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IDENTIFICATION OF DEGRADANTS OF THERMAL AND OXIDATION STRESS STUDIES OF EMPAGLIFLOZIN AND LINAGLIPTIN TABLETS BY HPLC-PDA AND LC-MS INSTRUMENTAL TECHNIQUES

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ABSTRACT

Objective of the manuscript is to identify the degradants observed in the thermal and oxidation degradation sample of Empagliflozin and Linagliptin tablets by using LC-MS and HPLC-PDA instrumental techniques. Thermal and oxidation degradation samples were injected in HPLC-PDA and LC-MS instruments. Mass of the degradants were detected by LC-MS technique, which were assigned to the probable structures correlating with the impurities mentioned in the literature. The assigned probable structures for the degradants were confirmed by procuring and confirming by injecting in HPLC-PDA detector and LC-MS instruments.

KEYWORDS: Empagliflozin and Linagliptin Tablets, Impurities, Degradants, HPLC-PDA, LC-MS.

1. INTRODUCTION

Linagliptin (LIN) and Empagliflozin (EMP) drugs are intended in the treatment of type-2 diabetes.[1-3] Linagliptin belongs to the class of drugs inhibiting the enzyme dipeptidyl-peptidase-4 (DPP-4). IUPAC name of Linagliptin is 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione.

Empagliflozin, a sodium-glucose cotransporter 2, (SGLT2) inhibitor, is the newest class of oral hypoglycemic agent.[4] IUPAC name of Empagliflozin is (1S)-1,5(1S)-1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3furanyl]oxy]phenyl] methyl]phenyl] / (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl) oxaane-3,4,5-triol.[1] Structures of Linagliptin and Empagliflozin are given in Figures 1-2. Literature survey indicates that Empagliflozin and Linagliptin has been estimated quantitatively from pharmaceutical dosage forms.[5-8] Here, this article talks about the assigning of the structures to the degradants generated in the thermal and oxidation degradation studies of Empagliflozin and Linagliptin tablets using HPLC-PDA and LC-MS instrumental techniques.

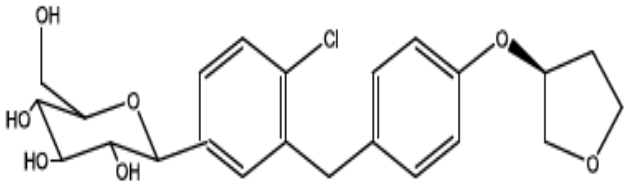


Figure 1: Empagliflozin.

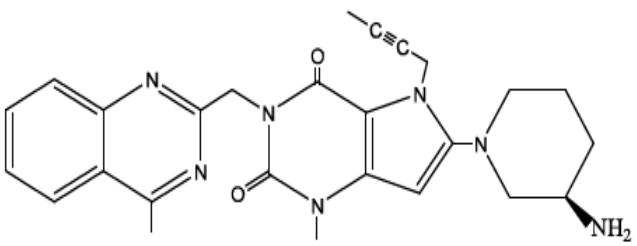


Figure 2: Linagliptin.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

An analytically pure sample of Empagliflozin, Linagliptin and their related impurities with purities greater than 90% were used for the study, and the tablet formulation was prepared in our Formulation R&D laboratory. Acetonitrile (HPLC grade of Standard make), Methanol (HPLC grade of Standard make), orthophosphoric acid (OPA) (Finar HPLC grade) and water (Milli Q) were used for the analysis.

2.2 Instrument

HPLC analysis was performed on Waters makes HPLC's having PDA detector capable of setting detection wavelength of 220 nm. A reverse phase X-Bridge shield RP18, (150 X 4.6 mm; 3.5µm), part number 186003045 is finalized for the method. The HPLC system was controlled with "EMPOWER" software. LCMS instrument used AB Sciex /QTRAP 4500. An electronic analytical weighing balance (0.1mg sensitivity, Sartorius make, ME5 model), Sonicator (Hwashin Make, Power sonic 420 model), Water purification system (Merck make, Milli-Q IQ 7000 model) and Centrifuge (Eppendorf make, 5810 model) were used for the analysis.

2.3 Chromatographic conditions

The developed method uses a reverse phase X-Bridge shield RP18 (150 X 4.6 mm; 3.5µm), part number 186003045, mobile phase-A consisting of pH 2.5 Phosphate Buffer : Acetonitrile in the proportion of 90:10 v/v, mobile phase-B consisting of Water : Acetonitrile in the proportion of 30:70 v/v, flow rate of 0.8 ml/min, injection volume of 10 µL, detection wavelength of 220 nm using a UV/PDA detector, setting column temperature and sample compartment temperature of 25°C and 5°C respectively and run time as 65 minutes for gradient program.

Time	Mobile phase A	Mobile phase B
0.0	82	18
6.0	82	18
28.0	70	30
40.0	60	40
50.0	30	70
58.0	30	70
60.0	82	18
65.0	82	18

2.4 Reagents solution preparation

Diluted Ortho Phoshoric acid solution preparation

Taken 10 mL of ortho Phoshoric acid into 100 mL volumetric flask made up to the volume with Milli-Q water and mixed well.

Mobile phase pH 2.5 Buffer Preparation

Weighed and transferred 2.72662 g of KH_2PO_4 into 1000 mL of Milli-Q water, sonicated to dissolved and mixed well. adjusted the pH 2.50 with diluted ortho-phosphoric acid solution and mixed well.

Mobile phase A

Mixed pH 2.5 Phosphate buffer and Acetonitrile in the ratio of 90:10 v/v respectively followed by degassing in a sonicator for 10 minutes.

Mobile phase B

Mixed water and acetonitrile in the ratio of 30:70 v/v respectively followed by degassing in a sonicator for 10 minutes.

Diluent Preparation

The diluent solution was prepared by mixing water, Acetonitrile and Methanol in the proportion of 20:20 :60 v/v/v respectively followed by degassing in a sonicator for 10 minutes.

Preparation of Linagliptin standard stock solution

Weighed accurately about 50 mg of Linagliptin and standard and transfer into a clean and dried 250 ml volumetric flask. Later add 170 mL of the diluent, sonicate to dissolve. Dilute to volume with the diluent and mix well.

Preparation of Empagliflozin standard stock solution

Weighed accurately about 50 mg of Linagliptin and standard and transfer into a clean and dried 200 ml volumetric flask. Later add 160 mL of the diluent, sonicate to dissolve. Dilute to volume with the diluent and mix well.

Preparation of standard solution

Further diluted 0.3ml of above Linagliptin standard stock solution and 1mL of Empagliflozin standard stock solution into a clean and dried 250 ml of volumetric flask, make up to the mark with diluent and mix well.

Preparation of sample solution

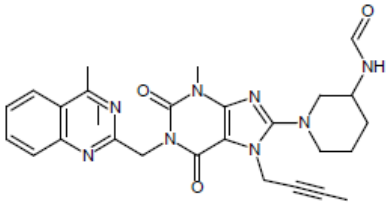
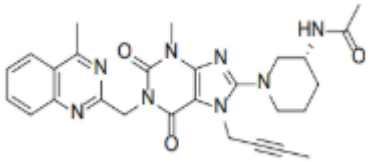
Drop 3 doses of Linagliptin and Empagliflozin tablets into a clean and dry 200 mL volumetric flask. Add 140 mL of the diluent and then sonicate for 30 minutes (maintain the temperature of water in sonicator between 20-25ºC) with intermittent shaking. Centrifuge a portion of the sample solution at 3000 rpm for 10 minutes. Inject the supernatant into HPLC.

3. RESULTS AND DISCUSSION

3.1 Identification by LC-MS and HPLC-PDA

Empagliflozin and Linagliptin tablets were subjected to the thermal stress at 105°C for 8 hours and for oxidation degradation conditions, later injected into HPLC-PDA detector and LC-MS instruments, as appropriate. Thermal stress sample gave 1 unknown impurity/degradant, whose M+1 fragment ion has m/z value of 501.1. To understand the probable structures corresponding to the masses of the degradants observed in the forced degradation study samples, retrieved the structures, molecular weights of the known degradants (**Table 1**), which were procured and injected in HPLC-PDA to understand the comparability of retention times, UV spectrums and / Mass.

Table 1: Summary Linagliptin impurities reported.[9]

Impurity Name	Structures	Molecular Weight
N-Formyl Linagliptin		**500**
N-Acetyl Linagliptin		**514.59**

Above known impurities were procured and injected in HPLC-PDA along with the thermal and oxidation stress samples and found that the retention times, UV spectrums match, thus concluded the structures of the degradants observed in the oxidation and thermal stress samples (**Figures 3-7**).

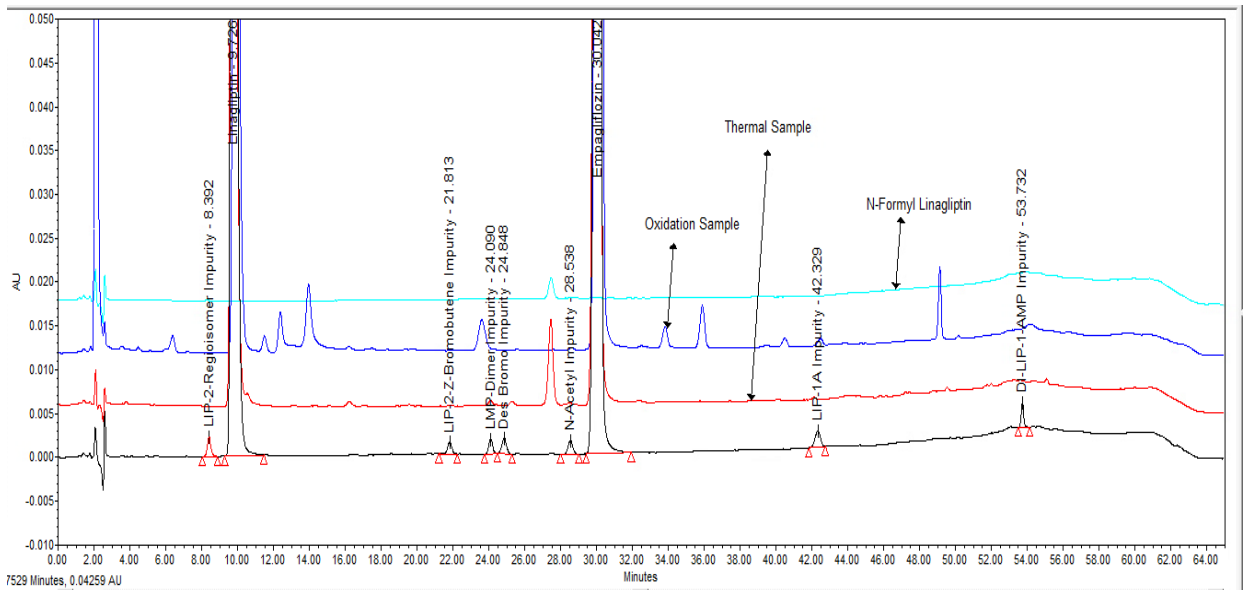
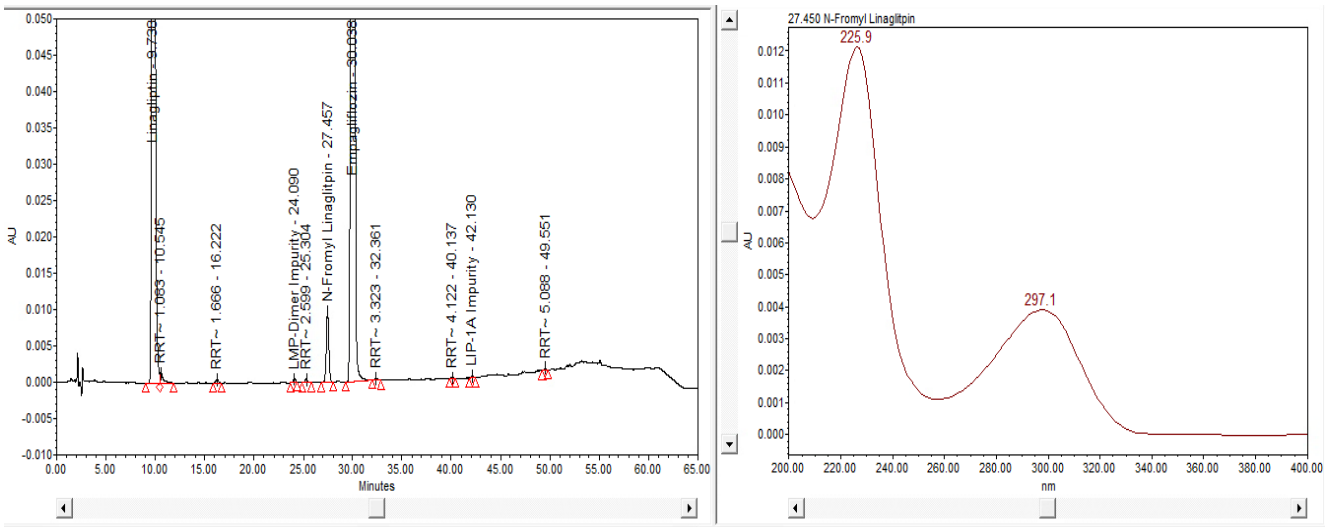


Figure 3: Overlay of chromatograms of thermal, oxidation stress degradation sample along with Impurities spiked sample (Retention time match)



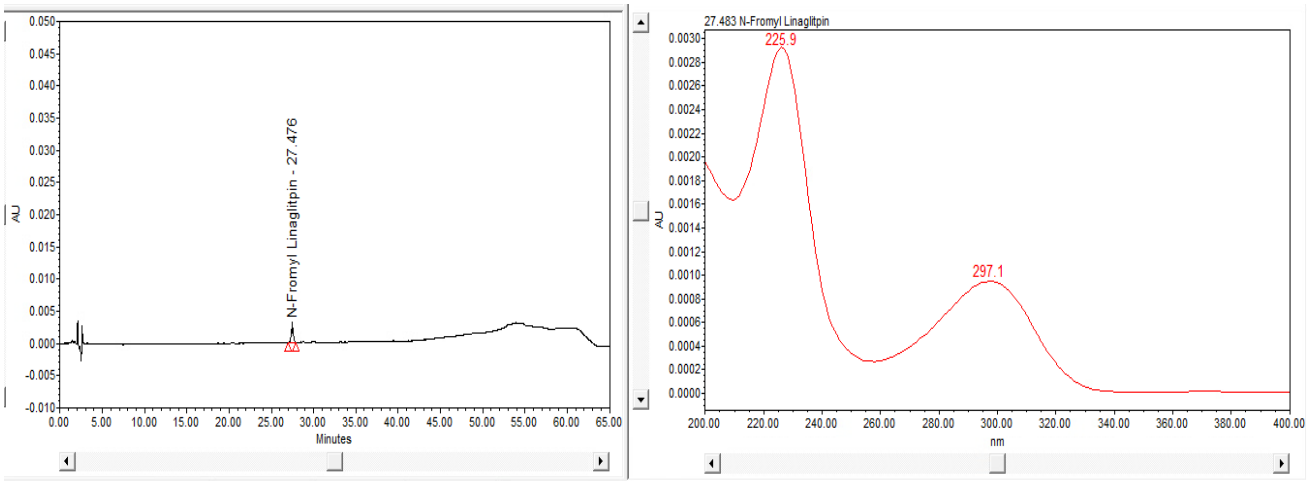


Figure 4: Comparison of HPLC-PDA and UV spectrum of the degradant of the thermal stress sample with individual known impurity (N-Formyl Linagliptin).

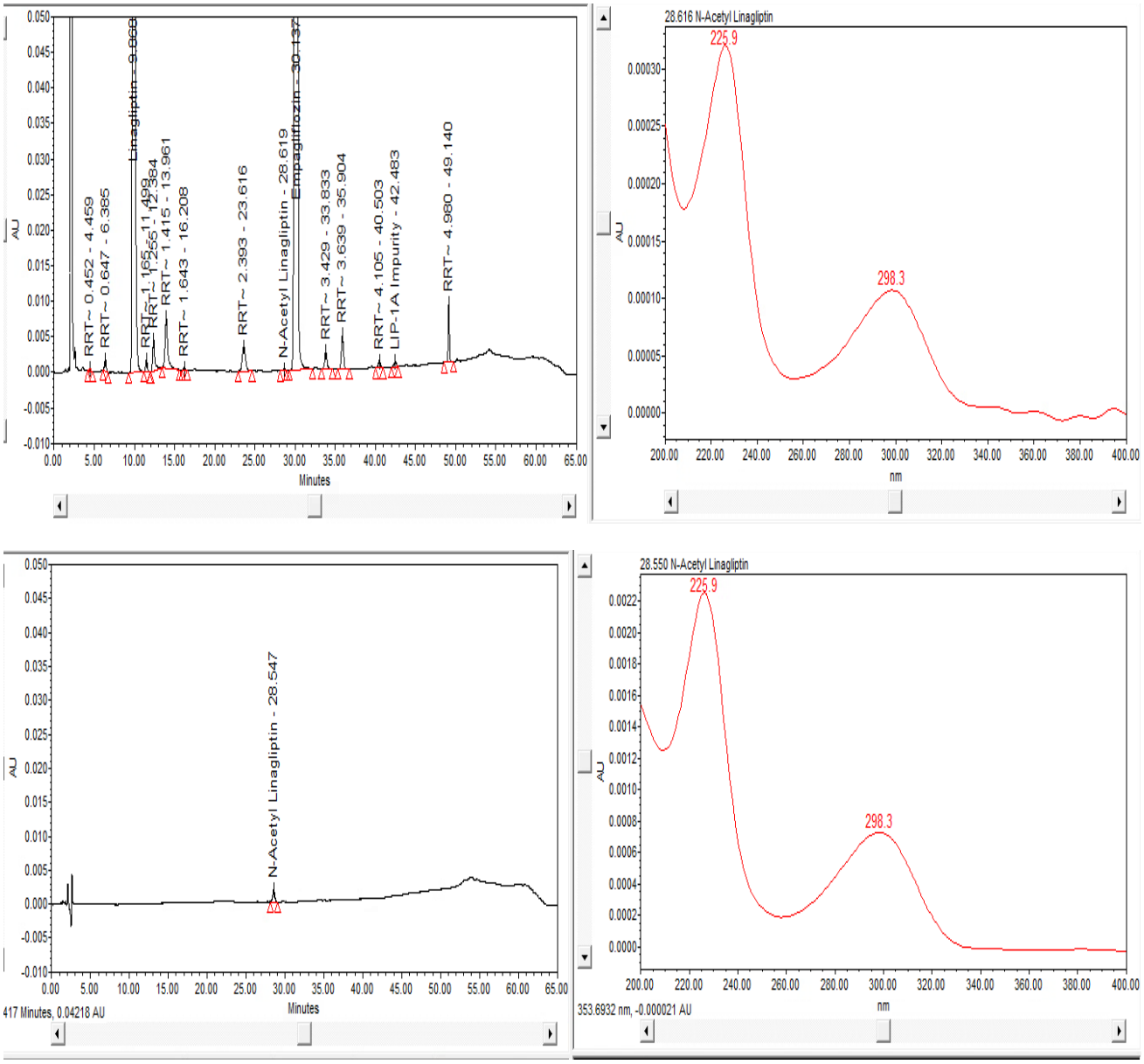


Figure 5: Comparison of HPLC-PDA and UV spectrum of the degradant of the Oxidation stress sample with individual known impurity (N-Acetyl Linagliptin).

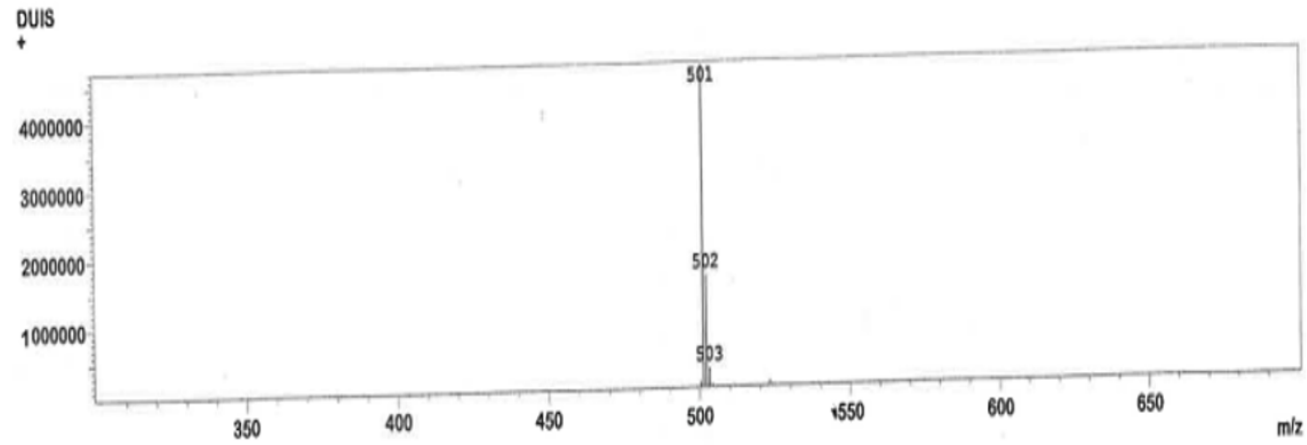


Figure 6: Mass Spectrum of procured N-Formyl linagliptin provided in COA.

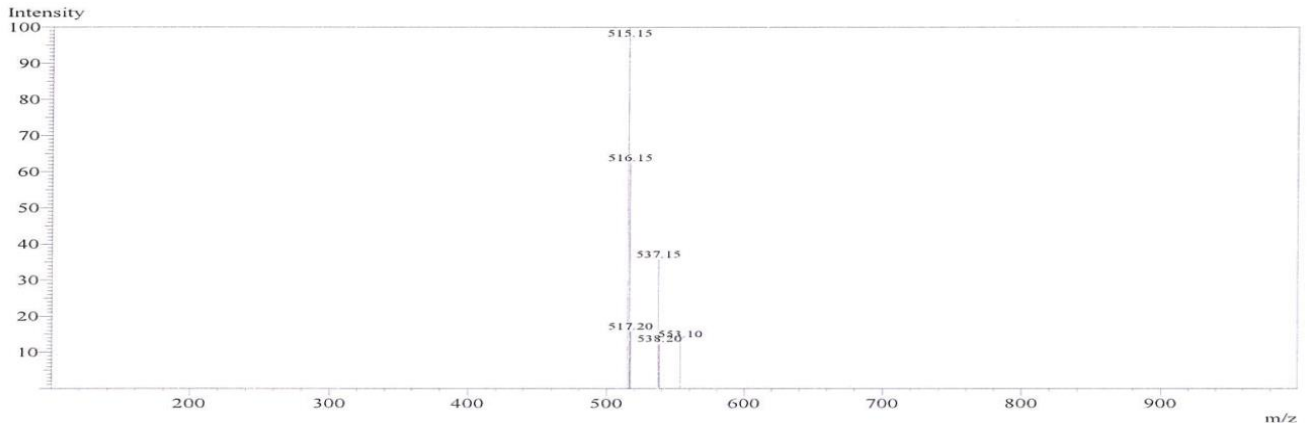


Figure 7: Mass Spectrum of procured N-Acetyl linagliptin provided in COA

4. CONCLUSION

Degradants observed in thermal and oxidation stress degradation samples were identified as N-Formyl Linagliptin and N-Acetyl Linagliptin basis to the retention times matching, UV spectrums concordance / mass values using HPLC-PDA and LC-MS techniques.

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