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Development and Validation of Stability Indicating Ultra Performance Liquid Chromatography Method for the Quantification of Teneligliptin hydrobromide hydrate and Characterisation of its Degradation products by Spectroscopic techniques.

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ABSTRACT

With the objective of reducing analysis time and maintaining good efficiency, a simple, rapid and specific stability indicating UPLC method has been developed and validated for the determination of Teneligliptin in pharmaceutical dosage form .Chromatographic separation was achieved on Thermo Hypersil C_{18} (50×2.1mm, 1.9µm) column using pH 2.5 orthophosphoric acid buffer and acetonitrile (85:15 *v/v*) as mobile phase in isocratic mode of elution at a flow rate of 0.3 mL/min. The column effluents were monitored by a variable wavelength UV detector at 248 nm. The newly developed method was validated according to ICH guidelines with respect to linearity, accuracy, precision and flowrate. Forced degradation studies of Teneligliptin were carried out under acidic, basic, oxidative, photo and thermal conditions for 48 hours. The degradation products were identified by UPLC and characterized by, NMR, IR andMass spectroscopic techniques. The toxicity of the degradation products was determined by Osiris software and suggestions have been made to remove the toxicity.

Keywords: Teneligliptin, Degradation, UPLC, Validation, Characterization

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INTRODUCTION

Teneligliptin (TNG) is a novel drug, which is used for the treatment of type 2 diabetes mellitus. It is an anti- diabetic drug that belongs to dipeptidyl peptidase-4 inhibitors or "gliptins". 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.The structure of Teneligliptin is shown in **Figure 1** TNG is a highly potent, competitive and long lasting dipeptidyl peptidase -4 inhibitor which degrades incretin, a hormone adjusting blood glucose level. Consequently, it enhances insulin secretion depending on blood glucose level and improves blood glucose control. The most common adverse reactions include stomatitis ,rashes, hypoglycemia, constipation,feeling of enlarged abdomen, nausea and eczema [1-4].Literature review revealed that HPLC method has been reported for the estimation of TNG in pure and tablet dosage form,[5] spectrophotometric determination has been developed for TNG in bulk and pharmaceutical formulations[6], LC-MS method has been developed for TNG in human plasma and its application to a pharmacokinetic study[7].

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 the following stages: (1) to develop and validate a Ultra performance Liquid Chromatography (UPLC) method for identifying TNG and its degradation products formed during various forced conditions[8-16] as per the ICH guidelines and (2) identification of the degraded products and their characterization by IR, NMR and Mass spectroscopic techniques (3) toxicity profiling using Osiris software.

EXPERIMENTAL

MATERIALS AND METHODS

HPLC grade acetonitrile (Lichrosolv®, Merck Life Science, Pvt. Ltd., Mumbai, India), Ortho phosphoric acid buffer, sodium perchlorate, sodium hydroxide (S D Fine-Chem. Ltd., Mumbai, India) were used for the study. Teneligliptin API was obtained from Pure Chem Limited, Ankleshwar, India. The tablet formulation, Ziten (20mg) was obtained from Abirami Medicals, Chennai.

Apparatus

The UPLC system (Thermo Scientific Company) with variable wavelength UV detector was used. Thermo Hypersil C_{18} (50 × 2.1 mm, 1.9µm particle size) column was used for chromatographic separation. The chromatographic and integrated data were recorded using Chromoquest 5 Version Software in computed system, consisting Accela 1250 pump and detected using photo diode array (PDA) detector.

Chromatographic conditions

Chromatographic separation was achieved on Thermo Hypersil C_{18} using the mobile phase consisting of a mixture of` pH 2.5 ortho phosphoric acid buffer and acetonitrile (85:15 *v*/*v*) under isocratic mode of elution. The mobile phase was filtered through membrane filter (0.45 µm) and sonicated for 30 min prior to use. Separation was performed at 0.3 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 248 nm. The 3D chromatogram of TNG is shown in **Figure 2.**

Standard solution preparation

Stock standard solution containing TNG was prepared by dissolving 37.5 mg of TNG in 25 ml of methanol. From this 5 ml was pipetted out and made up to 100ml with methanol. The different dilutions of TNG were injected and their peak area was measured.

Sample preparation

The developed method was used to quantify TNG in tablet formulation. For the analysis of the formulation, 20 tablets (Ziten-20mg) were weighed and the average weight was determined. Powder equivalent to a weight of 450 mg of TNG was transferred to a 100ml volumetric flask and dissolved in about 30

ml of mobile phase. The solution was shaken for five minutes and then ultrasonicated for 15-20 minutes and filtered through 0.45µ whatmann filter paper. The residue was washed with the mobile phase and the combined filtrate was made up to mark with the solvent. 5ml of the solution was diluted to 100 ml with methanol to get the final concentration. The solution was then injected for the quantitative analysis. The identity of the compound was established by comparing retention time of sample solution with that of standard solution and the amount of TNG was calculated.

Degradation studies

Acid degradation

TNG 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

TNG 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 degradation

Forced degradation of TNG was studied under the influence of (3 %) hydrogen peroxide maintained at 35 °C for 48 h. The stressed sample was diluted with methanol and filtered through a 0.45-µm membrane before its analysis.

Photolytic degradation

The influence of UV light on the stability of TNG 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 0.45µm membrane before its analysis.

Thermolytic degradation

The effect of increased temperature on TNG was studied by heating the sample at 100 °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.

Stability studies

TNG was stressed under different conditions, and the samples were subjected to UPLC separation. Significant degradation product peaks were observed in basic and oxidative conditions. The TNG was found to be stable under acidic, photolysis and Thermal conditions as no degradation peaks were observed. The chromatograms of pure drug and its stressed samples are shown in figures. **Figure 3** shows the degradation behavior of TNG under the various stress conditions. **Figure 4** represents the Blank and Placebo of TNG.

Validation

System suitability

The system suitability was determined by five injections of TNG. The developed method was found to be suitable for use as the tailing factor and peak resolution for TNG were within the limits.

Linearity

Linearity was obeyed in the concentration range of 50-100 µg/ml. The calibration curve of peak area versus concentration was plotted and correlation coefficient and regression line equation were determined.

Precision

The precision of the method was determined by injecting the standard solution for six ($n = 6$) injections of TNG (300 µg/mL) and the % RSD of peak areas were calculated. The obtained RSD was within the range (≤2). The results of precision study is furnished in Table-1.

Accuracy

The accuracy of the method was carried out by adding known amount of drug corresponding to the three concentration levels and the percentage recovery was calculated as shown in Table-2.

Characterization of degradation product

The degradation product was characterized by Infrared spectrophotometry, Nuclear Magnetic Resonance and Mass Spectroscopic techniques to get the structural insight.

Toxicity Profiling

The toxicity of the degraded products formed under the various stressed conditions was checked by Osiris software.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Initially, 25:55:20:0.3% *v/v* of water: acetonitrile: methanol: acetic acid was used as a mobile phase on Thermo Hypersil C_{18} at 0.3 mL/min flow rate. No elution was observed. Then, 75:25 % *v*/v water: methanol pH 2.5 was tried as a mobile phase on the same column at 0.3 mL/min flow rate. The peak was eluted with poor resolution and low intensity for high concentration of the drug. Then, 85:15 % *v/v* pH 2.5 orthophosphoric acid buffer (acidic) and acetonitrile was tried as mobile phase on Thermo Hypersil-C_{18}. Sharp peak with good intensity, and good retention time were observed in isocratic mode of elution. Linearity with 0.99 regression coefficient was observed in the concentration range of 100–500 µg/ml. The method was validated for the parameters, precision, accuracy and flow rate, as per ICH guidelines.

Degradation study

TNG was stressed under different conditions, and the samples were subjected to UPLC separation. Significant degradation product peaks were observed in basic and oxidative conditions. The TNG was found to be stable under acidic, photolysis and Thermal conditions as no degradation peaks were observed.

Characterization of degradation products and Toxicity profiling

The IR data of samples are shown in **Figures 5 to 9.** The interpretation of IR is furnished in separate Table-3 to 5.The ^{1}H and ^{13}C NMR Spectrums of TNG are shown in **Figures 10 to 14** and **15 to 19.** The Mass spectrums of TNG are shown in **Figures 20 to 24**. The mass fragmentation is shown in **Figures 25to 29.**Toxic products were identified during fragmentation and their toxicity was determined by using Osiris Software. The toxicity could be removed by changing and replacing their substituents as shown in Table-6 and 7.

Table 1: Precision data

INJECTIONS	RT	AREA
1	2.492	984896
2	2.493	978313
3	2.500	980589
4	2.497	978673

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	5	2.497	977873
	6	2.497	975784
Standard deviation			**0.1733**
Relative standard deviation			**0.000017695**

Table 2: Results of Accuracy study

Sample	Retention time	Percentage recovery
100%	2.502	100%
100%	2.502	100.01%
100%	2.443	100%
110%	2.497	100%
110%	2.487	99.99%
110%	2.498	100%
120%	2.490	100%
120%	2.505	100.01%
120%	2.508	100%
130%	2.505	100%
130%	2.512	99.99%
130%	2.503	100%

Table 3: IR interpretation of TNG API

TNG API STRETCHING (cm^{-1})	
3446.79	N-H stretching (secondary Amine)
2956.87	C-H stretching (methyl)
1570.06	C-C stretching (Aromatic rings)
1645.28	C=0 stretching (Ketone)
1261.45	C-N stretching
692.44	C-S stretching

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Table 4: IR interpretation of TNG API and formulation under Base stress

TNG API (cm^{-1})	FORMULATION	STRETCHING (cm^{-1})
3317.78	3296.35	N-H Stretching (secondary amine)
1635.64	1635.64	C=O Stretching (Ketone)
1365.53	1367 .53	C-H bending
1215.15	1215.15	C-N stretching

Table 5: IR interpretation of TNG API and TNG Formulation under hydrogen peroxide stress

TNG API (cm^{-1})	FORMULATION	STRETCHING (cm^{-1})
3288.63	3354.21	N-H Stretching (secondary amine)
1635.64	1636.64	C=O Stretching (Ketone)
1365.53	1265 .53	C-H bending
1215.15	1265.45	C-N stretching

Table 6: Toxicity profile of TNG API and Formulation under Base hydrolysis

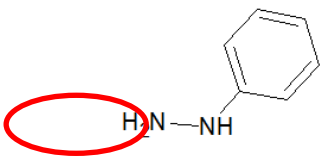
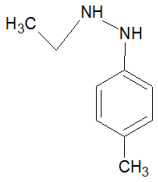
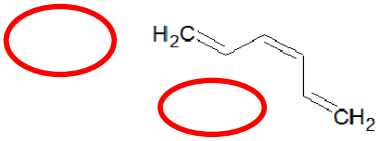
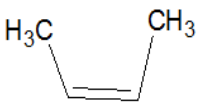
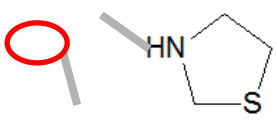
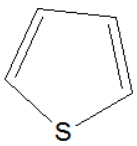
Degraded Fragments	Toxicity Effects Toxicity API	Substitution To Reduce
	Phenyl hydrazine Mutagenic, tumorigenic , reproductive effect	
	Hexa-1,3,5-triene Irritant	
	1,3 thiazolidine Mutagenic	

Table 7: Toxicity profile of TNG API and formulation under Oxidative stress

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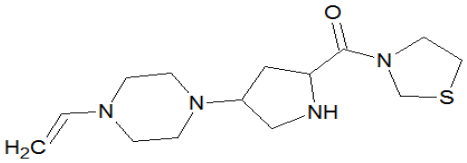
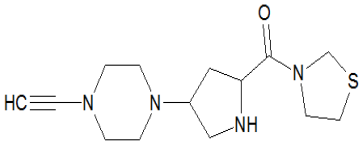
Degraded Fragments | Toxicity Effects | Substitution To Reduce Toxicity

API

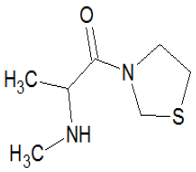


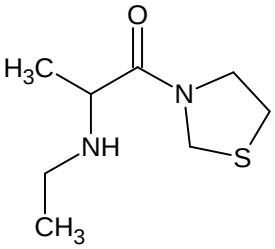


Tumorigenic

[4-ethenylpiperazin-1-yl)pyrrolidin-2-yi] (1,3-thiazolidin-3-yl)methanone

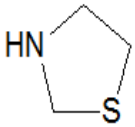
FORMULATION

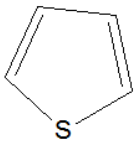




Irritant

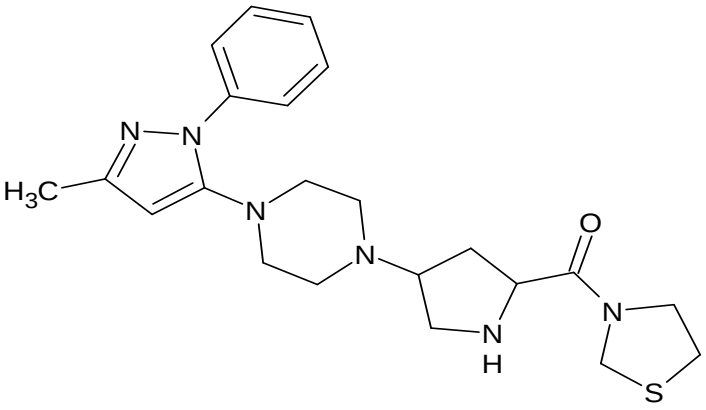
2-(methylamino)-1-(1,3-thiazolidin-3-yl) propan-1-0ne





Mutagenic

1,3 thiazolidine



.21/2 HBr.H_2O

Figure 1: Structure of teneligliptin hydrobromide hydrate

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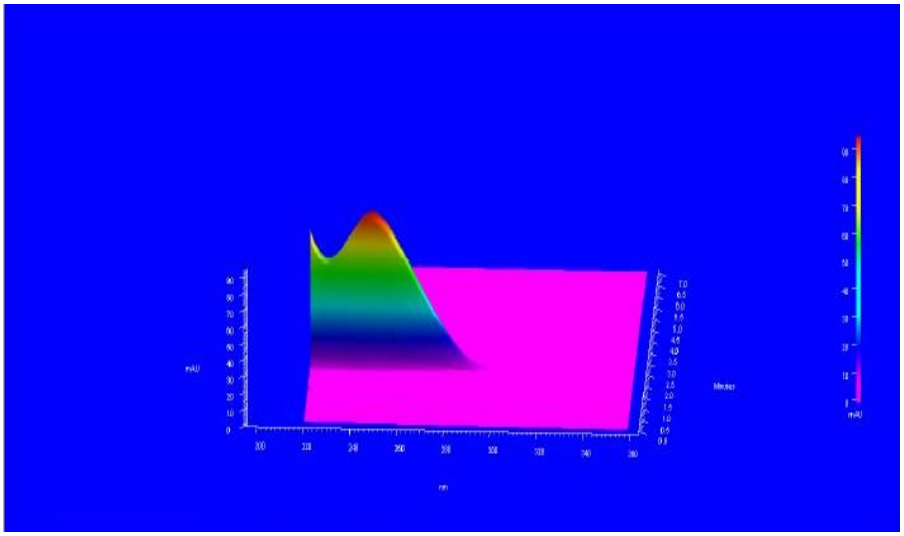

Figure 2: 3D Chromatogram of TNG

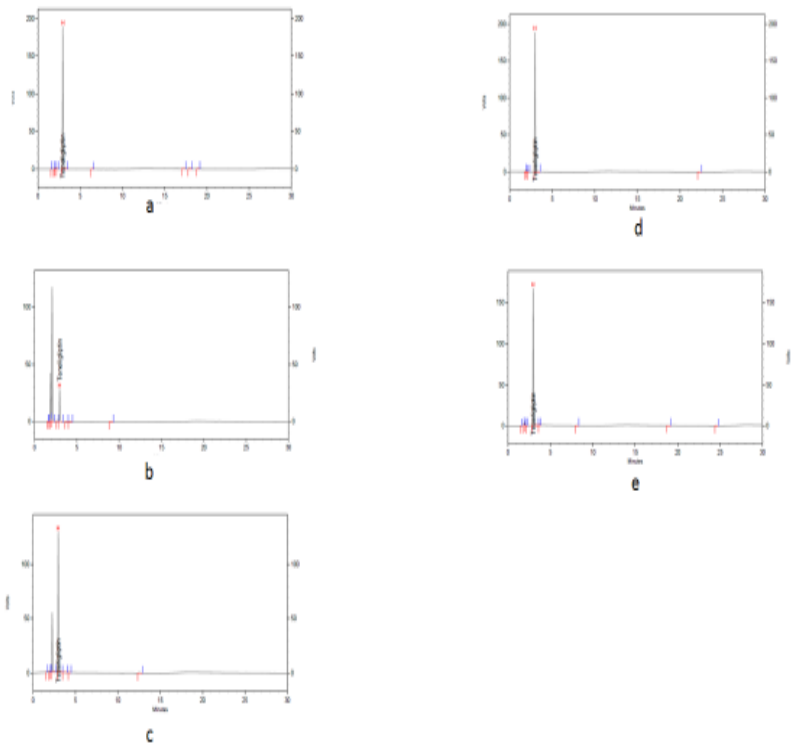

Figure 3: Degradation of raw material (a) Acid degradation (b) Base degradation (c) Hydrogen peroxide degradation (d) Photolytic degradation (e) Thermal degradation

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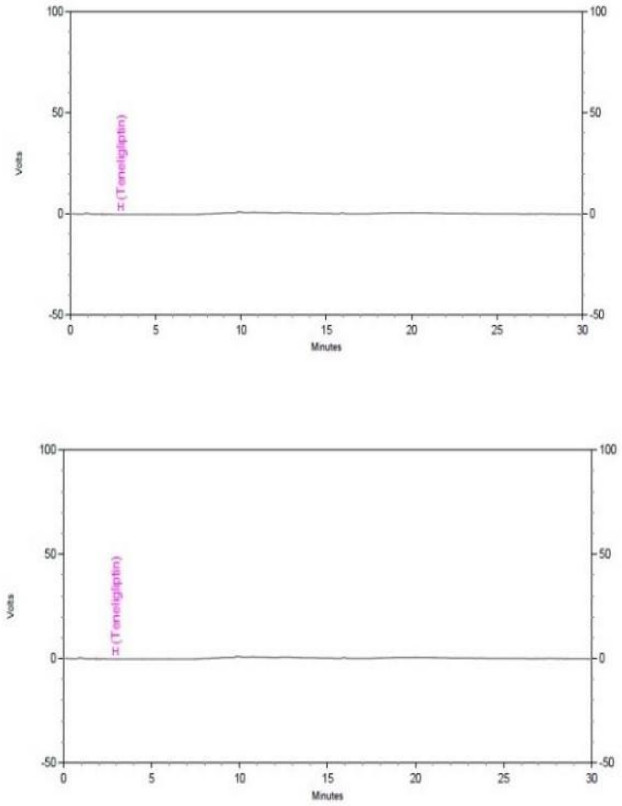

a b

Figure 4: (a) Blank (b) Placebo

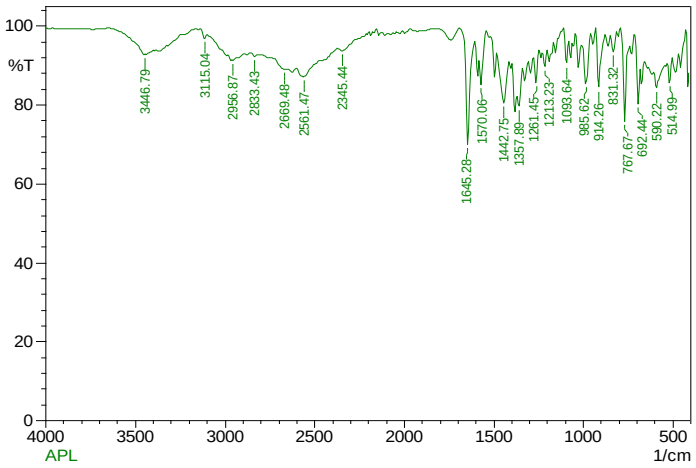

Figure 5: IR Spectrum of TNG API

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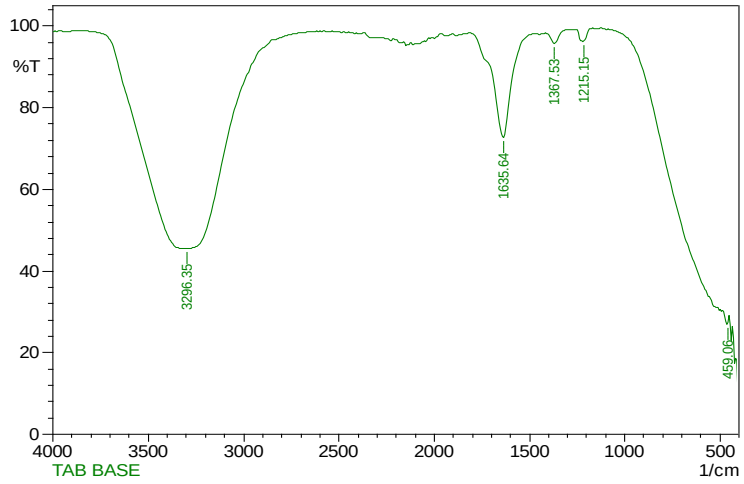

Figure 6: IR Spectrum of TNG formulation under base condition

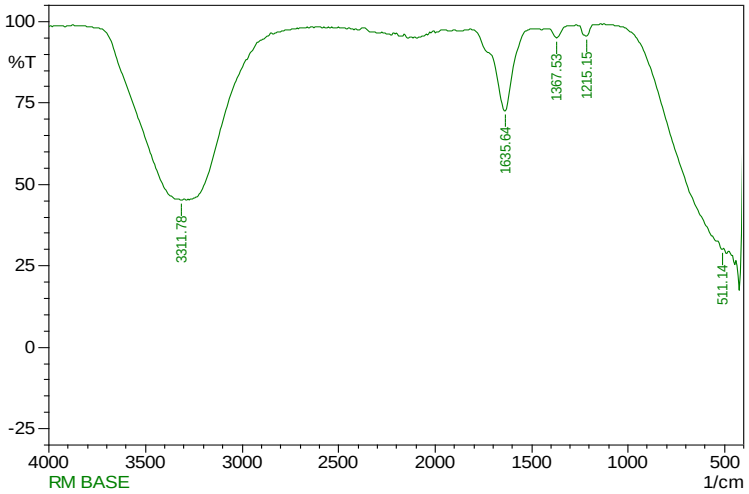

Figure 7: IR Spectrum of TNG API under base condition

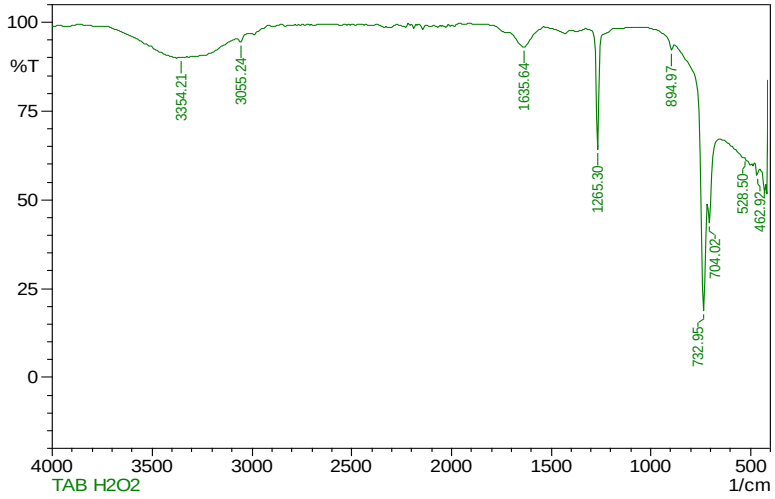

Figure 8: IR Spectrum of TNG formulation under oxidative condition

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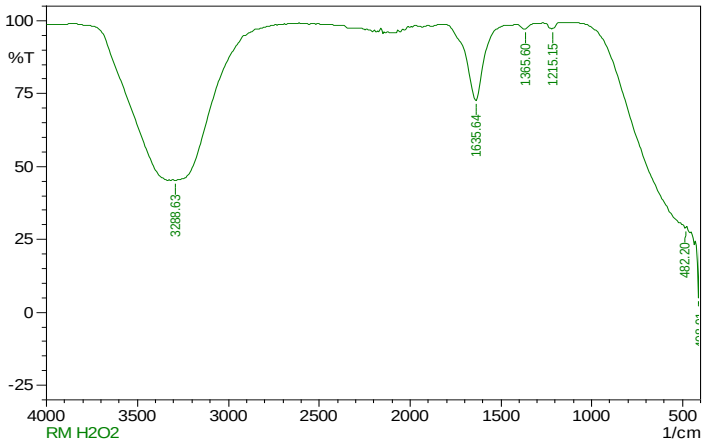

Figure 9: IR Spectrum of TNG API under oxidative condition

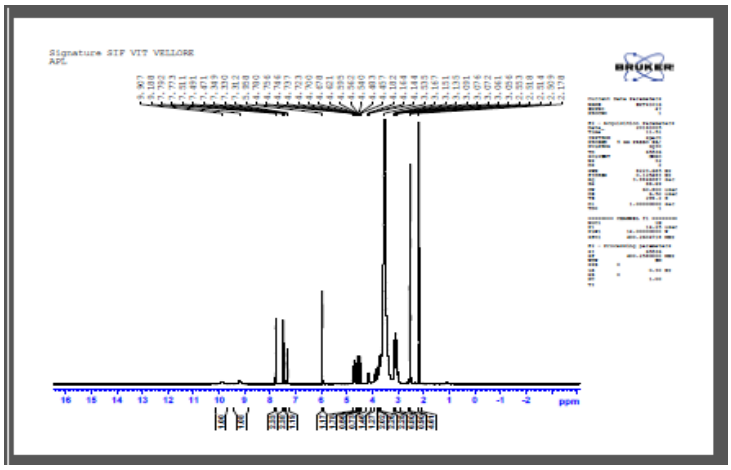

Figure 10: ^{1}H NMR Spectrum of TNG API

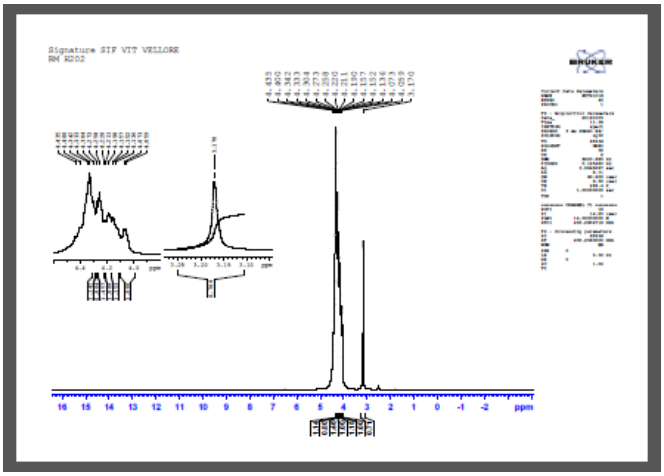

Figure 11: ^{1}H NMR Spectrum of TNG API under Hydrogen peroxide stress

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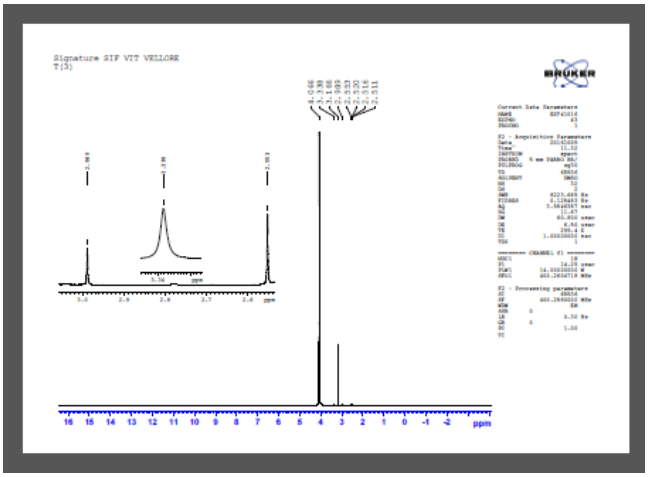

Figure 12: ^{1}H NMR Spectrum of TNG formulation under hydrogen peroxide stress

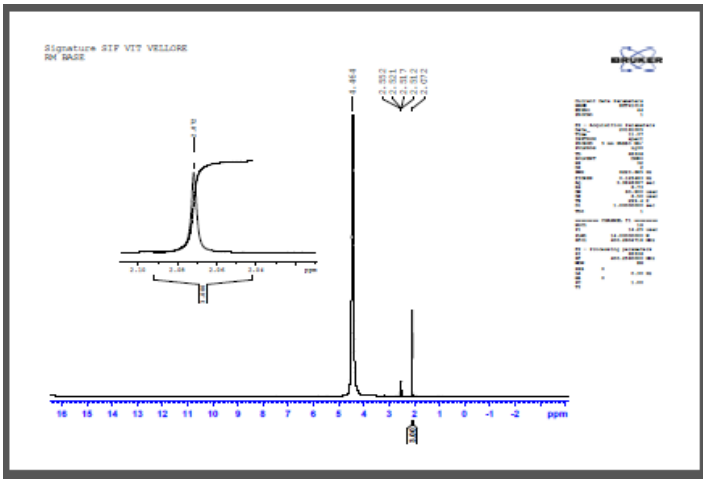

Figure 13: ^{1}H NMR Spectrum of TNG API under base stress

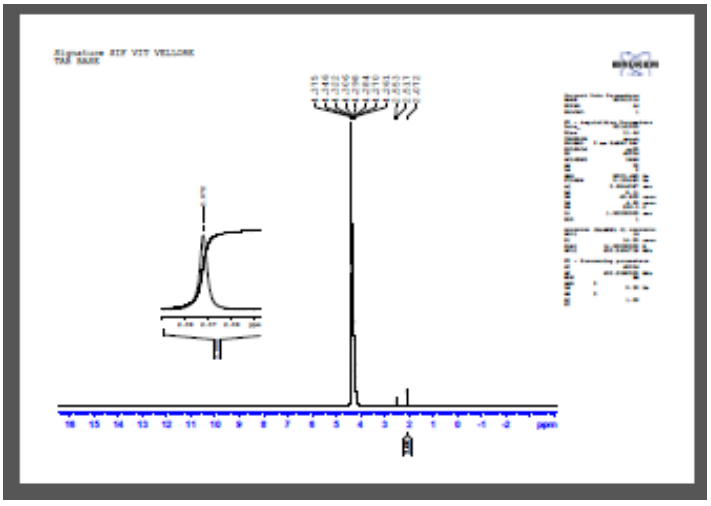

Figure 14: ^{1}H NMR Spectrum of TNG formulation under base stress

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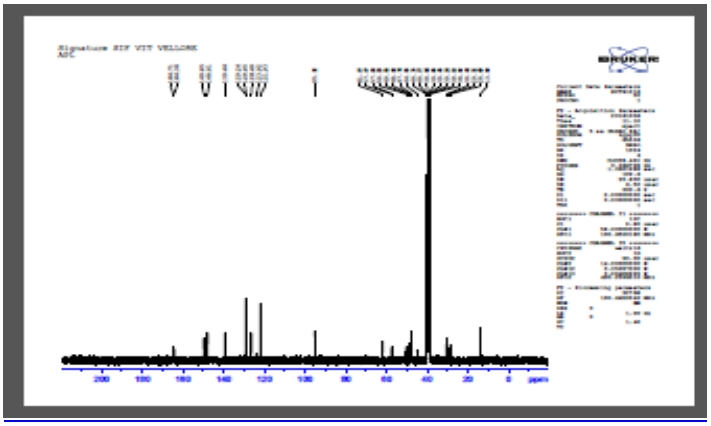

Figure 15: ^{13}C NMR Spectrum of TNG API

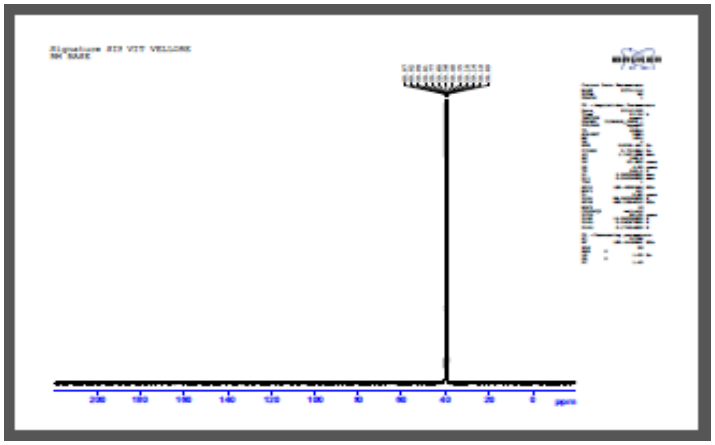

Figure 16: 13C NMR Spectrum of TNG APIunder base stress

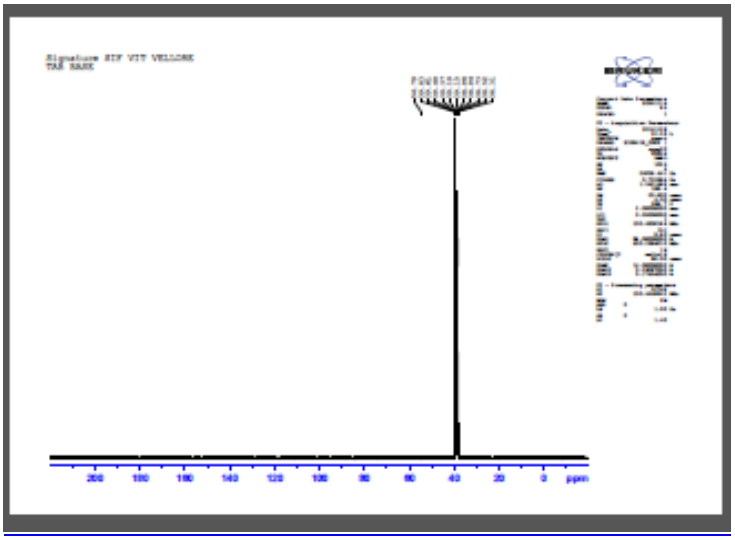

Figure 17: ^{13}C NMR Spectrum of TNG formulation under base stress

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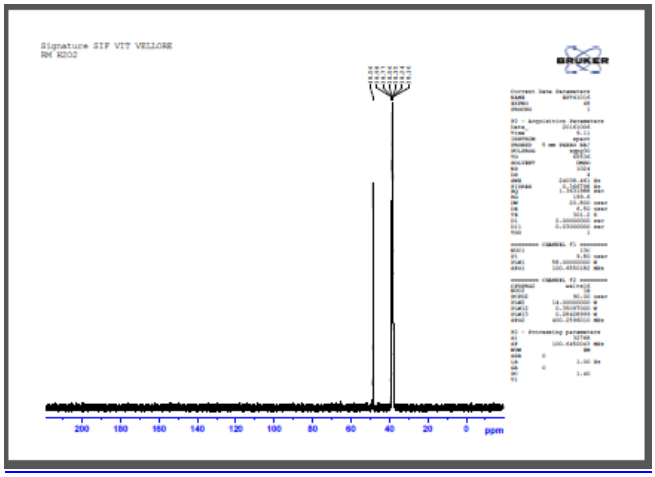

Figure 18: ^{13}C NMR Spectrum of TNG API under hydrogen peroxide stress

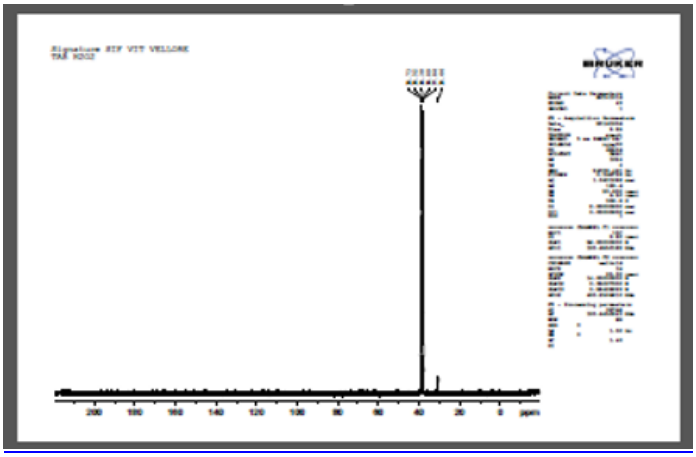

Figure 19: ^{13}C NMR Spectrum of TNG formulation under hydrogen peroxide stress

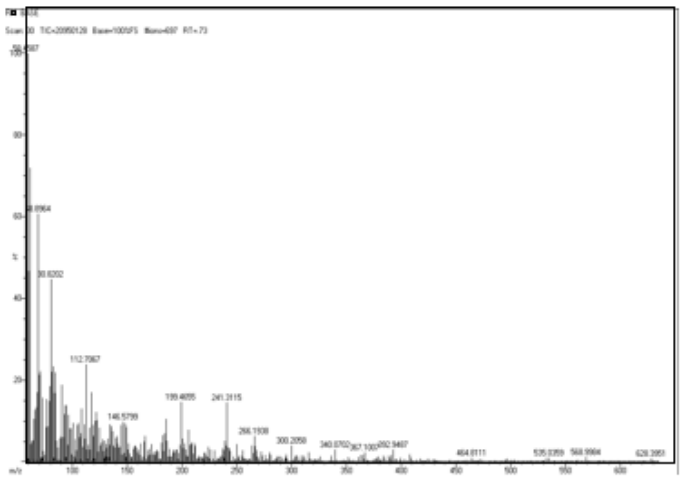

Figure 20: Mass Spectrum of TNG API

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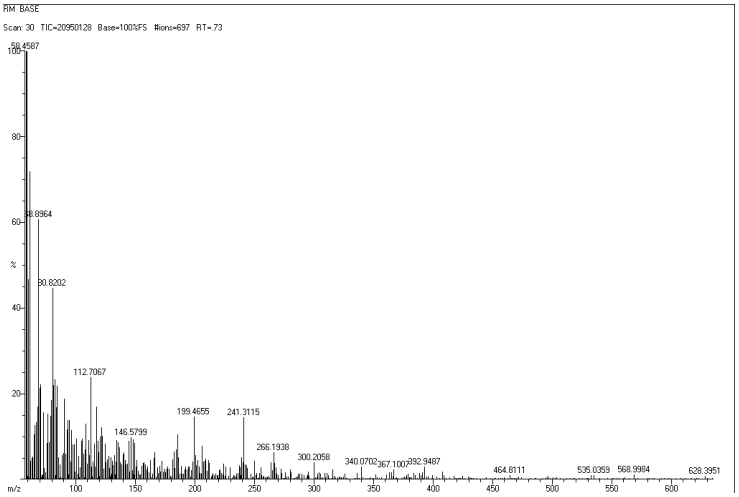

Figure 21: Mass Spectrum of TNG API under base hydrolysis

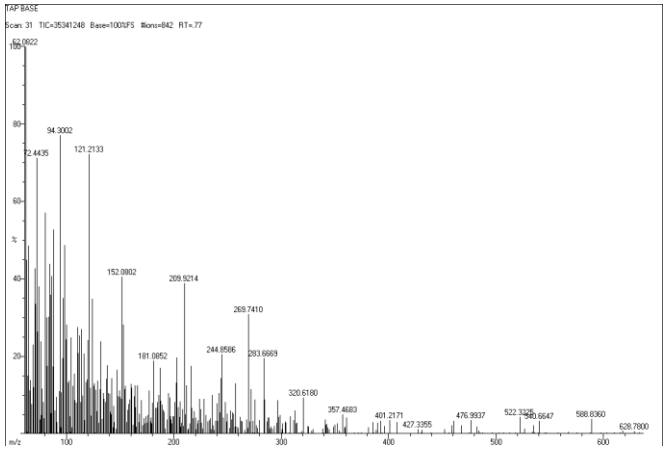

Figure 22: Mass Spectrum of TNG formulation under base stress

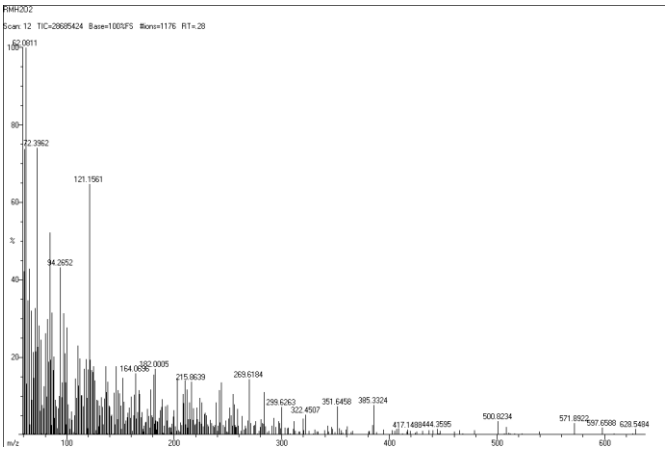

Figure 23: Mass Spectrum of TNG API Hydrogen peroxide stress

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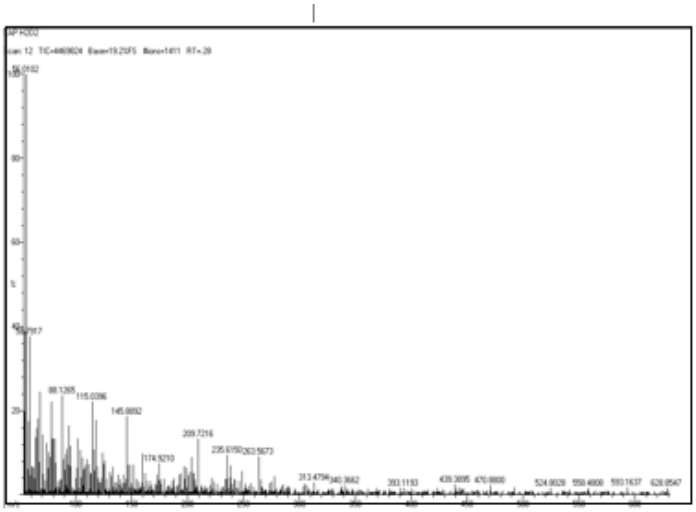

Figure 24: Mass Spectrum of TNG formulation under Hydrogen peroxide stress

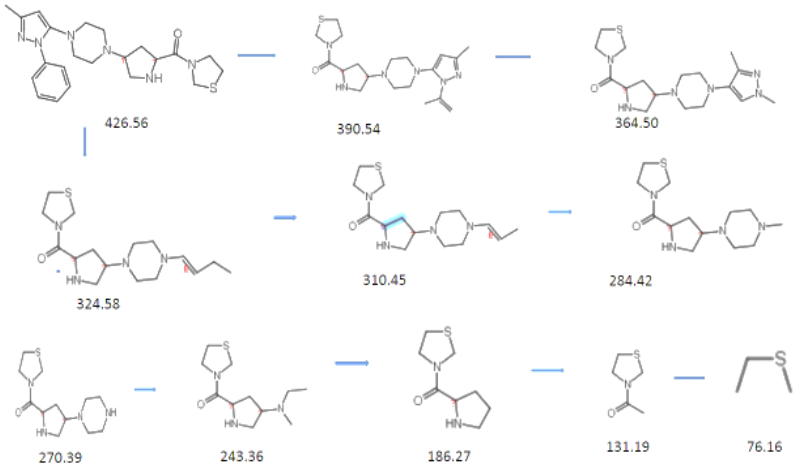

Figure 25: Fragmentation pattern of TNG API

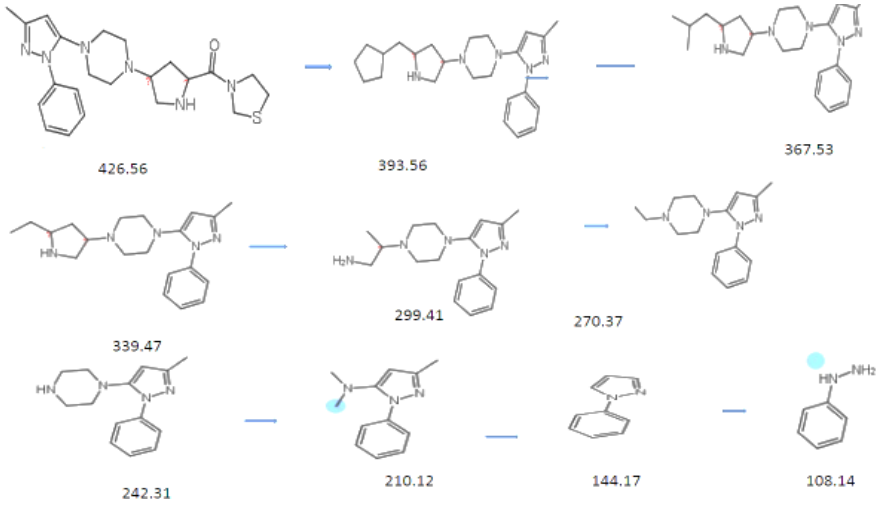

Figure 26: Fragmentation pattern of TNG API under base stress

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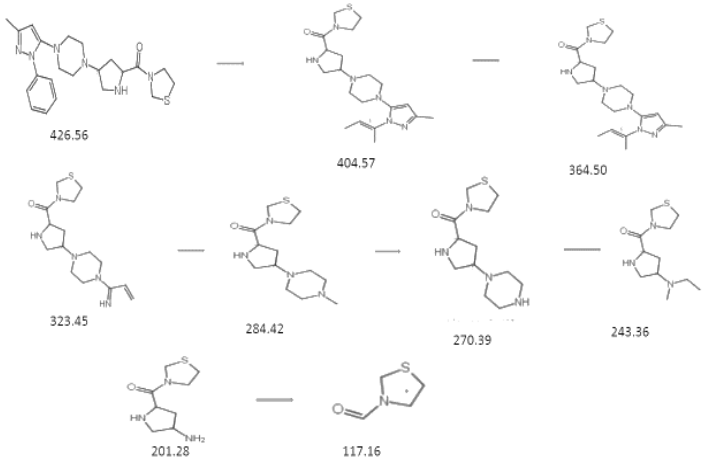

Figure 27: Fragmentation pattern of TNG formulation under base stress

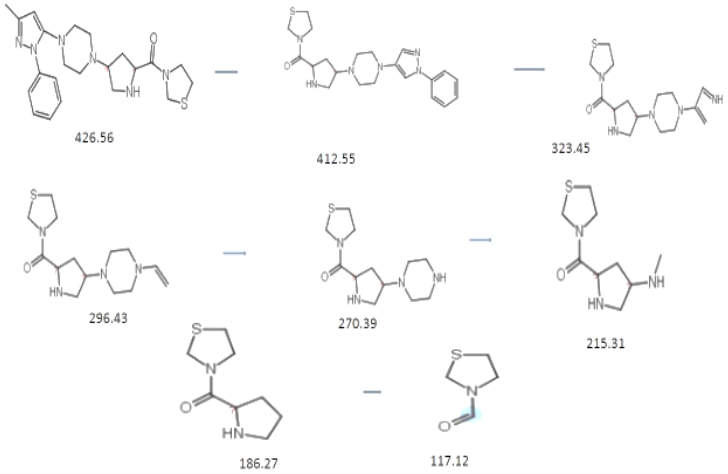

Figure 28: Fragmentation pattern of TNG API under oxidative degradation

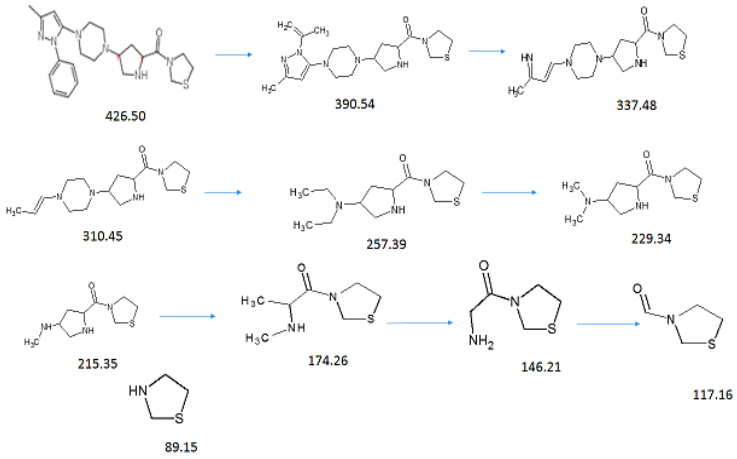

Figure 29: Fragmentation pattern of TNG Formulation under oxidative degradation

CONCLUSION

In the present study UPLC method was developed and validated for TNG API and formulation. The developed method was validated according to ICH guidelines. TNG was stressed under acid, base, oxidative, photolytic and thermal conditions. The UPLC analysis of the stressed samples has shown that no degradation

occurred under the influence of acid, UV light and thermal conditions. But the base and peroxide stressed samples showed the presence of degraded products, which was observed as separate peaks in UPLC. The obtained degraded samples were further analyzed by NMR, IR and Mass to identify the products formed. From the data, it is observed that degradation products formed are toxic by using Osiris software. Substitutions have been suggested to remove the toxicity of the possible degraded products.

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