Research Article
CODEN (USA): IJPLCP

ISSN: 0976-7126
John & Khan, 13(4):1-6, 2022

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

Simple cost effective Method Development for the estimation of combined Anti-diabetic drugs in Marketed Formulation

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Article info

Received: 10/03/2022

Revised: 26/03/2022

Accepted: 27/04/2022

www.ijplsjournal.com

Abstract

Metformin and Teneligliptin is a combination of two antidiabetic medicines: Metformin and Teneligliptin. Metformin is an antidiabetic medication (biguanide). It works by lowering glucose production in the liver, delaying glucose absorption from intestines and increasing the body's sensitivity to insulin. Teneligliptin is a dipeptidyl peptidase-4 inhibitor which works by increasing the release of insulin from pancreas and decreasing the hormones that raise blood sugar levels. This reduces both fasting and postmeal sugar levels. Together, they provide better control of blood sugar. The separation was achieved on Thermo C_{18} analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using Acetonitrile: Water in the ratio of 80:20v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication.

Flow rate employed for analysis was 1.0 ml/min. The total chromatographic analysis time per sample was about 20min with eluting at retention time of about 3.458±0.001min and 5.125±0.002 for metformin and Teneligliptin respectively. The method was validated for accuracy, precision, specificity, linearity and sensitivity. Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The developed and validated method was successfully applied for the quantitative analysis of metformin and Teneligliptin tablet formulation. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of metformin and Teneligliptin in marketed formulation.

Key words: Analytical method development, Reversed phase HPLC method, ICH guidelines, Tablet dosage forms, Accuracy and precision

Introduction

Analytical method Development and validation for newly introduced pharmaceuticals is of importance, as drug or drug combination may not be official in pharmacopoeia and so analytical method for quantification is not available. To check and ensure the quality standards of drug molecules and their formulation various analytical methods are employed. Most of the drugs in single or multi component dosage forms can be analyzed by HPLC method because of the associated advantages like speed, greater sensitivity, improved resolution, specificity, accuracy, precision, reusable columns and ease of automation in this method [1, 2, 3].

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Metformin hydrochloride chemically, N,N-dimethylimidodicarbonimidic diamide hydrochloride[4] is an antidiabetic agent[5]. In literature cited for analysis of metformin hydrochloride, few HPLC [6, 7] methods have been reported but the retention time has been found to be very high and none have been reported from the microspheres. (Figure 1).

Teneligliptin (Figure 2) is chemically [(2S,4S)-4-[4-(5-methyl-2-phenylpyrazol-3- yl)piperazin-1-yl]pyrrolidin-2-yl]-(1,3-thiazolidin-3-yl)methanone. It is highly effective in lowering blood glucose levels. This drug inhibits the enzyme dipeptidyl peptidase-4 (DPP4) which degrades incretin, a hormone adjusting blood glucose control. It is effectively used to treat type-II diabetes mellitus [8, 9]. Several methods have been employed for the estimation of metformin and teneligliptin alone and combination with other drugs by UV and RP-HPLC methods in bulk drug and plasma samples. But there is no simple and easy method for the analysis of metformin and teneligliptin. Hence, it is necessary to develop a rapid, accurate and validated RP-HPLC method for the determination of metformin and teneligliptin in bulk and marketed formulation. This paper describes the development and validation of reliable, simple, robust, time and money saving reversed phase HPLC method, using UV detection, for the estimation of Teneligliptin and Metformin in bulk and tablets formulation. The developed method validated according to ICH guidelines [10].

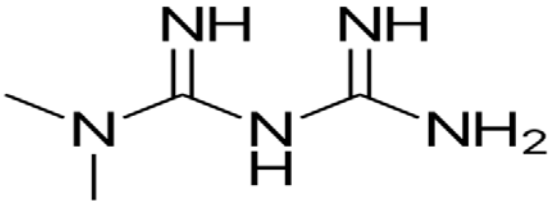

Fig. 1: Chemical structure of metformin

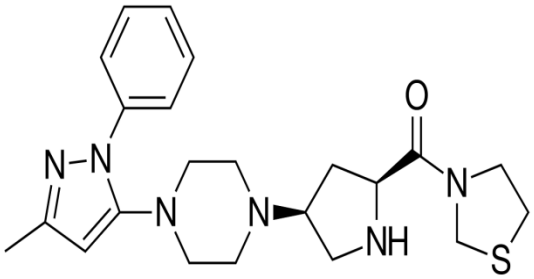

Fig. 2: Chemical structure of teneligliptin

Material and Method

Instrumentation

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

Reagents and chemicals

Analytically pure sample of metformin and teneligliptin was a generous gift from Pharmaceutical Company. Acetonitrile (HPLC Grade) was purchased from E. Merck Ltd. Worli, Mumbai, India. All other chemical used were of analytical grade. Triple distilled water was used for whole experiment was generated in house. Marketed formulation tablets were purchased from local market.

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials acetonitrile was used as diluents.

Selection of Mobile Phase

Initially to estimate Metformin and Teneligliptin in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Water in the ratio of 80:20v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and

then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock Solution:

Accurately weighed 10 mg API of METF and TNLG was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (acetonitrile) to give concentration of 100µg/ml of METF and TNLG respectively (Stock-B).

Preparation of Different Solution

1.0ml, 2.0ml, 3.0ml, 4.0ml and 5.0ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (acetonitrile). This gives the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml, for METF. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml of TNLG also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 10-50µg/ml for METF and 1-5µg/ml for TNLG were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 250.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Validation of developed Method [10]

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 10 to 50µg/ ml for METF) and (1 to 5µg/ ml for (TNLG) and areas for each concentration were recorded three times and mean area was calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 10, 20, 30, 40 and 50µg/ml for METF and 1, 2, 3, 4 and 5µg/ml for TNLG indicates the precision under the same operating condition over short interval time.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for METF and TNLG respectively.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Water (80:20 % v/v) to (85:15% v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 50 mg of METF and 2mg of TNLG was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture

was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 50µg/mL METF and 2µg/mL TNLG respectively. The amounts of METF and TNLG in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

Results and Discussion

The RP-HPLC method was developed for estimation of Metformin (METF) and Teneligliptin (TNLG) in combined formulation by isocratically using Acetonitrile: Water in the ratio of 80:20 v/v as mobile phase, Prontosil C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 280nm. Then developed method was validated by using various parameters.

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard METF 10µg/ml for METF and 2µg/ml TNLG were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in table 1.

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 2.

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 3.

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 4.

The robustness of developed method was checked by changing in the deliberate variation in solvent. Result of robustness shown in table 5.

The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drugs 7.

Table 1: Results of system suitability parameters

Parameters	% MEAN±SD*	
	METF	TNLG
No. of Theoretical Plates	2541.500±43.353	2644.500±19.907
Tailing Factor	1.182±0.051	1.048±0.019
Retention time	3.458±0.001	5.125±0.002

Table 2: Results of linearity of Metformin (METF) and Teneligliptin (TNLG)

Parameter	METF	TNLG
Concentration (µg/ml)	10-50	1-5
Correlation Coefficient (r^2)*	0.997	0.997
Slope (m)*	31.48	203.6
Intercept (c)*	30.89	18.00

*value of six replicate

ISSN: 0976-7126
John & Khan, 13(4):1-6, 2022

Table 3: Results of recovery study

% Level	% MEAN±SD*	
	METF	TNLG
80%	98.98±0.129	98.66±0.561
100%	98.90±0.561	96.35±0.195
120%	98.77±0.494	98.21±0.798

***** Value of three replicate and three concentrations.

Table 4: Results of precision

Parameter	% MEAN±SD*	
	METF	TNLG
Repeatability	99.002±0.117	96.423±0.086
Day To Day	99.171±0.147	97.634±0.051
Analyst to Analyst	98.127±0.093	97.653±0.030

***** Value of five replicate and five concentrations

Table 5: Results of Robustness

Parameter	% MEAN±SD*	
	METF	TNLG
Robustness	99.446±0.134	96.230±0.076

***** Value of five replicate and five concentrations

Table 6: Results of LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
METF	0.35	0.95
TNLG	0.25	0.75

Table 7: Analysis of tablet formulation

	METF*	TNLG*
Label Claim (mg)	500mg	2mg
% Found (mg)	498.85	1.95
% Assay	99.77	97.50
% RSD	0.074	0.085

*Average of three determination

Conclusion

The present study was conducted to develop and validate a simple, sensitive and reproducible RP-HPLC method for quantitative determination of Metformin (METF) and Teneligliptin (TNLG). The developed chromatographic assay fulfilled all the requirements to be identified as simple, specific, selective and reliable, including accuracy, linearity, and recovery and precision data. The data generated from the studies enabled the evaluation of Metformin (METF) and Teneligliptin (TNLG) validate under a variety of ICH recommended conditions. These data are valuable for the safety and potency assessment of a drug product. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Metformin (METF) and Teneligliptin (TNLG) in pharmaceutical formulations without any interference from the excipients. It was thus, concluded that the proposed method is new, simple, accurate, safe, precise and can be successfully employed in the routine analysis. The simplicity, rapidity reproducibility and economy of the proposed methods completely fulfill the objective of this research work.

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Cite this article as:
John S. and Khan N. (2022). Simple cost effective Method Development for the estimation of combined Anti-diabetic drugs in Marketed Formulation. *Int. J. of Pharm. & Life Sci.,* 13(4): 1-6.

Source of Support: Nil
Conflict of Interest: Not declared
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