloride standard stock solution (5000 g/mL): Metformin hydrocloride 50 mg was weighed and placed to a 10 mL flask. Mobile phase was used to maintain the difference in volume.
- Development of a workable solution tartrate of evogliptin (5 g/mL) 1-mL of evogliptin tartrate stock solution was transferred to a 10 mL flask and the volume was brought up to the mark with mobile phase.

- Construction of a working solution hydrochloride metformin (500 g/mL) take 1 mL of the metformin hydrocloride is stock solution volume, which has been increased to 10 mL by mobile phase.
- Mixed solution preparation: Weighing of 0.5 mg of evogliptin tartrate or 50 mg of metformin hydrocloride into a 10 mL flask. Methanol was used to maintain the difference in volume. Take 1 mL of the above-mentioned solution volume, which has been increased to 10 mL by mobile phase. This yields 5 g/mL tartrate and 500 g/mL Metformin hydrocloride.
- Mobile Phase (diluent): 250 mL water and 750 mL acetonitrile are combined and sonicate.

Wavelength Selection

Using a UV-visible spectrophotometer, a standard solution of metformin hydrocloride and evogliptin tartrate (1.0 mg/mL) was scanned between 200–400 nm. Both solutions have been scanned between in 200 and 400 nm. The wavelength can be choose by the overlaying spectrum of the preceding samples.

Mobile Phase Selection

The trail has multiple mobile phases that are assumed to be methanol, water, and acetonitrile in varying proportions with or without buffers and in varied volumes at different flow rates. Based on several trails, the Water: Acetonitrile (25:75) mixture at 1.2 mL/min flow rate Outperformed the other combination in terms of shape of peak , theoretical plate count, and dis-symmetry.

Table 4: Data of Linearity for metformin hydrochloride and evogliptin tartrate.

Sr. No	*Concentration (µg/mL)*	*Mean Area (n=2) Metformin*
1	250	523.52
2	375	792.24
3	500	1054.68
4	625	1312.8
5	750	1576.1

Sr. No	*Concentration (µg /mL)*	*Mean Area (n=2) Evogliptin*
1	2.5	816.45
2	3.75	1232.79
3	5	1634.73
4	6.25	2066.76
5	7.5	2446.35

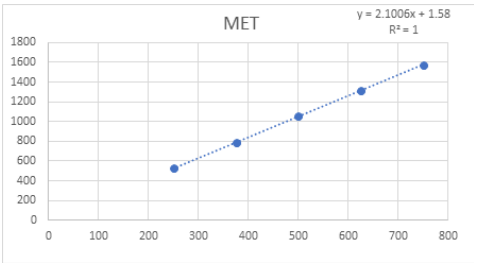

Figure 2: Calibration Curve of Metformin Hydrochloride (µg/mL).

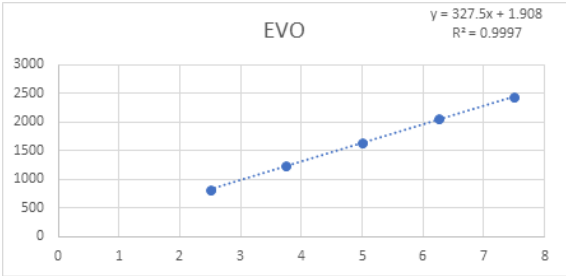

Figure 3: Calibration Curve of Evogliptin Tartrate (µg/m).

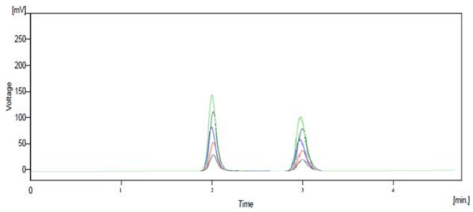

Figure 4: Linearity Overlay Chromatogram.

Chromatographic Separation

Standard solutions of 1 mg/mL metformin hydrocloride and 1-mg/mL evogliptin tartrate were used for chromatographic separation is injected into the column using a 20l micro syringe loop. The chromatogram was conducted for the specified number of minutes with water: Acetonitrile (25:75). Initially, the detection was performed at 255 nm. After full separation, the chromatogram was stopped. Peak data such as area, height, retention duration, resolution, and so on are captured using

Table 5: Metformin Hydrochloride % recovery

Sr. No.	*Concentration (%)*	*Sample amount (microgram/mL)*	*Conc. taken (microgram/mL)*	*Recovered Sample (microgram/mL)*	*Recovery in %*	*% RSD*
1		500	400	405.5	101.37	
2	80 %	500	400	401	100.17	0.98
3		500	400	397.66	99.41	
4		500	500	494.23	98.85	
5	100 %	500	500	492.43	98.49	0.95
6		500	500	501.38	100.28	
7		500	600	610.84	101.81	
8	120 %	500	600	609.25	101.54	0.95
9		500	600	600.11	100.02	

Table 6: Evogliptin Tartrate recovery data.

Sr. No.	*Concengtration (%)*	*Sample amount (microgram /mL)*	*Amount Added (microgram/mL)*	*Amount recovered (microgram/mL)*	*Recovery in %*	*% RSD*
1		5	4.0	4.06	102	
2	80.0 %	5	4.0	4.06	101.39	0.40
3		5	4.0	4.03	100.77	
4		5	5	4.91	98.18	
5	100 %	5	5	4.97	99.42	1.26
6		5	5	5.03	101	
7		5	6	5.92	98.65	
8	120 %	5	6	5.95	99.22	0.29
9		5	6	5.94	98.99	

the software. For improved response, the wavelengths 210 and 230 nm were used for dual detection mode for metformin hydrochloride and evogliptin tartrate, respectively.

Test for System Suitability Check

It is a fundamental component of the chromatographic strategy. These tests are used to ensure that the framework's organization and repeatability are sufficient for the examination to be done. Framework reasonableness checks are founded on the assumption that the tools, hardware, scientific processes, and testing give a fundamental framework that may be assessed.

RESULT AND DISCUSSION

Detection of Isobestic Point

For the detection of isobestic point solutions of drug metformin hydrochloride and evogliptin (50 ppm) were obtained by using mobile phase ratio acetonitrile: Methanol (50:50). The maximum absorbance of drug solutions were measured by UV in 200–400 nm region (Figure 1).

Solvent Selection

Selection of Mobile phase for Metformin Hydrochloride and Evogliptin Tartrate.

On the basis of various trials, acetonitrile and water with a proportion of 75:25 v/v, respectively, were found to be wise selection than the other mobile phase proportion for different variables like peak shape, Plate number and asymmetry. Different efforts of good selection of solvent are reviews in given Table 1.

Linearity

Correlation co-efficient for metformin hydrochloride was 1 and evogliptin tartrate was 0.9997.

Metformin hydrochloride, y=2.1006x+1.58 and Evogliptin, y=327.5x+1.908

The calibration curve for metformin hydrochloride and evogliptin is shown in (Figure 2 and 3) respectively. The linearity overlay chromatogram is shown in Figure 4.

Precision

Repeatability

The repeatability data for metformin hydrochloride and evogliptin tartrate (500 µg/mL and 5µg/mL respectively) obtained by six repeated injection of sample solution. The % RSD for metformin hydrochloride was 0.18 and for evogliptin was 0.20.

Intraday Precision

The % RSD was calculated. The % RSD in intraday precision for metformin hydrochloride was between 0.41-0.92 and for evogliptin was between 0.76–1.59.

Interday Precision

The %RSD was calculated. The % RSD in intraday precision for metformin hydrochloride and evogliptin tartrate was in range of 0.33–1.11 and 0.4–0.63, respectively

Accuracy

Recovery was observed in range of 98–102% for metformin hydrochloride and evogliptinat each level.

Limit of detection and Limit of Quantitation

The standard deviation (SD) of the intercepts was calculated.

LoD of metformin hydrochloride was 0.55 µg/mL, while evogliptin tartrate was 0.006 µg/mL.

LoQ of metformin hydrochloride evogliptin tartrate was 1.67 µg/mL while evogliptin tartrate was 0.017 µg/mL.

Robustness

The effect of possible variables was detected on system suitability for standard preparation as given below.

- Flow rate change by ± 0.2 mL/min.
- Solvent proportion was changed by ± 2 %
- Column oven temperature changed by ± 5°C

The % RSD for metformin hydrochloride and for evogliptin tartrate in the acetonitrile mobile phase was 0.12 and 0.57, respectively.

CONCLUSION

A sophisticated, most suitable, appropriate and highly precise method was established and confirmed with respect to standard of ICH for quantitation of evogliptin tartrate and metformin hydrochloride in solid dosage form. In terms of variables, linearity, accuracy, robustness, precision, system applicability, and specificity were all validated. It can be effectively implemented for tiresome analysis of evogliptin tartrate and metformin hydrochloride in pharmaceutical dosage form without any interference from common excipients and impurities. The proposed method can utilize for regular

investigation/analysis for evogliptin tartrate and metformin hydrochloride in any solid formulation.

REFERENCES

1. Panigrahy UP, Reddy S. RP-HPLC-DAD Method for the Simultaneous Estimation of Metformin Hydrochloride and Canagliflozinin Bulk and Pharmaceutical Tablet Dosage form with Forced Degradation Studies. Oriental journal of chemistry. 2015; 31(3):1489-1507 Available from: http://dx.doi.org/10.13005/ojc/310328
2. Mahrouse MA, Lamei NT. Ion pair RP-HPLC method development. Micro chemical Journal. 2019; 147:691-706. Available from: https://doi.org/10.1016/j.microc.2019.03.038.
3. Pulla RP, Sastry BS, Prasad YR, Appala RA. Simultaneous estimation of Metformin Hcl and Sitagliptin Phosphate in tablet dosage form by RP-HPLC. Research Journal of Pharmacy and technology. 2011; 4:646- 649. https://rjptonline.org/AbstractView.aspx?PID=2011-4-4-32
4. Santhosha A. Ravinranath H, Sundari C. Validated method for simultaneous estimation of metformin hydrochloride and vildagliptin by RP HPLC in bulk and the pharmaceutical dosage form. International research journal of pharmaceutical and applied sciences. 2012; 2(3):22-28. Available online: www.uptodateresearchpublication.com
5. Gattu MP, Muthukumaran M, Krishnamoorthy B. Development and validation of simultaneously of vildagliptin and metformin hydrochloride by RP HPLC in bulk and oral dosage form. International journal of advanced pharmaceutical genuine research. 2014; 2(1):24-33. Available from: Corpus ID 97153488
6. Devi R, Mohammad H. Stability Indicating RP-HPLC Method for the Simultaneous Determination of Atorvastatin Calcium, Metformin Hydrochloride and Glimepiride in Bulk and Combined Tablet Dosage Form. International Scholarly Research Notices. 2014; 55(21):374–407. Available from: https://doi.org/10.1155/2014/754695.
7. Kumar V, Rao JV. A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms. International Research Journal of Pharmacy and Medical Sciences; 2581-3277.
8. Shirode AR, Maduskar PD, Deodar MS, Kadam VJ. RP-HPLC and HPTLC Methods for Simultaneous Estimation of Metformin Hydrochloride and Vildagliptin from Bulk and Marketed Formulation. Journal of Pharmaceutical Research International. 2014; 2370-2386. Available from: https://doi.org/10.9734/BJPR/2014/12820
9. Urooj A, Shyam Sundar PS, Vasanthi R, Dutt KR, Rao KN. Development and validation of Rp-hplc method for simultaneous estimation of dapagliflozin and metformin in bulk and in synthetic mixture. World journal of pharmacy and pharmaceutical sciences. 6(7):2139-2150. Available from: https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1579347816.pdf
10. Sarvana K, Bandhakavi S. Development and validation of stability-indicating RP-HPLC method for the simultaneous determination of ertugliflozin pidolate and metformin hydrochloride in bulk and tablets. Future Journal of pharmaceutical sciences. 2020; 6:66 Available from: https://doi.org/10.1186/s43094-020-00079-1
11. Mishra K, Soni H, Nayak G, Patel S. Method Development and Validation of Metformin Hydrochloride in Tablet Dosage Form. Hindavi Journal of Chemistry. 2011; 8(3): 1309-1313. Available from: https://doi.org/10.1155/2011/768014
12. Jani BR, Shah KV, Kapupara PP. Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Hydrochloride in Synthetic Mixture. Journal of Bio equivalence Studies. 2015; 1(1):1-8. Available from: 10.15744/2575-551X.1.102
13. Rao DN, Rao MP, Naga HS, Sumanoja S, Rao L. Method development and validation of forced degradation studies of metformin hydrochloride by using UV spectroscopy. International Journal of Pharmaceutical, Chemical & Biological Sciences. 2013; 3(3): 546-553. Available from: Corpus ID: 99124949.
14. Kavitha R, Arivukkarasu R, Development and validation of HPTLC method for simultaneous estimation and stability indicating study of metformin HCl and linagliptin in pharmaceutical formulation. World journal of pharmaceutical sciences. 2014; 2(4):259-421. Available from: https://wjpsonline.com/index.php/wjps/article/view/hptlc-method-metformin-linagliptin-estimation
15. Thomas AB, Patil SD, Nanda RK, Kothapali LP, Deshpande AV. Stability-indicating HPTLC method for simultaneous determination of nateglinide and metformin hydrochloride in pharmaceutical dosage form. Saudi Pharmaceutical journal. 2011; 19: 221. Available from: https://doi.org/10.1016%2Fj.jsps.2011.06.005.
16. Modi DK, Parejiya PB, and Patel BH, A Simple and Sensitive HPTLC Method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in Tablet Dosage Form. Hindavi Journal of Chemistry. 2013, 1-5. Available from: https://doi.org/10.1155/2013/139561
17. Sakhare RS, Pekamwar SS, Mohkare DP. Development and Validation of Stability Indicating HPTLC Method for the Determination of Metformin Hydrochloride and Benfotiamine in Bulk and Combined Dosage Form. Indian Journal of Pharmaceutical Education and Research. 2017; 51(2). Available from: 10.5530/ijper.51.2s.44.
18. Avani K, John B. Brijesh D. Spectrophotometric Method for Simultaneous Estimation of Telmisartan and Metformin Hydrochloride in their Combined Pharmaceutical Dosage Form. International Journal of Pharmaceutical Quality Assurance. 2020; 11(1). Available from: 10.25258/ijpqa.11.1.9.
19. Patel A, Patel R, Yadav P. Development and Validation of RP-HPLC Method for estimation of Evogliptin in Pharmaceutical Dosage Form. International Journal of Pharmaceutical Research and Applications. 2021; 6(2):775-781. Available from: 10.35629/7781-0602775781.
20. Kyunghee C, Koung Joung S, Sookie L, Young Lee D. Bio analytical Validation for the Determination of Evogliptin and Its Metabolites M7/M8 In Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry. Drug Metabolism and Pharmaco kinetics. 2017; 32. Available from: https://www.researchgate.net/publication/31217965
21. Rathod HC, Desai SP. Review on Analytical Method for Quantitative Estimation of Metformin Hydrochloride and Evogliptin Tartrate in Pharmaceutical Dosage Form. World Journal of Pharmaceutical Research. 2021; (4) :418-435. Available from: https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1617174180.pdf

22. Agrawal YP, Agrawal M, Jadhav SB, Shinde RJ. Development and Validation of Novel UV Spectrophotometric Method for the Determination of Evogliptin Tartrate in Pharmaceutical Dosage Form. Indian Journal of Pharmaceutical Education and Research. 2020; 54(4):1174-1179.
23. Indian Pharmacopoeia 2018 Volume 3 and 4, Govt. of India Ministry of Health and Family welfare, Indian Pharmacopoeia Commission, Ghaziabad. 2018. Available from: https://www.ipc.gov.in.
24. United State Pharmacopoeia 42 National Formulary 37, Volume 2, The United States Pharmacopoeia Convention 2019. 2278-2281. Available from: https://www.usp.org.