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

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## 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 60F254 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 teneza was 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:*** ***Teneligliptin; HPTLC; Development and validation***

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## 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) methanogen (FIG. 1), having molecular formula:C17H27NO4, with a molecular mass of 309.40 g/mol. It is a white fine powder which is freely soluble in water,

4 inhibitor, an enzyme widely distributed in the body. 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.

Various method are 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. The objective 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.

## 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.

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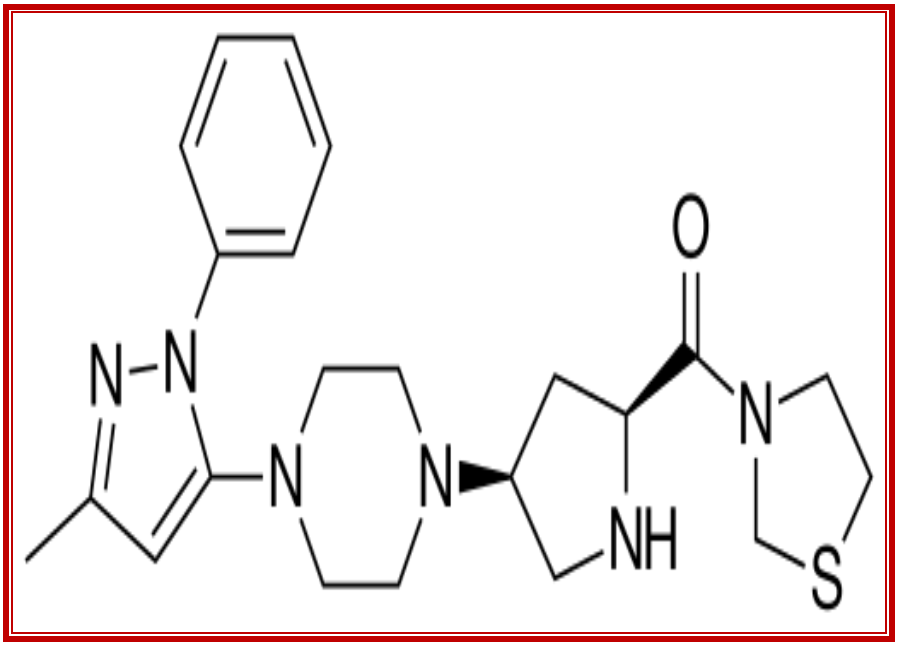


FIG. 1. **Chemical Structures of Teneligliptin.**

**HPTLC instrumentation**

the sample were spotted in the form of band 6 mm with a Camag microliter syringe on precoated silica gel aluminium plate $60F_{254}$ (20 cm × 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.

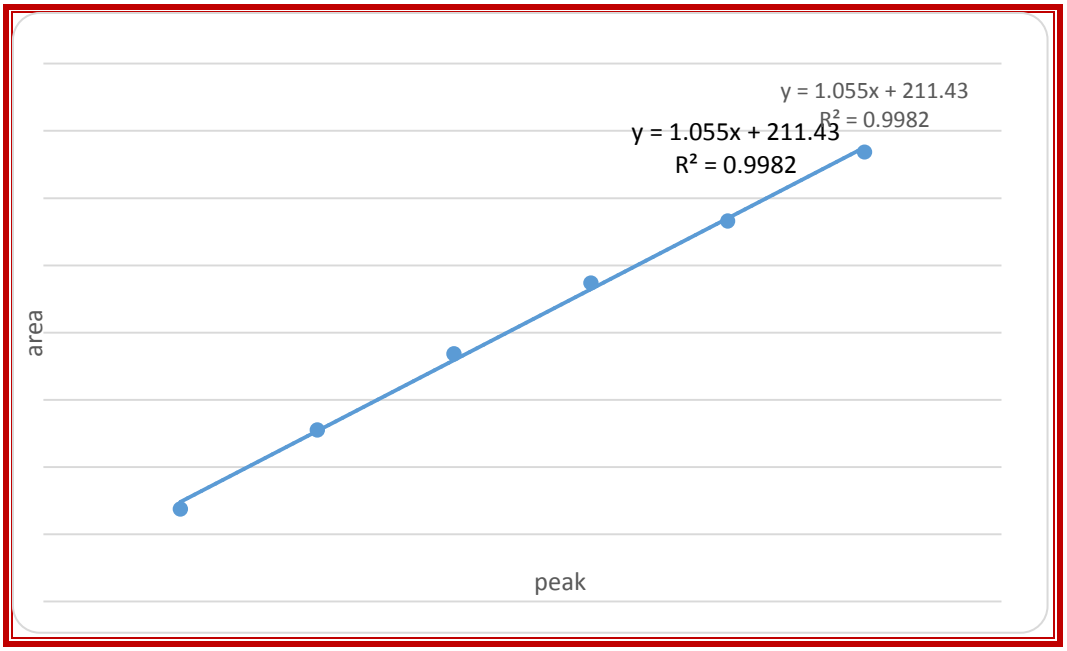


FIG. 2. **Calibration curve of Teneligliptin.**

**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 determination were performed.

**Robustness**

The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variation in methanol 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 deviations of responses were 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 20 mg tablet were accurately weighed, average weight determined and crushed into fine powder. A quantity of powdered drug equivalent to 10 mg 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 (FIG. 3).

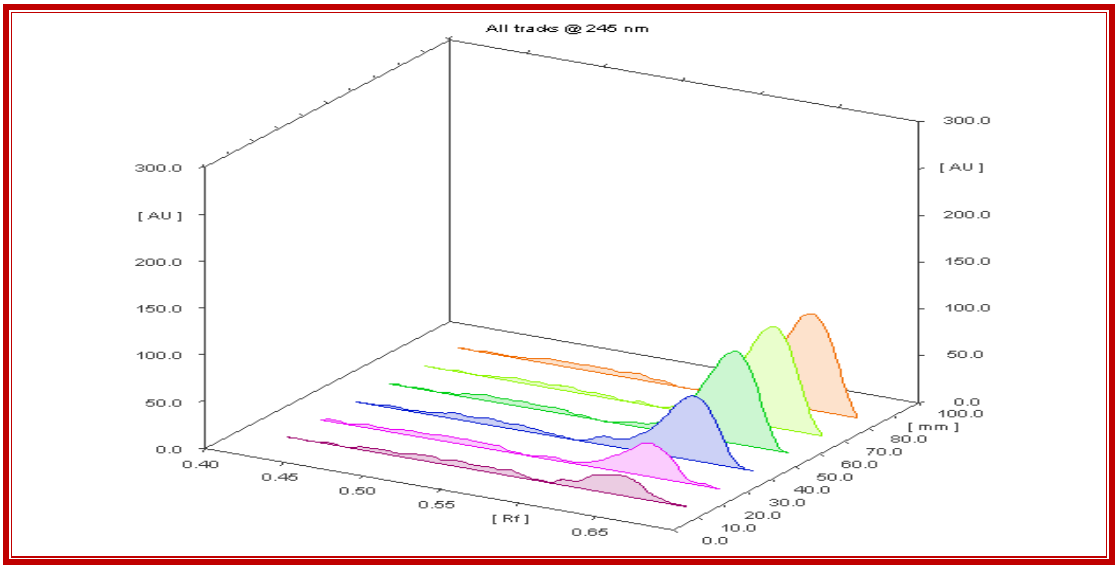


FIG. 3. **3-D linearity chromatogram of Teneligliptin.**

## Result and Discussion

### Development of optimum mobile phase

Different ratios of methanol, toluene and triethylamine were 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 $R_f$ value of 0.63 for teneligliptin (TABLE 1).

TABLE 1. **Linearity of 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 |

### Calibration curve

The linear regression data for the calibration curves showed good linear relationship over the concentration range 500-3000 ng/spot. 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%.

TABLE 1. **Precision study.**

| **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 |

**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.

TABLE 3. **Recovery study.**

| **% 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 |

**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.

TABLE 4. **Recovery study.**

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

**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.

TABLE 5. **Ruggedness study.**

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

**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.

TABLE 6. **LOD and LOQ.**

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

**Analysis of tablet formulation**

A single spot at $R_f$ 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 calculated. The low %RSD value indicated the suitability of this method for the routine analysis of teneligliptin in pharmaceutical dosage forms (TABLE 7).

TABLE 7. **Analysis of tablet formulation.**

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

## Conclusion

The proposed HPTLC methods have been developed and successfully validated for quantitative estimation of Teneligliptin in tablet dosage form. 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 in quality control laboratories, where economy and time are essential.

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