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Archives of Natural and Medicinal Chemistry

Patil PM, et al. Arch Nat Med Chem: ANMC-108.
DOI: 10.29011/ANMC-108.000008

Research Article

Development and Validation of High-Performance Thin-Layer Chromatography Method for Estimation of Teneligliptin in Bulk and in Pharmaceutical Formulation

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Citation: Patil PM, Jain PS, Surana SJ, Yeole SD (2017) Development and Validation of High-Performance Thin-Layer Chromatography Method for Estimation of Teneligliptin in Bulk and in Pharmaceutical Formulation. Arch Nat Med Chem: ANMC-108. DOI: 10.29011/ANMC-108.000008

Received Date: 28 August, 2017; **Accepted Date:** 28 September, 2017; **Published Date:** 5 October, 2017

Abstract

A high-performance thin layer chromatographic method for determination of Teneligliptin was developed and validation as per ICH guidelines. HPTLC separation was performed on aluminum plates precoated with silica gel $60F_{254}$ and Methanol: Toluene: Triethylamine (1:3:1% v/v) as optimized mobile phase at detection wavelength of 245 nm. The retardation factor (Rf) value for Teneligliptin were 0.63 respectively. Accuracy for the marketed formulation were found to be 98.31-100.51%. The percent relative standard deviation for repeatability and intermediate precision studies was found to be < 2%. The propose development HPTLC method can be applied for identification and quantitative determination of Teneligliptin.

Keywords: Development; HPTLC; Teneligliptin; Validation

Introduction

Teneligliptin is chemically {(2s,4s)-4-[4-(3-Methyl-1-phenyl-1-H pyrazole-5-yl) piperazin-1-yl] pyrrolidin-2-yl}(1,3-thiazolidin-3-yl) methanone (Figure1), having molecular formula: C22H28N6OS, with a molecular mass of 309.40 g/mol. It is a white fine powder which is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol, and insoluble in acetonitrile. Teneligliptin is a Type-2 diabetis mellitus drug that belongs to dipeptidyl peptidase-4 inhibitors or "Gliptins". DPP-4 inhibitor degradation, increasing the concentration of active GLP-1 in the blood, which stimulates glucose dependent insulin secretion and at the same time, suppresses glucagon secretion, thereby exhibiting a glucose lowering effect [1].

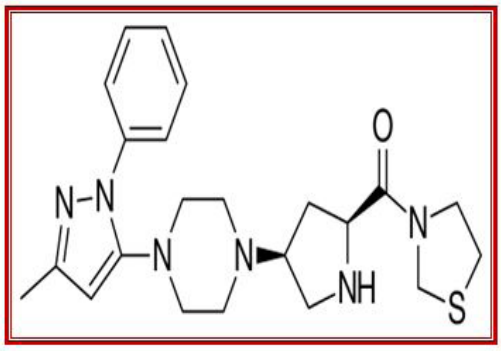

Figure 1: Chemical structures of Teneligliptin.

Various method is reported for the analysis of individual drug as HPLC and LC MS/MS but no HPTLC method is reported estimation of drug in pharmaceutical dosage form [2-7]. The ob-

jective of this research work was therefore to develop a simple, rapid, precise and accurate HPTLC method for quantitative analysis of Teneligliptin to validation the method in accordance with ICH guidelines [8,9].

Material and Methods

Teneligliptin was supplied by gift sample Ipca laboratory ltd. (Mumbai). All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

HPTLC Instrumentation

the sample were spotted in the form of band 6 mm with a Camag microliter syringe on precoated silica gel aluminium plate 60F254 (20 cm X 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat 5 (Switzerland). A constant application rate of 200 nl/s was employed and space between two bands was 8 mm. the slit dimension was kept 6 mm X 0.45 mm micro, 20 mm/s scanning speed was employed. The mobile phase consisted of methanol: toluene: triethylamine (1:3:1 v/v). Linear ascending development was carried out in twin through glass chamber saturated with mobile phase. The length of chromatogram run was approximately 75 mm. subsequent to the development; TLC plate was dried in a current of air-dryer. Densitometric scanning was performed on Camag TLC scanner 3 in the absorbance mode at 245 nm. The source of radiation utilized was tungsten lamp.

Preparation of Standard Solution and Linearity Study

A standard stock solution of teneligliptin2000 µg/mL was prepared in methanol. From the stock solution 1.0 mL was taken in 10 mL volumetric flask and the vol. was adjusted with methanol to give 100 µg/mL. From this 0.5, 1, 1.5, 2, 2.5 and 3 µl of the solution were spotted on TLC plate to obtain concentration of 500, 1000, 1500, 2000, 2500 and 3000 ng per spot of teneligliptin, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

Method Validation

Precision: Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (2000 ng per spot of teneligliptin). The intra-day and inter-day precision for the determination of teneligliptin was carried out at three different concentration levels of 1000, 2000 and 2500 ng per spot.

Recovery: Recovery studies were carried out by applying the method to drug samples, in which known amount of teneligliptin correspondence to 80, 100 and 120% were spiked. For each stated level, six determinations were performed.

Robustness: The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variation in various parameters.

Ruggedness: Ruggedness of the method was checked by analyzing 2000 ng (n=6) if teneligliptin with the help of two analysts and the variations in the results were checked.

Limit of Detection and Limit of Quantification: To determine detection and quantification limit, teneligliptinin the lower levels of the linear range of the calibration curve were use. Teneligliptin solution of 500, 600, 700, 800, 900 and 1000 ng/spot were applied in triplicate. The amount of teneligliptin versus average response (peak area) were plotted in a linear regression equation was determined. The standard deviation of responses was calculated. The average of standard deviation was calculated. Detection limit was calculated by (3.3XA.S.D)/b and quantification limit was calculated by (10XA.S.D.)/b, where "b" corresponds to the slope obtained in the linearity study of method.

Application of Proposed Method to Tablet Formulation: Twenty Teneza 20mg tablet were accurately weighed, average weight determined and crushed into fine powder. A quantity of powdered drug equivalent to 10 mg of teneligliptin was transferred into 100 mL volumetric flask containing 80 mL methanol, shaken manually for 15 min; volume was adjusted to mark using same solvent. The solution was then filtered through Whatmann filter paper no.41. An appropriate concentration 2000 ng/spot of teneligliptin was spotted on TLC plates. The plates were developed and scanned as described above. The concentration of the drug was assessed from the linearity curve.

Result and Discussion

Development of optimum mobile phase: Different ratios of methanol, toluene and triethylaminewere tried as mobile phase was tried but, tailing of spot, less persistent spots were observed in most of the attempts. In order to overcome the problems, methanol: toluene: triethylamine (1:3.1 v/v/v) was tried and result is good resolution, sharp and symmetrical peak with Rf value of 0.63 for teneligliptin.

Concentration (ng/band)	Mean peak area ± SD	%RSD
500	683.6 ± 21.56	0.848
1000	1283.6 ± 15.55	0.870
1500	1852.6 ± 15.69	0.847
2000	2363.5 ± 13.43	0.359
2500	2843.9 ± 20.36	0.716
3000	3347.5 ± 13.85	0.215

Table 1: Linearity of teneligliptin.

Calibration curve: The linear regression data for the calibration curves showed good linear relationship over the concentration range 500-3000 ng/spot (Figure 2), (Figure 3).

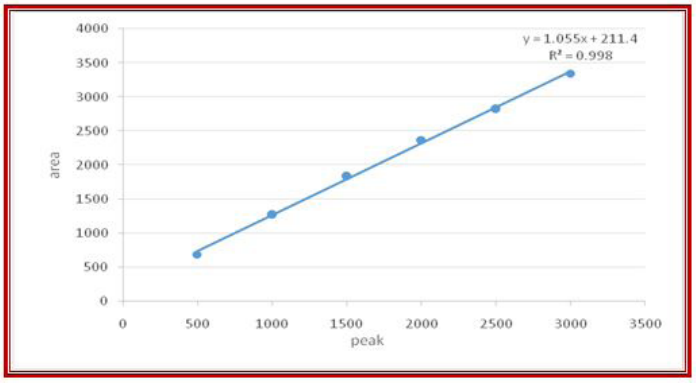

Figure 2: Calibration curve of Teneligliptin Area Vs ng/ml.

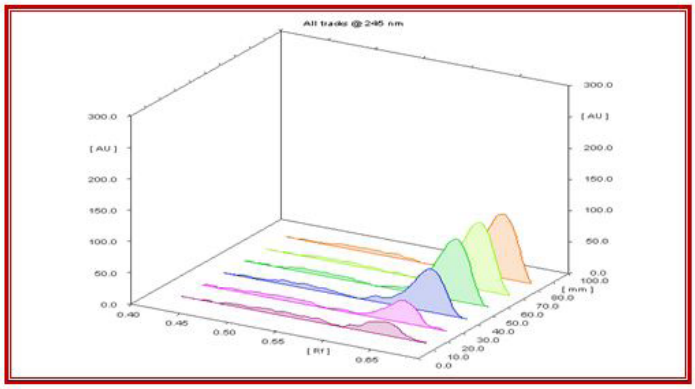

Figure 3: 3-D linearity chromatogram of Teneligliptin.

Linear regression was found to be y=1.055x + 211.4, slop = 1.055, intercept = 211.4, correlation coefficient = 0.998.

Method validation

Precision: The repeatability of sample application and measurement of peak area were expressed in terms of %R.S.D. and results are depicted in (Table 2). The intra and inter-day variation of teneligliptin at three different concentration levels of 1000, 2000 and 2500 ng per spot was to be <2%.

Concentration ng/band (n=3)	Intra-day Amount found Area ±SD	%RSD	Inter-day Amount found Area ± SD	%RSD
1000	1272.7 ± 4.06	0.31926	1274.6 ± 7.20	0.565
2000	2369.23 ± 3.30	0.13963	2373.6 ± 5.51	0.232
2500	2840.97 ± 1.53	0.05388	2831.7 ± 7.76	0.274

Table 2: Precision study.

Recovery: The proposed method when used for extraction and estimation of teneligliptin from pharmaceutical dosage form after spiking with 80, 100 and 120% of additional drug afforded recovery of 98.31-100.51% as listed in (Table 3).

% Amount	Initial Amount (ng/band)	Amount Added (ng/band)	% Recovery (N=3)	%Rsd
80	2000	1600	98.31	0.281
100	2000	2000	96.63	0.428
120	2000	2400	100.58	0.127

Table 3: Recovery study.

Robustness studies: The robustness of the method was established by introducing small changes in mobile phase composition and chromatograms were run. The amount of mobile phase, chamber saturation time, time from spotting to chromatography and from chromatography to scanning (±10 min). The %RSD calculated as shown in (Table 4).

Parameters	± SD of peak area (n=3)	% RSD
Mobile phase composition (± 0.5 mL)	8.712	0.489
Mobile phase volume (± 5ml)	13.28	0.603
Duration of saturation (± 5 min.)	35.46	0.932

Table 4: Robustness study.

Ruggedness: Ruggedness of the method was pre-formed by applying 2000 ng for teneligliptin, respectively by two different analyst keeping same experimental and environmental conditions. The results summarized in (Table 5).

Analyst	Peak area ± SD	%RSD
1	2371.18 ± 8.988	0.379
2	2369.62 ± 9.716	0.410

Table 5: Ruggedness study.

LOD and LOQ: The S/N 3:1 and 10:1 was considered as LOD and LOQ. The LOD and LOQ were found to be 67.42 and 204.42.the result summarized in (Table 6).

LOD (ng/band)	LOQ (ng/band)
67.46	204.42

Table 6: LOD and LOQ.

Analysis of tablet formulation: A single spot at Rf 0.63 was observed in the chromatogram of the drug sample extracted from tablets. There was no interference from the excipients commonly present in the tablet. The %drug content and %RSD were calcu-

lated. The low %RSD value indicated the suitability of this method for the routine analysis of teneligliptin in pharmaceutical dosage forms (Table 7).

Conc. (ng/ band)	Amount found area (ng/band)	%Amount found	%RSD
2000	2196.34	109.817	0.340

Table 7: Analysis of tablet formulation.

Conclusion

The proposed HPTLC methods have been developed and successfully validated for quantitative estimation of teneligliptin in tablet dosage form and in bulk drug. The results of the validation tests indicated that the developed methods were accurate, precise, robust and reproducible. Hence, the developed HPTLC methods are suitable for routine determination of Teneligliptin in pharmaceutical formulation inquality control laboratories, where economy and time are essential.

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