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# Method development, validation, and stability studies of teneligliptin by RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy

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## Abstract

**Background:** Teneligliptin is a new FDA approved drug for treating Diabetes Mellitus. There are no reported evidences for their identified degradation products and their effects on humans.

**Methods:** A simple and new stability indicating RP-HPLC method was developed and validated for identification of Teneligliptin and its degradants on Kromasil 100- 5C18 (250×4.6mm, 5μm) column using pH 6.0 phosphate buffer and acetonitrile (60:40 *v/v*) as a mobile phase in isocratic mode of elution at a flow rate of 1.0 mL/min. The column effluents were monitored by a variable wavelength UV detector at 246 nm. The method was validated as per ICH guidelines. Forced degradation studies of Teneligliptin were carried out under acidic, basic, neutral (peroxide), photo and thermal conditions for 48 hours at room temperature. The degradation products were identified by HPLC and characterized by UPLC with tandem mass spectroscopy (LC/MS-MS).

**Results:** UPLC MS/MS data shown major peaks, observed at 375.72, 354.30, 310.30, 214.19, 155.65, 138.08 and 136.18 m/z.

**Conclusion:** Degradation was observed in base, peroxide and thermal stressed samples, but not in acid and photolytic stressed samples.

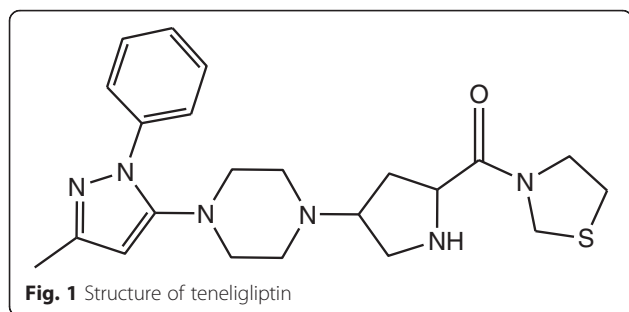**Keywords:** Teneligliptin, Degradation, RP-HPLC, LC/MS

## Background

Teneligliptin is a novel drug, which is used for the treatment of type 2 diabetes mellitus. It is an antidiabetic drug that belongs to dipeptidyl peptidase-4 inhibitors or "gliptins" (Kishimoto 2013). Chemically, it is {(2S, 4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-1-piperazinyl]-2-pyrrolidinyl} (1, 3-thiazolidin-3-yl) methanone (Fig. 1). Teneligliptin exerts its activity for 24 h, with elevation of activated glucagon-like peptide 1 (GLP-1) levels by suppressing postprandial hyperglycemia after the meals (Goda and Kadowaki 2013; Ideta et al. 2015; Bronson et al. 2013). Significant decrease in hemoglobin A1c (HbA1c), fasting blood glucose, and postprandial blood glucose levels was observed in type 2 diabetic patients taking teneligliptin for 12 weeks (Goda and Kadowaki 2013). This drug showed a promising effect in stabilizing the glycemic fluctuations throughout the day and suppressing the diabetic complications (Ideta et al. 2015). Teneligliptin is approved for use in India, Japan, and Korea in 2012 (Bronson et al. 2013). Although the drug entered the market, there is no much information available about its degradation studies and its degraded products. Few have reported its metabolism and pharmacokinetic studies (Halabi et al. 2013; Reddy and Rao 2014; Luhar et al. 2016; Shanthikumar et al. 2015). Identification of the degraded products helps in future metabolic studies and also related impurity determination during its bulk synthesis. In the present study, we have focused our research into two stages: (1) to develop and validate a reversed-phase HPLC (RP-HPLC)

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**Fig. 1** Structure of teneligliptin

method for identifying the teneligliptin and its degradation products formed during various forced conditions as per the ICH guidelines (Asian Guideline for Validation of Analytical Procedure Adopted from ICH guideline, Q2A27, and ICH Q2B 1994) and (2) identification of the degraded products obtained and their characterization using ultra-performance liquid chromatography (UPLC) with tandem mass spectroscopy (LC/MS/MS).

## Methods
### Materials and reagents
HPLC grade acetonitrile (Lichrosolv®, Merck Life Science, Pvt. Ltd., Mumbai, India), HPLC water (Lichrosolv®, Merck Life Science, Pvt. Ltd., Mumbai, India), formic acid and potassium dihydrogen *o*-phosphate (Thermo Fisher Scientific Pvt. Ltd., Mumbai, India), and sodium hydroxide (S D Fine-Chem. Ltd., Mumbai, India) were used for the study. Teneligliptin pure drug and its tablet formation were obtained from Ajanta Pharma Limited, Mumbai, India.

### Apparatus
The HPLC system (Agilent Technologies, Compact LC-G4286A made in Germany) with variable wavelength UV detector was used. Reversed-phase Kromasil® 100-5-C18 (250 × 4.6 mm, 5-μm particle size) column was used for chromatographic separation. The chromatographic and integrated data were recorded using EZChrom Elite Compact Software in computed system (Version 3.30B, Sr. no. 08051601100, Scientific Software, Inc.). For the LC/MS

**Table 1** System suitability of teneligliptin

| Concentration (µg/mL) | Peak height (mAv) | Retention time (min) |
|---|---|---|
| 300 | 1288 | 4.04 |
| 300 | 1273 | 4.04 |
| 300 | 1282 | 4.03 |
| 300 | 1266 | 4.03 |
| 300 | 1257 | 4.04 |
| 300 | 1280 | 4.05 |
| Average | 1274.33 | |
| Standard deviation | 11.3959 | |
| % RSD | 0.894 | |

**Table 2** Linearity of teneligliptin

| Concentration (µg/mL) | Peak height (mAv) | Retention time (min) |
|---|---|---|
| 100 | 0452 | 4.09 |
| 200 | 0854 | 4.06 |
| 300 | 1246 | 4.06 |
| 400 | 1691 | 4.05 |
| 500 | 2052 | 4.04 |

studies, UPLC system consisting of gradient mode pump with column Acquity UPLC@ BEH C18 (1.7 μm, 2.1 × 50 mm) detected using photo diode array (PDA) detector range 200–400 nm was used. The mass spectrum with electrospray ionization (ESI) mode of ionization was used for the study (LC/MS/MS (Water, XEVO-TQD).
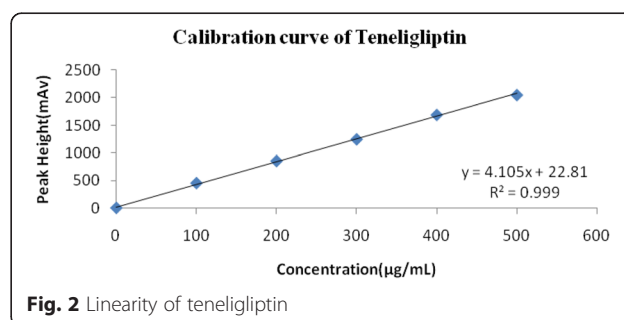
### Chromatographic conditions
Chromatographic separation was achieved on Kromasil® 100-5-C18 using mobile phase consisting of a mixture of pH 6.0 phosphate buffer and acetonitrile (60:40 *v/v*) under isocratic mode of elution. The mobile phase was prepared and filtered through membrane filters (0.45 μm) and sonicated for 30 min prior to use. Separation was performed using 1 mL/min flow rate at room temperature, and the run time was 25 min. The injection volume was 20 μL and the detection wavelength set at 246 nm.

### LC/MS conditions
Chromatographic separation was achieved on Acquity UPLC@ BEH C18 1.7 μm, 2.1 × 50 mm using the gradient mobile phase consisting of A (10 % acetonitrile in water with 0.1 % formic acid) and B (90 % acetonitrile with 0.1 % formic acid). A flow rate of 0.3 mL/min is maintained for the study. The eluted components were detected using PDA at a range of 200–400 nm. The products were ionized by ESI mode for their mass data.

### Sample preparation
1000 μg/mL solution of teneligliptin was prepared by dissolving the required amount of the drug in methanol. The solution was adequately diluted with methanol for accuracy, precision, linearity, limit of detection, and quantification studies.



**Fig. 2** Linearity of teneligliptin

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**Table 3** Precision results of teneligliptin

| Concentration (μg/mL) | Peak height (mAv) | Retention time (min) |
|---|---|---|
| 300 | 1308 | 4.04 |
| 300 | 1273 | 4.04 |
| 300 | 1298 | 4.06 |
| 300 | 1299 | 4.11 |
| 300 | 1284 | 4.04 |
| 300 | 1280 | 4.05 |
| Average | 1290.33 | |
| Standard deviation | 13.366 | |
| % RSD | 1.035 | |

### Stability sample preparation
The collected samples of acid and base hydrolysis were neutralized with sodium hydroxide and hydrochloric acid, respectively. Further dilution was carried out with methanol and the remaining stressed samples also diluted with methanol. All the samples were filtered before analysis.

### Degradation studies
#### Acid degradation
The teneligliptin was subjected to forced degradation by acid hydrolysis using 0.1 N HCl maintained at 35 °C for 48 h. The sample after the stress was neutralized with sodium hydroxide and diluted with methanol and filtered through a 0.45-μm membrane before its analysis.

#### Base degradation
The teneligliptin was subjected to forced degradation by base hydrolysis using 0.1 N NaOH maintained at 35 °C for 48 h. The sample after the stress was neutralized with hydrochloric acid and diluted with methanol and filtered through a 0.45-μm membrane before its analysis.

#### Hydrogen peroxide (neutral) degradation
Forced degradation of teneligliptin was studied under the influence of (3 %) hydrogen peroxide maintained at 35 °C for 48 h. The stressed sample was diluted with

**Table 4** Accuracy results of teneligliptin

| Recovery level (%) | Test concentration (μg/mL) | Standard concentration (μg/mL) | Total concentration (μg/mL) | Peak intensity | % recovery |
|---|---|---|---|---|---|
| 80 | 200 | 100 | 300 | 1276 | 102.5 |
| 100 | 200 | 200 | 400 | 1632 | 96.5 |
| 120 | 200 | 300 | 500 | 2089 | 101.8 |

**Table 5** LOD and LOQ results of teneligliptin

| Calibration equation | SD of the lowest concentration in linearity | LOD (μg/mL) | LOQ (μg/mL) |
|---|---|---|---|
| $Y = 4.105x + 22.81$ | 5.03 | 4.04 | 12.259 |

methanol and filtered through a 0.45-μm membrane before its analysis.

### Photolytic degradation
The influence of UV light on the stability of teneligliptin was studied by exposing the sample in UV light at 365 nm for 48 h. The stressed sample was diluted with methanol and filtered through a 0.45-μm membrane before its analysis.
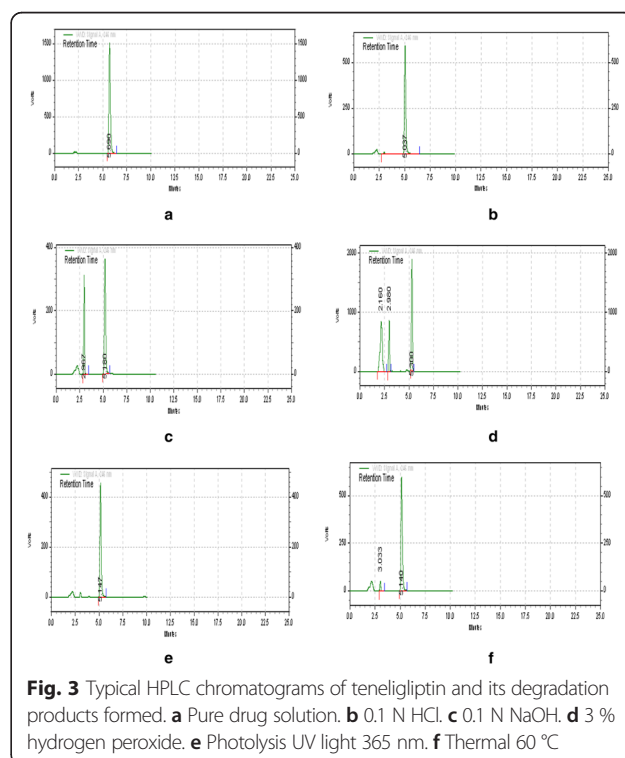
### Thermolysis degradation
The effect of increased temperature on teneligliptin was studied by heating the sample at 69 °C for 48 h in a refluxing apparatus. The stressed sample was diluted with methanol and filtered through a 0.45-μm membrane before its analysis.

### Validation
#### System suitability
The system suitability was determined by six injections of teneligliptin (300 μg/mL). The developed method was found to be suitable for use as the tailing factor and peak resolution for teneligliptin were within the limits.



**Fig. 3** Typical HPLC chromatograms of teneligliptin and its degradation products formed. **a** Pure drug solution. **b** 0.1 N HCl. **c** 0.1 N NaOH. **d** 3 % hydrogen peroxide. **e** Photolysis UV light 365 nm. **f** Thermal 60 °C

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**Table 6** HPLC data of degradation studies

| S. no. | Stress condition | Stress parameters used | Time (h) | Drug peak | | Degraded product peaks | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Rt | Peak intensity | Peak 1 | | Peak 2 | |
| | | | | | | Rt | Intensity | Rt | Intensity |
| 1 | Acid | 0.1 N HCl | 48 | 5.035 | 594.73 | – | – | – | – |
| 2 | Base | 0.1 N NaOH | 48 | 5.177 | 364.46 | 2.966 | 312.129 | – | – |
| 3 | Oxidative | 0.3 % $H_2O_2$ | 48 | 5.299 | 1899.8 | 2.519 | 846.61 | 2.979 | 857.45 |
| 4 | Light | UV light | 48 | 5.150 | 457.29 | – | – | – | – |
| 5 | Temperature | 60 °C | 48 | 5.140 | 600.41 | 3.033 | 46.65 | | |

*Rt* retention time

### Linearity

The linearity of teneligliptin was studied from the standard concentrations ranging from 100 to 500 μg/mL. The calibration curve of peak intensity versus concentration was plotted, and correlation coefficient and regression line equation were determined.
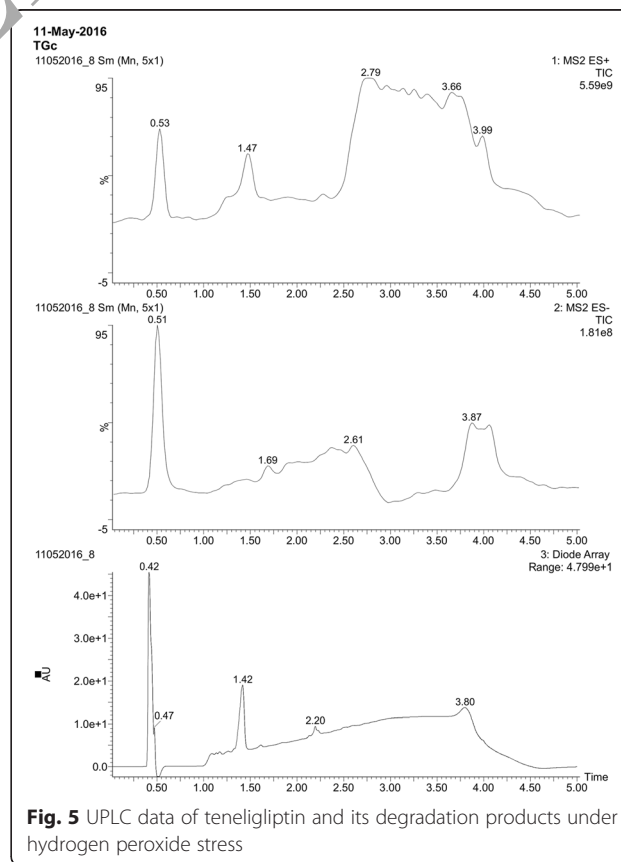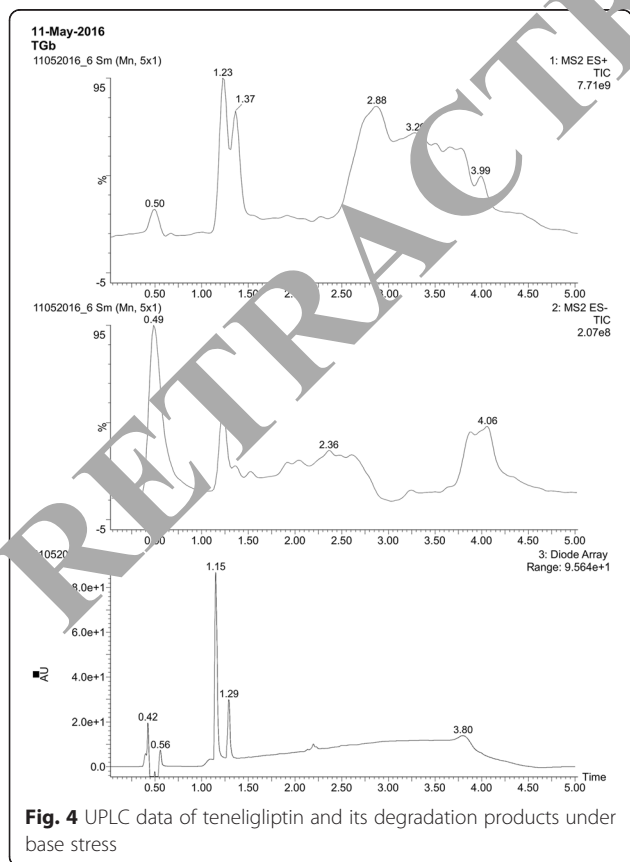
### Precision

The precision of the method was determined by six ($n = 6$) injections of teneligliptin (300 μg/mL), and the % RSD of peak areas were calculated. The obtained RSD was within the range ($\leq$2).

### Accuracy

The recovery of the method was determined by adding a known amount of the drug to the standard concentration. The recovery was performed at three levels of 80, 100, and 120 % of teneligliptin standard concentration. The three samples were prepared for each recovery level, and % recoveries were calculated.

### Limits of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ are the lowest level and lowest concentration of the analyte, respectively, in a sample that would yield signal-to-noise ratios of 3.3 for LOD and 10



**Fig. 4** UPLC data of teneligliptin and its degradation products under base stress



**Fig. 5** UPLC data of teneligliptin and its degradation products under hydrogen peroxide stress

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for LOQ. These are determined from the standard deviation of the peak response and the slope of the calibration curve.
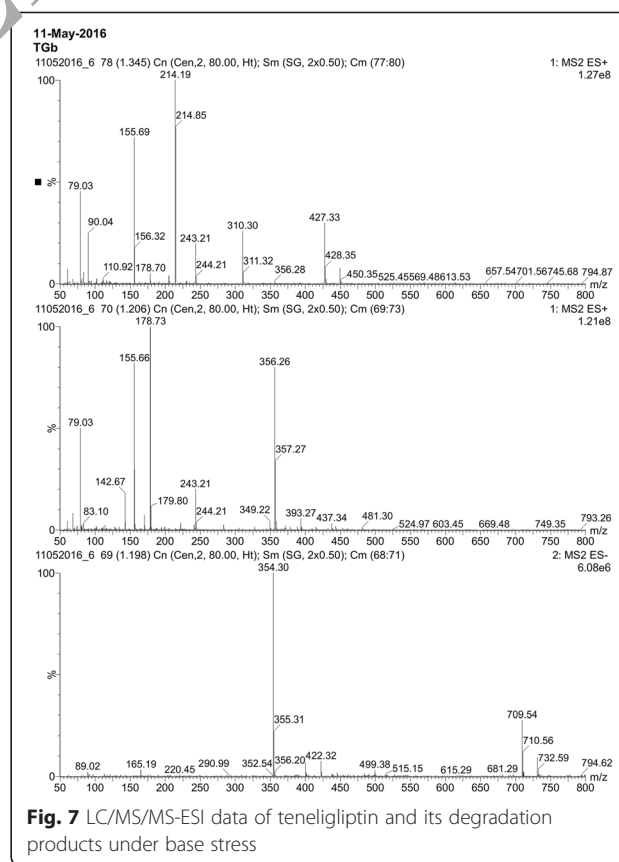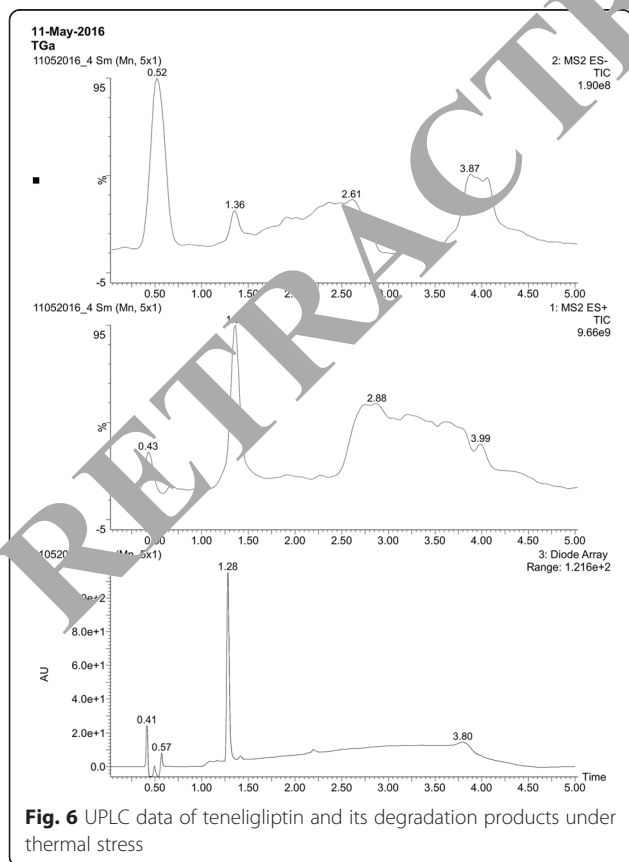
## Results

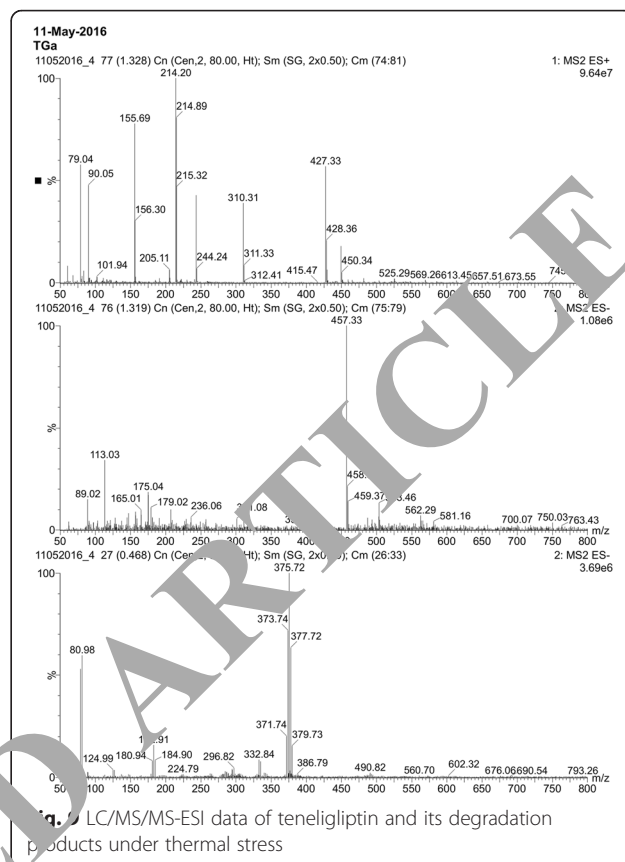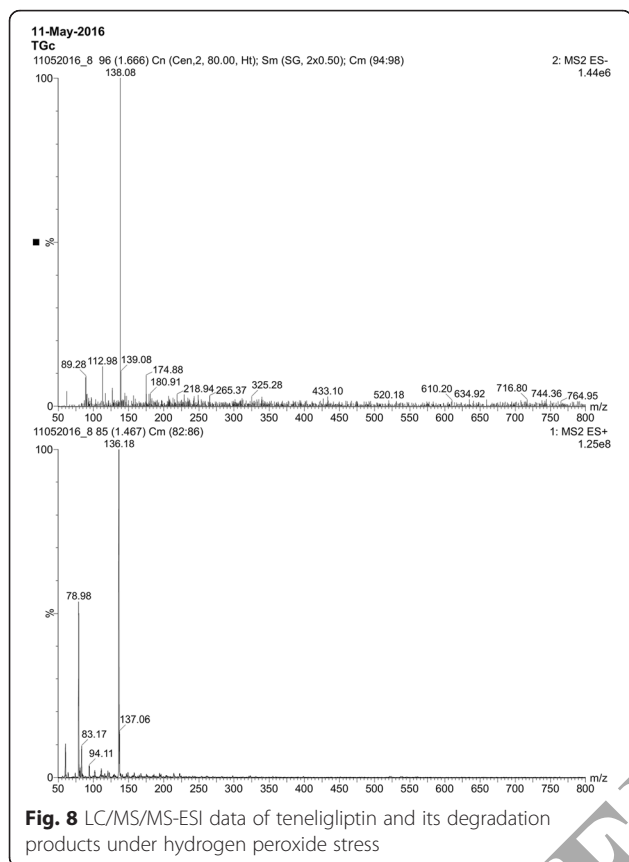### Method development and optimization of chromatographic conditions

Initially, 50:50 % *v/v* of methanol and water was tried on Kromasil® 100-5-C18 as a mobile phase at 1 mL/min flow rate and we observed that the peak was not eluted in this mobile phase. Then, 50:50 % *v/v* pH 7.4 phosphate buffer (basic) and acetonitrile was tried as a mobile phase on the same column at 1 mL/min flow rate. The peak was eluted with poor resolution and low intensity for high concentration (1000 μg/mL) of the drug. Then, 50:50 % *v/v* pH 6 phosphate buffer (acidic) and acetonitrile was tried as a mobile phase on Kromasil® 100-5-C18, and greater intensity and peak broadening were observed. Then, for better resolution, 60:40 % *v/v* pH 6 phosphate buffer and acetonitrile was modified as a mobile phase at 1 mL/min flow rate. Sharp peak, good intensity, and good retention time were observed in isocratic mode of elution. The system suitability was performed, and the results were found to be within the limits (Table 1).

Linearity with 0.99 regression coefficient was observed at the concentration range of 100–500 μg/mL tabulated in Table 2, and the calibration curve is depicted in Fig. 2. The method was validated for the parameters, precision, accuracy, and LOD-LOQ as per the guidelines and tabulated the results in Tables 3, 4, and 5, respectively. The results of validation were within the limits.

### Stability studies

Tenegliptin was stressed under different conditions, and the samples were subjected to HPLC separation. Significant degradation product peaks were observed in basic, neutral (hydrogen peroxide), and thermolysis conditions. The teneligliptin was found to be stable under acidic and photolysis conditions. The chromatograms of pure drug and its stressed samples are shown in Fig. 3. The peak intensities and retention times of the degraded product peaks during stress conditions are tabulated in Table 6. The UPLC data is depicted in Figs. 4, 5, and 6 for the base-, hydrogen peroxide-, and thermally stressed samples, respectively. The respective mass spectra are shown in Figs. 7, 8, and 9. From the mass spectral data, the fragmentation pattern of teneligliptin was depicted. The fragmentation patterns of the base-, peroxide-, and



**Fig. 6** UPLC data of teneligliptin and its degradation products under thermal stress



**Fig. 7** LC/MS/MS-ESI data of teneligliptin and its degradation products under base stress

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**Fig. 8** LC/MS/MS-ESI data of teneligliptin and its degradation products under hydrogen peroxide stress



**Fig. 9** LC/MS/MS-ESI data of teneligliptin and its degradation products under thermal stress

thermally stressed teneligliptin are depicted in Figs. 10, 11, and 12, respectively.

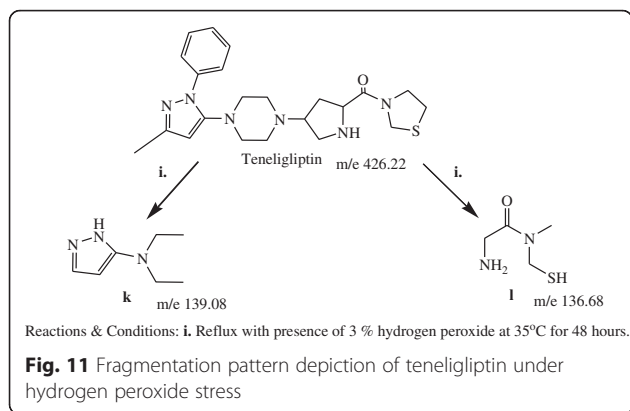## Characterization of the degradation products

The molecular ion peak for teneligliptin was observed at 427.22 in ESI mode. In the base-stressed sample, the fragments of 354.30 at a retention time of 1.195 min, 310.30 and 214.19 at a retention time of 1.345 min, and 178.73 and 155.64 at a retention time of 1.205 min were observed. In the peroxide-stressed sample, the fragments of 138.08 and 136.18 were observed at a retention time of 1.666 min and 1.467 min, respectively. In the thermally stressed sample, the fragments of 375.72 at a retention time of 0.455 min and 214.20, 310.31, and 155.69 at a retention time of 1.325 min were observed. There are no degradation peaks for teneligliptin in acid- and UV-stressed conditions.

## Discussion

Teneligliptin is an antidiabetic drug recently approved by the FDA. There are no reports available for the stability of the drug and their possible degraded products till date. In the present research work, we aimed to perform stability studies on teneligliptin and develop and validate

a method for its estimation and identification by RP-HPLC. A new RP-HPLC method was developed and validated for teneligliptin as per the ICH guidelines and used as a stability-indicating method. The teneligliptin pure drug was used for the study and stressed under



Reactions & Conditions: **i.** Reflux under basic medium with 0.1 N NaOH at 35°C for 48 hours.

**Fig. 10** Fragmentation pattern depiction of teneligliptin under base stress

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**Fig. 11** Fragmentation pattern depiction of tenegliptin under hydrogen peroxide stress
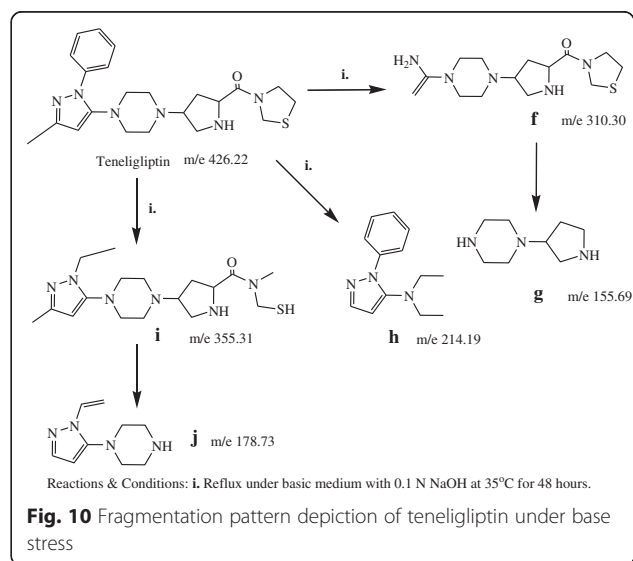
acid, base, neutral (hydrogen peroxide), UV photolysis, and thermal conditions. The HPLC analysis of the stressed samples has shown that no degradation occurred under the influence of acid and UV light. But the stressed samples under base, peroxide, and thermal have presence of the degraded products, which was observed as separate peaks in HPLC other than the tenegliptin. The obtained degraded samples were further analyzed by UPLC/MS/MS, to identify the products formed. The major molecular ion fragments formed for all the three stress conditions are different except 310.30 ((4-(4-(1-aminovinyl)piperazin-1-yl)pyrrolidin-2-yl)(thiazolidin-3-yl)methanone), 214.19 (N,N-diethyl-1-phenyl-1H-pyrazol-5-amine), and 155.65 (1-(pyrrolidin-3-yl)piperazine) were observed in both base and thermal stress conditions. A characteristic 354.30 (4-(4-(1-ethyl-3-methyl-1H-pyrazol-5-yl)piperazin-1-yl)-N-(mercaptomethyl)-N-methylpyrrolidine-2-carboxamide) and 375.72 ((4-(4-(3-methyl-1-vinyl-1H-pyrazol-5-yl)piperazin-1-yl)pyrrolidin-2-yl)(thiazolidin-3-yl)methanone) molecular ion peak was observed in base condition and thermal condition,
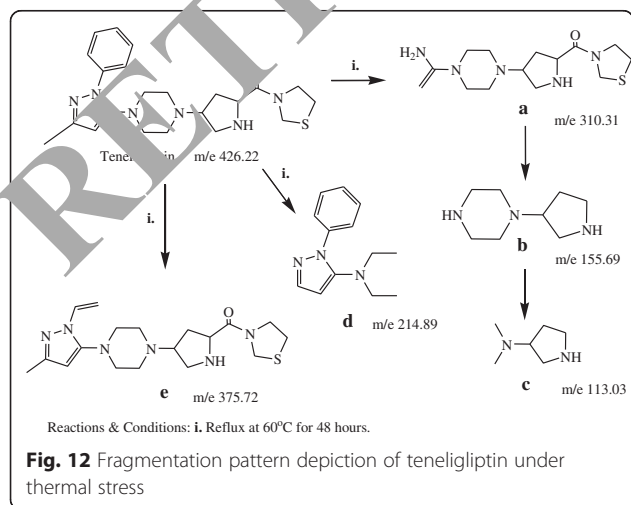
respectively. The products formed with photolytic stress were completely different with molecular ions at 138.08 (N,N-diethyl-1H-pyrazol-5-amine) and 136.18 (2-amino-N-(mercaptomethyl)-N-methylacetamide), which are not observed in other stress conditions. From the data, it is observed that comparatively less degradation occurred for photolysis stress than for base and thermal stress. The fragmentation pattern shows that the degraded products are similar for the base and thermal stress samples. Further study is required for determining the degraded products' toxicity by quantifying the samples.

## Conclusions

The present study helps in identifying the degraded products of tenegliptin in bulk and formulations, during their storage and transport conditions. This research work is the first to report its stability studies with degraded product identification, which is helpful for determining the toxicity of the degraded products and also to caution the storage conditions. The products formed could also be the starting materials during its synthesis, which has to be studied. Further study is required for establishing the toxicity profile of the degraded products, which is under process.

**Authors' contributions**
TNVGK conceived the main idea and implementation of the work by selecting the drug and performed the wet lab study. TNVGK also analyzed the degradation products and interpreted the results from the LC/MS/MS data. SV helped in analyzing the results of the RP-HPLC method development and validation. NAN performed the LC/MS study of the samples and helped in the UPLC method development. YSS performed the wet lab study of the hydrogen peroxide stress and thermal stress work for the drug sample. YSS also helped in adjusting the pH of the mobile phase during the HPLC study. MRL performed the wet lab study of collecting and dilutions of the sample after stress conditions for the drug sample. MRL also helped in the mobile phase preparation during the HPLC study. All authors read and approved the final manuscript.

**Competing interests**
The authors declare that they have no competing interests.

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**Fig. 12** Fragmentation pattern depiction of tenegliptin under thermal stress

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