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Development and Validation of Stability Indicating Analytical Method for Estimation of Azelnidipine and Telmisartan and Characterization of Some Degradant Impurities Using LC-MS/MS

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ABSTRACT

The objective of this study was to explore the degradation behavior of Azelnidipine and Telmisartan under acidic, basic, oxidative, photolytic and thermal stress conditions as per prescribed International Conference on Harmonization (ICH) guidelines. Azelnidipine was found to be liable under acidic and oxidative stress conditions, whereas it was stable under basic, photolytic and thermal stress conditions. Similarly, Telmisartan was found to be liable under acidic and oxidative stress conditions, whereas it was stable under basic, photolytic and thermal stress conditions. A total of two degradation products (DPs) were characterized for azelnidipine and two degradation products (DPs) were characterized for Telmisartan, and their chromatographic separation was accomplished on Hypersil, BDS, C18, (150mm x 4.6mm, 5µm) column using a mobile phase consisting buffer (pH-5) : methanol in isocratic elution mode. The ion transitions were quantified in positive mode with MRM transition of 583.300→496.200 Da for Azelnidipine and 515.100→499.500 Da for Telmisartan. All the stressed sample were subjected to ESI-MS/MS and LC-MS/MS analysis. Azelnidipine and Telmisartan and its degradation products were characterized based on MRM scan mode and fragmentation patterns were obtained from ESI-MS/MS spectra. Structural elucidation of DPs of Azelnidipine and Telmisartan was achieved by comparing their fragmentation patterns with that of Azelnidipine and Telmisartan. The developed method has been validated for specificity, linearity, accuracy, precision and robustness as per ICH guideline. The method provided good linearity over the range of 0.4-1.2 µg/ml for Azelnidipine and 2.0-6.0 µg/ml for Telmisartan with short run time of 10 min. The proposed method was successfully applied for the estimation of Azelnidipine and Telmisartanin its pharmaceutical dosage form. The LC-MS/MS method were found to be simple, accurate, robust and reproducible. The assay can be successfully applied for routine QC analysis.

Keywords: Azelnidipine, Telmisartan, LC-MS/MS method, Validation, ICH Q2 (R1) guideline.

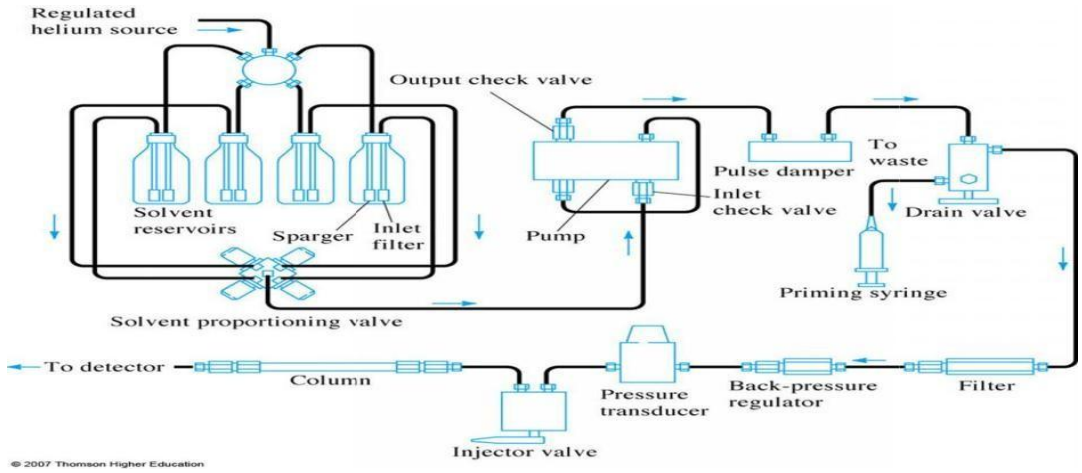
INTRODUCTION OF DRUG

Azelnidipine and **Telmisartan** both drug use of Hypertension. There are many classes of antihypertensive, which lower blood pressure by different means. Among the most important and most widely used medications are thiazide diuretic, calcium channel blocker, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers. Prevention of the important end points of hypertension, such as heart attack, stroke and heart failure. Thiazide diureties as the First-line treatment of choice for high blood pressure when drugs are necessary.

Introduction to Chromatography[2-5]:
Chromatography may be defined as method of separating a mixture of components into individual component through equilibrium distribution between two phases. Essentially, the technique of chromatography is based on the differences in the rate at which components of mixture moves through a porous medium (called stationary phase) under the influence of some solvent or gas (called mobile phase). Chromatography can be used for the quantitative and qualitative analysis.

Introduction of HPLC method[2]:
HPLC is an analytic caltechniquewidely used for identification, separation, detection and quantification of various

drugs and its related degradants. Most of the drugs as well as other compounds can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method.

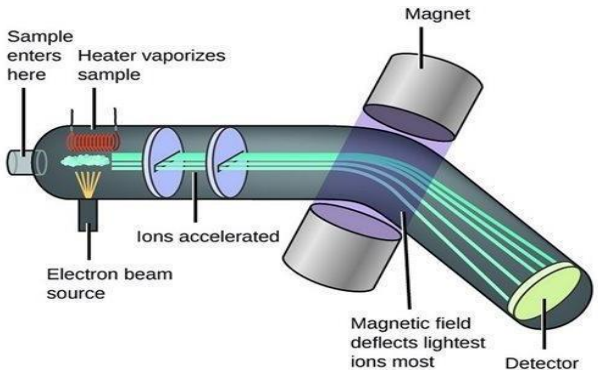


Principle of separation:
The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity to wards the stationary phase, the components are separated.

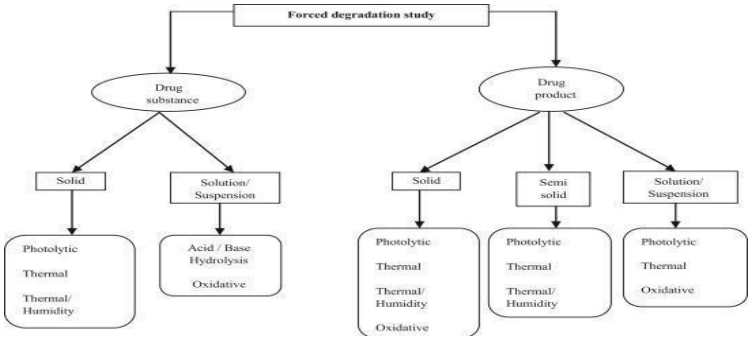
Normal phase: phase is nonpolar in nature. In this technique, nonpolar compounds travel faster and are eluted first. This is because of the lower affinity between the nonpolar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase.

Reversed phase mode: Reversed phase mode is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is nonpolar hydrophobic packing with octyl or octadecyl functional group bonded to silicagel and them obile phase is polar solvent.

Introduction to Massspectrometry[4-7]:
Mass spectrometry (MS) is an analytical technique that is used to measure the mass-to-charge ratio of ions. The results are presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures. A mass spectrum is a type of plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds.



Introduction of Force Degradation Study [4-7]:
Force Degradation is a process that involves degradation of drug products and drug substance at condition More severe than accelerated conditions and thus generates degradation product that can be studied to determine the stability of the molecule. The sample generated from forced degradation can be used to develop the stability indicating method which can be applied latter for the analysis of samples generated from accelerated and long term stability studies.



Introduction To Validation

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. Method validation provides an assurance of reliability during normal use, and is sometime referred to as "the process for providing documented evidence that the method does what it is intended to do."

- **Validation characteristics which should be considered are listed below :**
- Accuracy
- Specificity
- Linearity
- Range
- Precision
- Ruggedness
- Limit of detection
- Limit of Quantitation
- Robustness
- System Suitability parameters

MATERIAL AND METHOD :

- DRUG IDENTIFICATION :

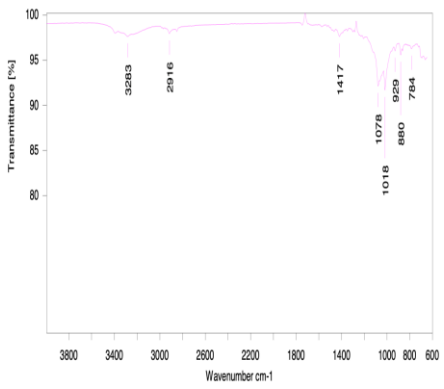
Drug Name	Reported (°C)	Observed(°C)
Azelnidipine	193-195°C	192°C
Telmisartan	261-263°C	262°C

- SOLUBILITY STUDY :

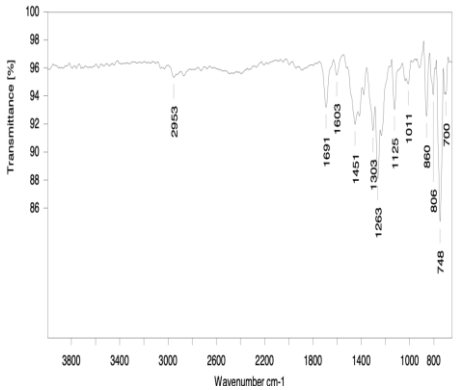
Solvent	Solubility of Azelnidipine	Solubility of telmisartan
Water	Very slightly soluble	Insoluble
Acetonitrile	Soluble	Slightly Soluble
Methanol	Freely soluble	Soluble

- IDENTIFICATION BY IR SPECTROSCOPY :

IR Spectra of Azelnidipine



IR Spectra of Telmisartan



IR Interpretation of Azelnidipine

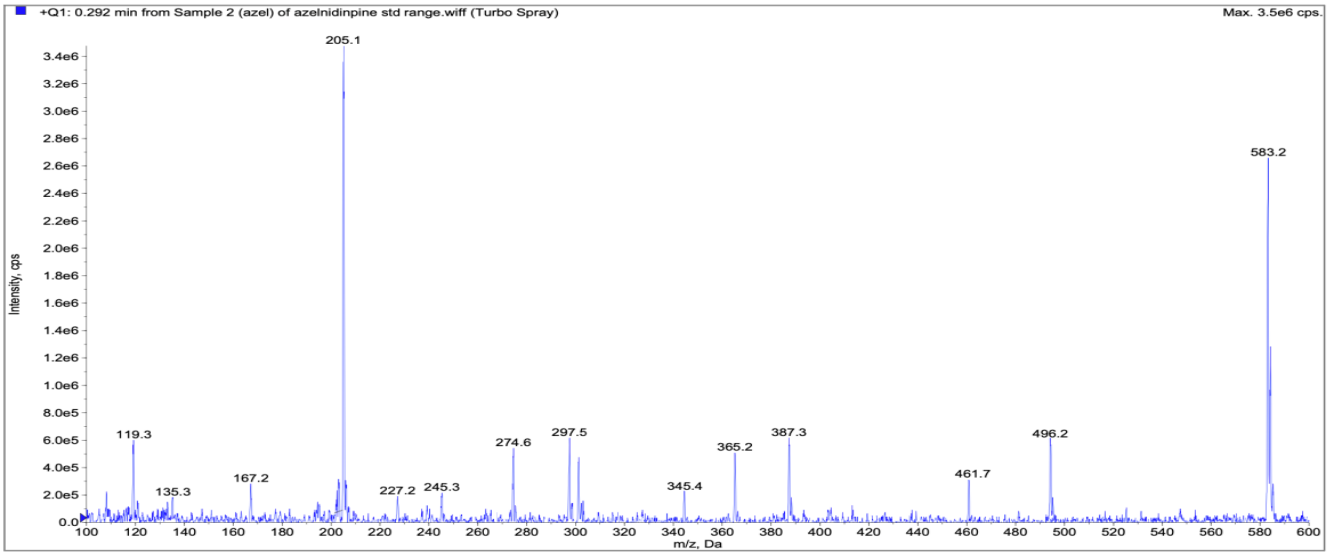
Functional Group	Frequency (cm^{-1})
N-H stretching	3283
N=O stretching	1417
C-Nstretching	1018-1078

IR Interpretation of Telmisartan

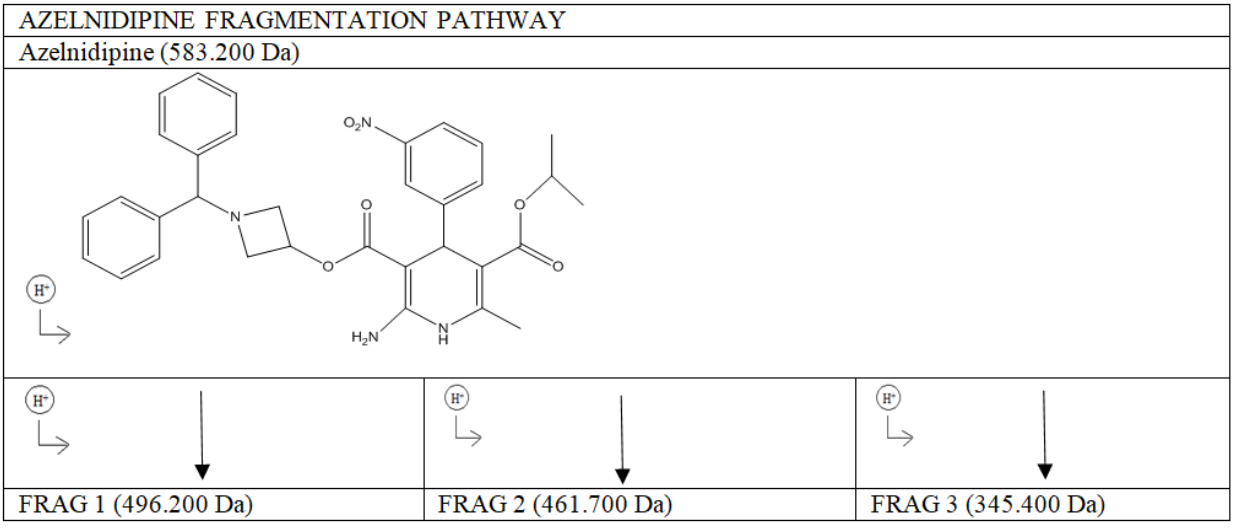
Functional Group	Frequency (cm^{-1})
O-H stretching	2953
C=O stretching	1691
C=Cstretching	1451-1603

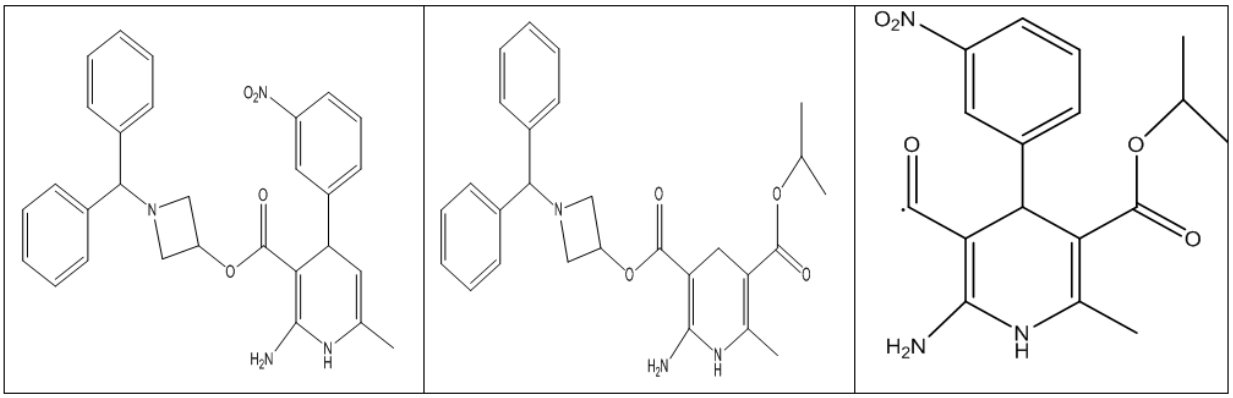
Mass Determination

Mass spectra of Azelnidipine

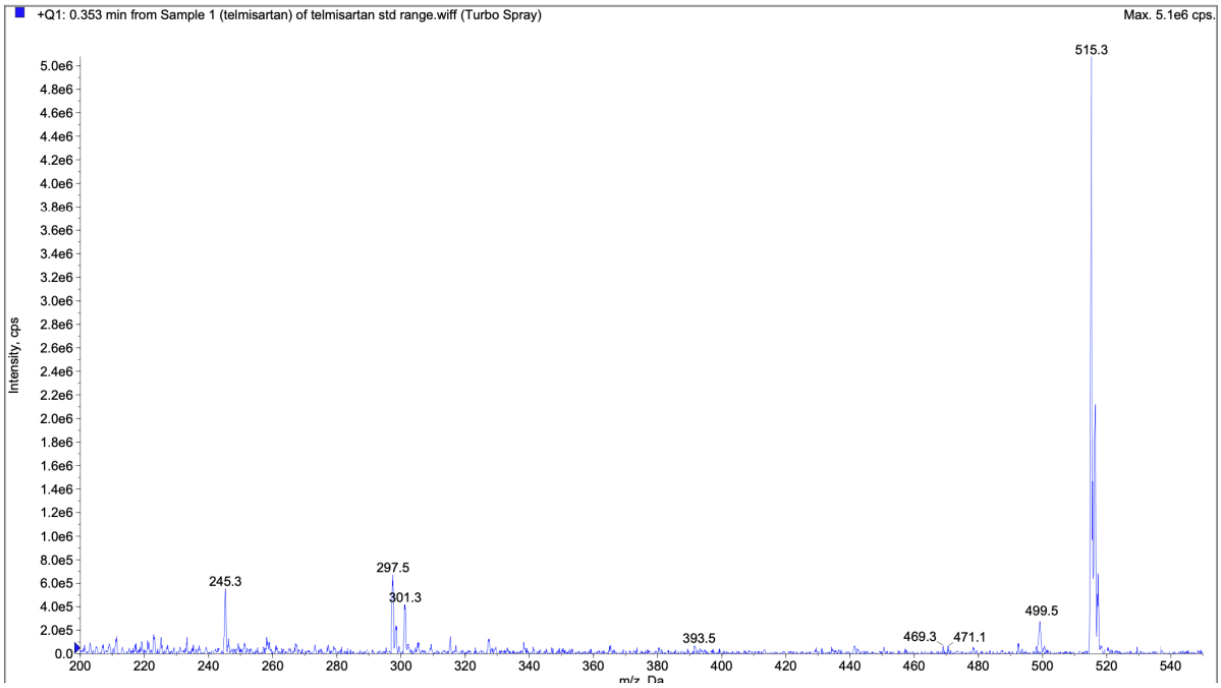


Fragmentation Pattern of Azelnidipine

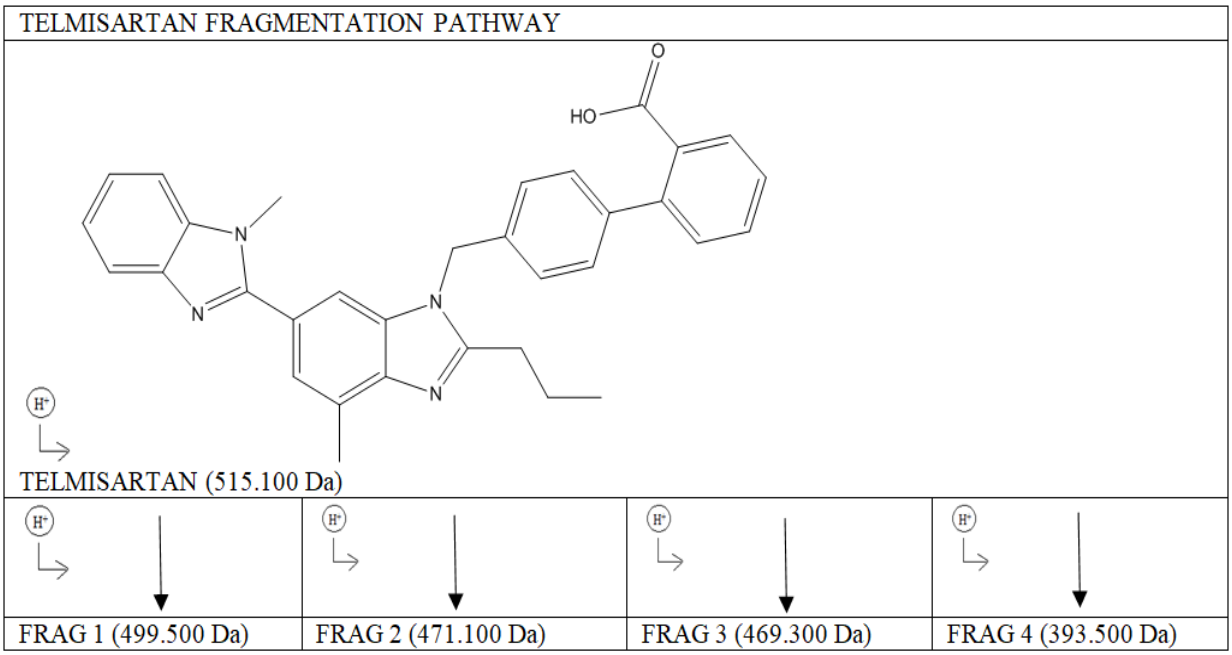


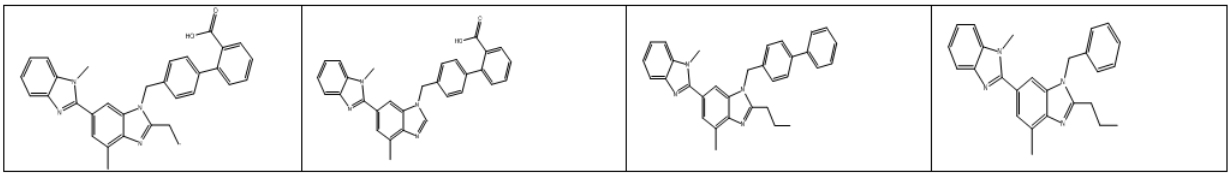


Mass spectra of Telmisartan



Fragmentation Pattern of Telmisartan





SOLUTION PREPARATION

Preparation of Standard Stock Solution of Azelnidipine
Accurately weighed separately quantity of 80.0mg Azelnidipine API were transferred into 1000 ml volumetric flask and dissolved in diluent using ultra sonication and diluted up to mark to give a stock solution having concentration of 80µg/ml Azelnidipine.

Preparation of Standard Stock Solution of Telmisartan
Accurately weighed separately quantity of 40 mg Telmisartan API were transferred into 1000 ml volumetric flask and dissolved in diluent using ultra sonication and diluted up to mark to give a stock solution having concentration of 400µg/ml Telmisartan.

Preparation of Working Standard Solution of Azelnidipine
From above Standard Stock Solution of Azelnidipine, 1 ml was taken in to 100 ml volumetric flask and was made up to the mark with the diluentto get 0.8 µg/ml of Azelnidipine.

Preparation of Working Standard Solution of Telmisartan
From above Standard Stock Solution of Telmisartan, 1 ml was taken in to 100 ml volumetric flask and was made up to the mark with the diluent to get 4.0 µg/ml of Telmisartan.

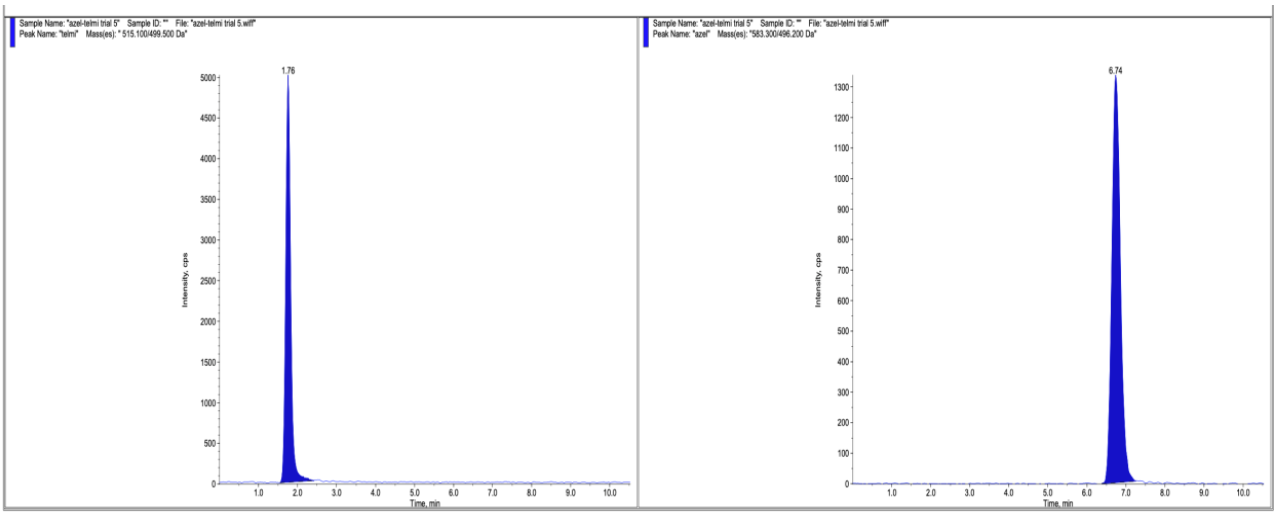
Combine Preparation of Working Standard Solution of Azelnidipine and Telmisartan
Take 1ml from azelnidipine stock solution and 1ml from telmisartan stock solution into 100ml volumetric flask and make up the volume with diluent to get 0.8 µg/ml of Azelnidipine and 4.0 µg/ml of Telmisartan.

Preparation of Mobile Phase
Prepare 10ml ammonium acetate buffer in water (pH 5.0) and methanol in the ratio of 50:50.Mix well and degas by sonication.

Preparation of Sample Stock Solution of Azelnidipine and telmisartan
The average weight of 10 tablets was determined and was ground in a mortar. Stock solution was prepared by dissolving tablet powder equivalent to 80.0mg of Azelnidipine and 40.0mg of telmisartan was transferred to 100ml volumetric flask. Then 50ml diluent was added and sonicated for 5 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluent. Filter the stock solution with 0.45µ Millipore filter and the final filtrate is collected as sample stock solution.

CHROMATOGRAPHY

Chromatogram of Azelnidipine and Telmisartan in buffer(pH 5) : Methanol (50:50v/v) (FINAL)



LC-MS/MS Chromatographic Condition :

Instrument	Liquid chromatography Mass spectrometer (API-2000) equipped with auto sample, auto injector, column oven, ion source ESI electron spray ionizer with Q1 and collision energy.		
Ion Source setting		***Scan setting***	
Ion source	ESI	Polarity	Positive ion
Curtain Gas	20psi	Scan type	MRM
Ion Spray Voltage	5200	Scan time	1-10 min
Temperature	400°C	Declustering Potential	60
Ion Source Gas(GS1)	50psi	Focusing Potential	300
Ion Source Gas(GS2)	50psi	Entrance Potential	10
Scan type	Telmisartan	MRM:(Q1)515.100 Da and (Q3) 499.500 Da	
	Azelnidipine	MRM:(Q1)583.200 Da and (Q3) 496.200 Da	

Chromatographic condition:

Column	:	Agilent, Zorbax, C18, (150mm x 4.6mm), 5µm			
Flow rate	:	1.0 mL/min	Injection volume	:	20 µL
Column oven temperature	:	35 °C	Run time	:	10 min
Column oven compartment	:	Ambient	Mode	:	Isocratic
Telmisartan R.T	:	About 1.7 min			
Azelnidipine R.T	:	About 6.8 min			

FORCE DEGRADATION STUDY :
Different Degradation Conditions for Azelnidipine

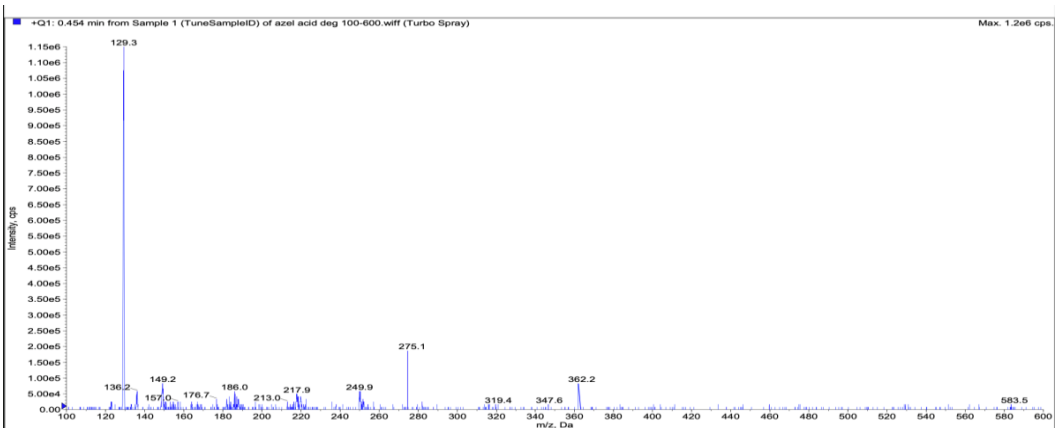
Sr. No.	**Stress Type**	**Stress Condition**
1	Acid Degradation	1 N HCl at 60^{o}C for4 hr.
2	Base Degradation	1 N NaOH at 60^{o}C for 3 hr.
3	Oxidative Degradation	30.0 % H_2O_2at 60^{o}Cfor 6 hrs.
4	Thermal Degradation	105^{o}C for 5 days
5	Photolytic Degradation	UV for 5 days

Different Degradation Conditions for Telmisartan

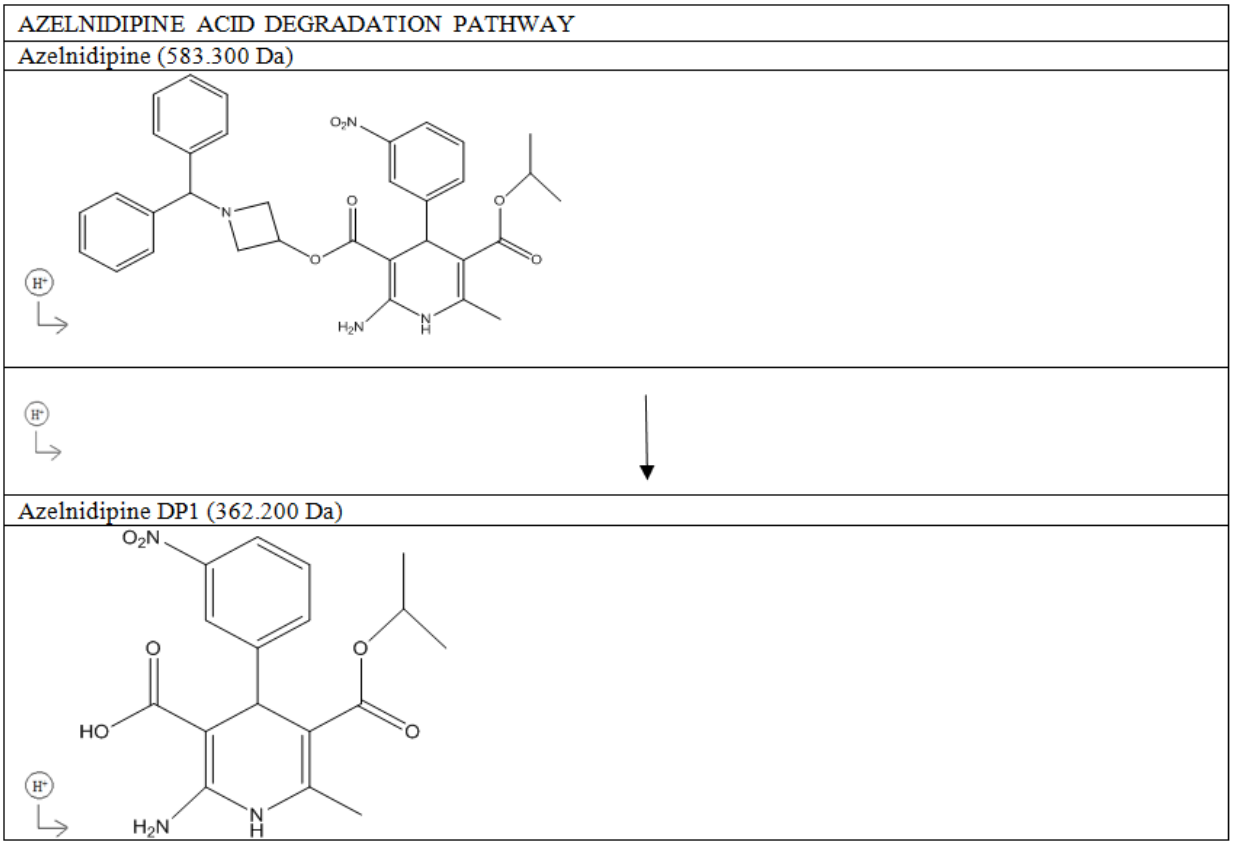
Sr. No.	Stress Type	Stress Condition
1	Acid Degradation	1 N HCl at 60°C for 2 hr.
2	Base Degradation	1 N NaOH at 60°C for 4 hr.
3	Oxidative Degradation	30.0 % H_2O_2 at 60°C for 5 hrs.
4	Thermal Degradation	105 °C for 5 days
5	Photolytic Degradation	UV for 5 days

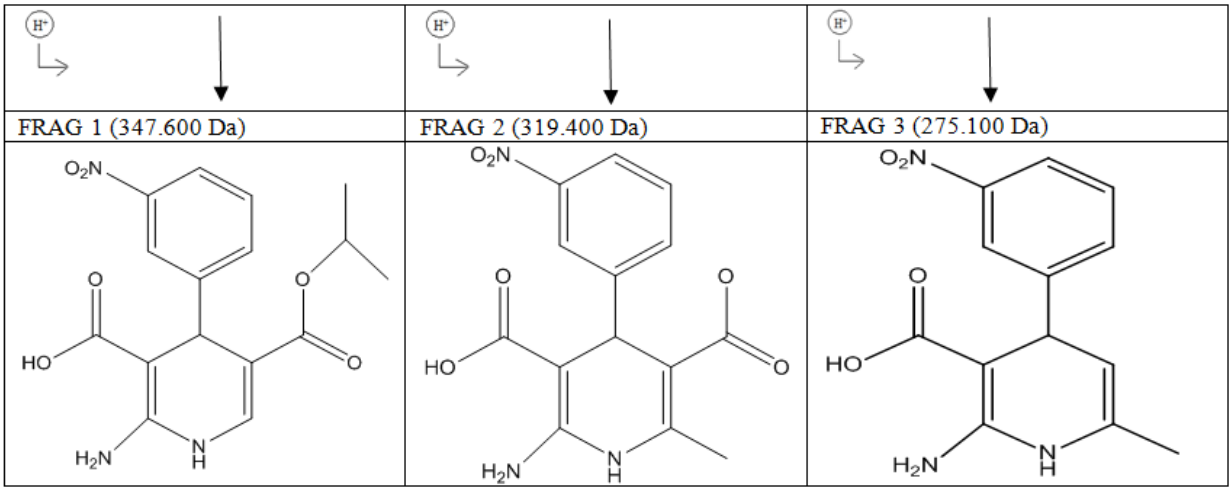
ESI-MS/MS Spectra and Fragmentation pattern of acidic Degradation :

Mass Spectra of acidic degradation solution of Azelnidipine

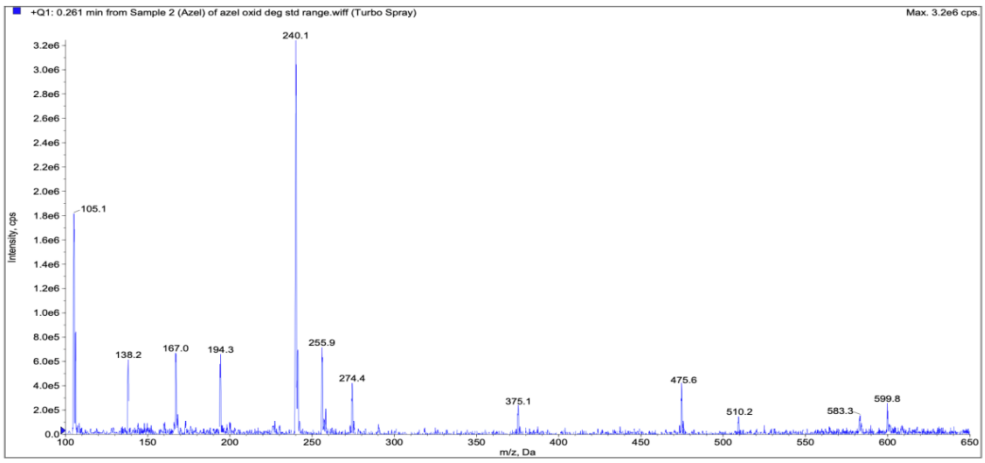


Azelnidipine acidic Degradation Pathway

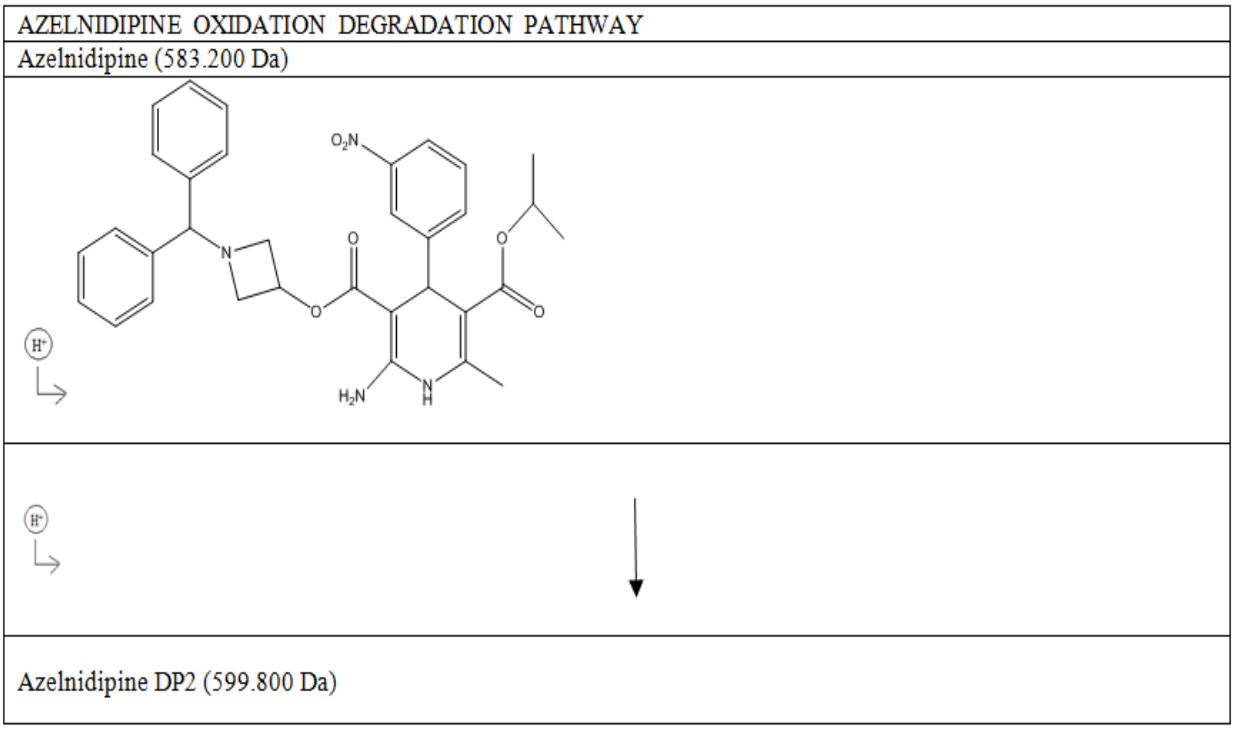


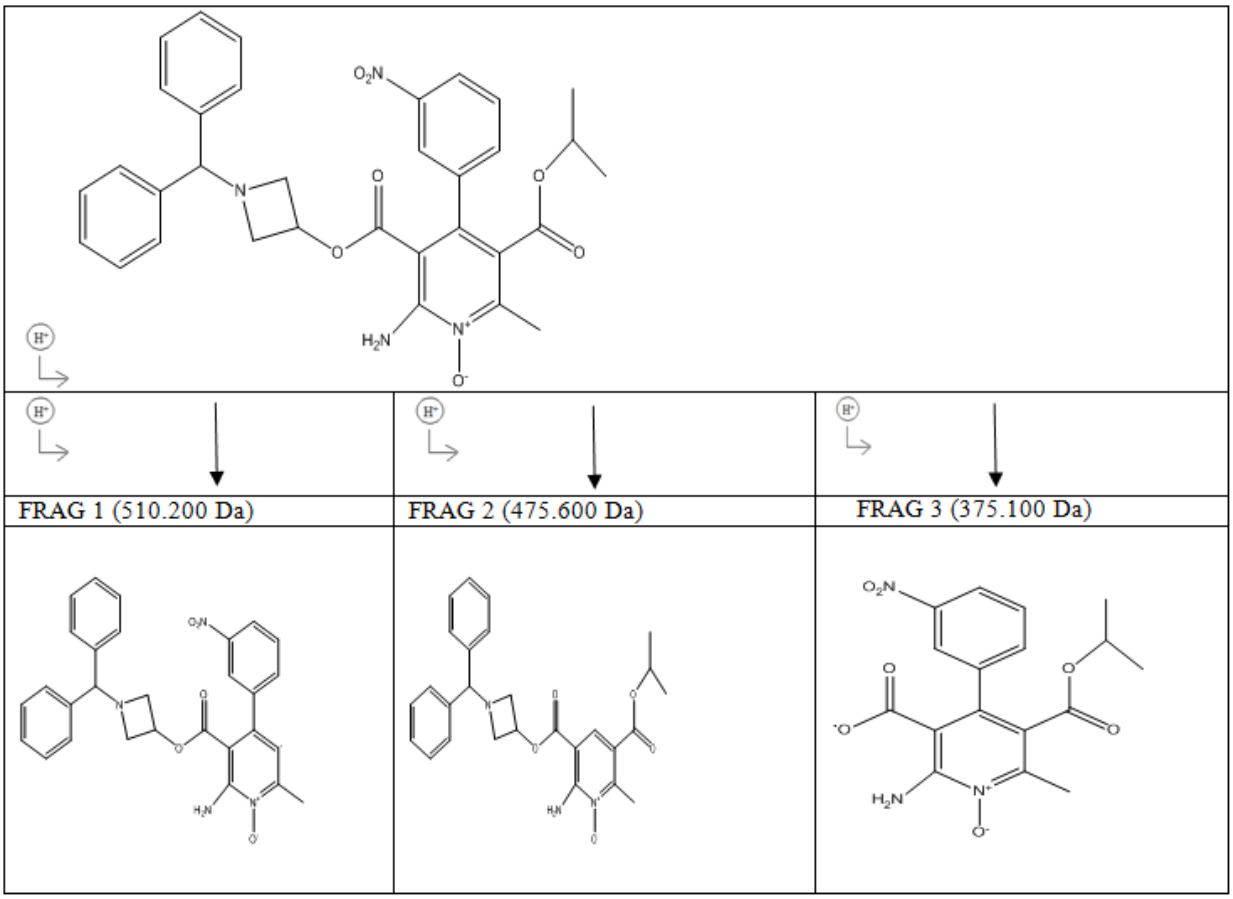


ESI-MS/MS Spectra and Fragmentation pattern of oxidative Degradation solution of Azelnidipine :
Mass Spectra of oxidative degradation solution of Azelnidipine



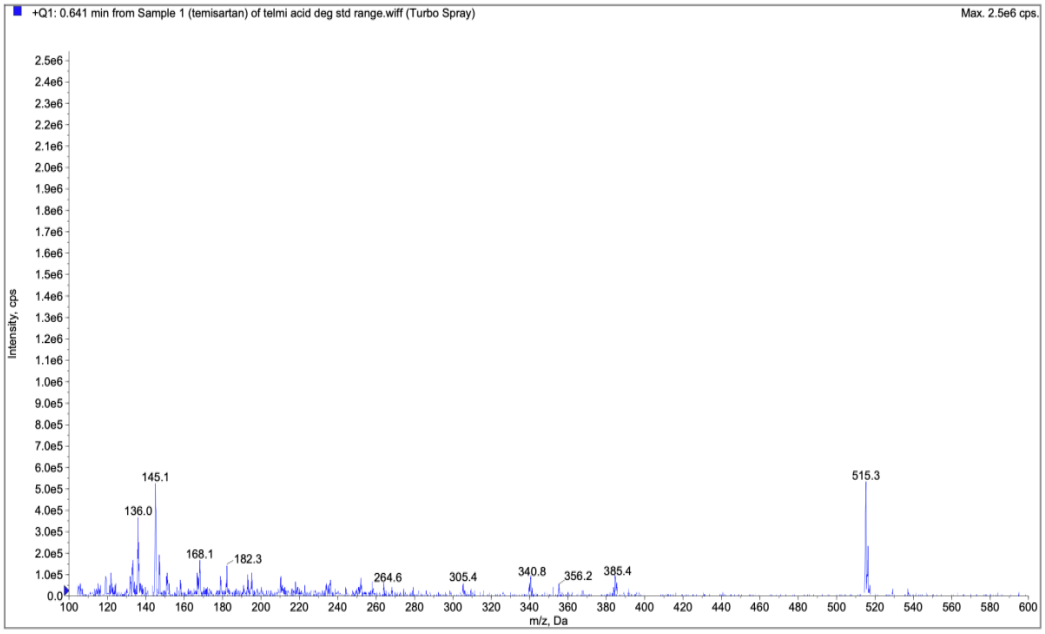
Azelnidipine oxidative Degradation Pathway





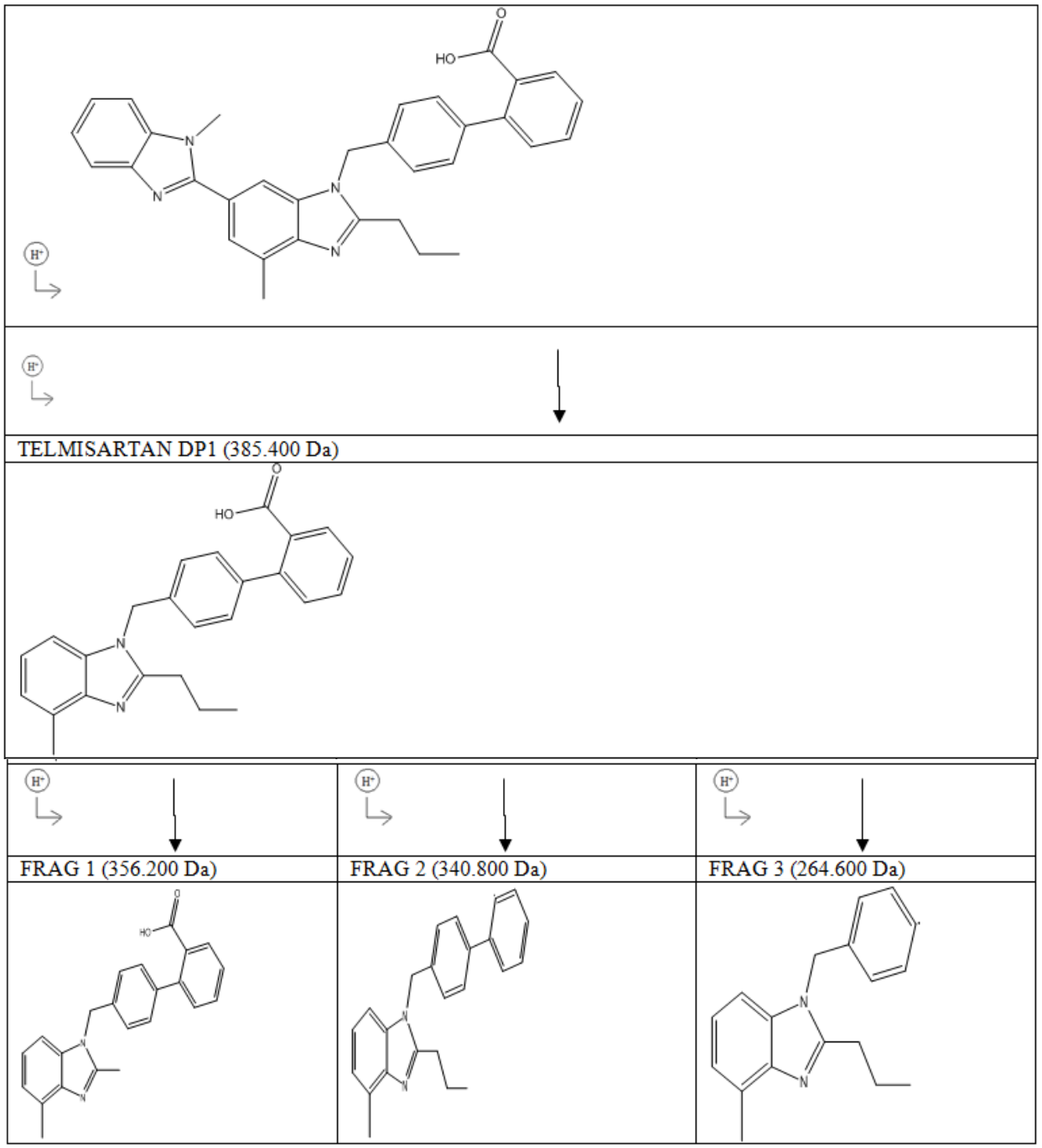
ESI-MS/MS Spectra and Fragmentation pattern of acidic Degradation solution of Telmisartan:

Mass Spectra of acidic degradation solution of Telmisartan

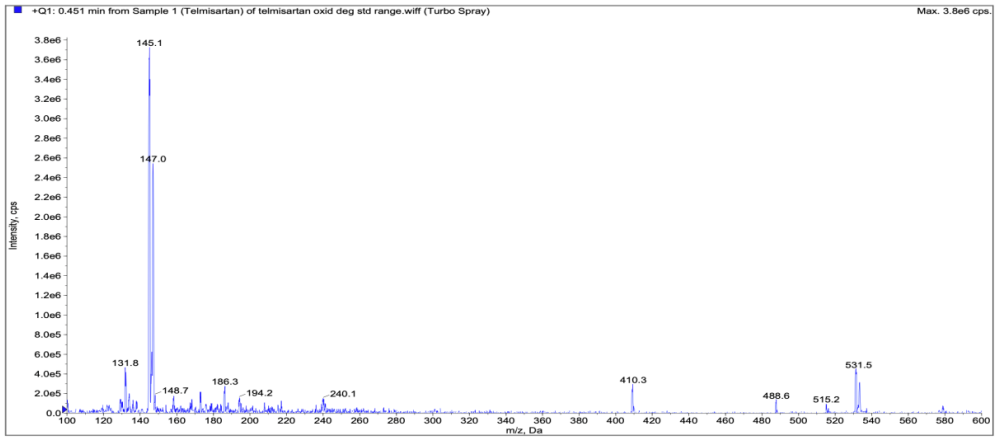


Telmisartan acidic Degradation Pathway

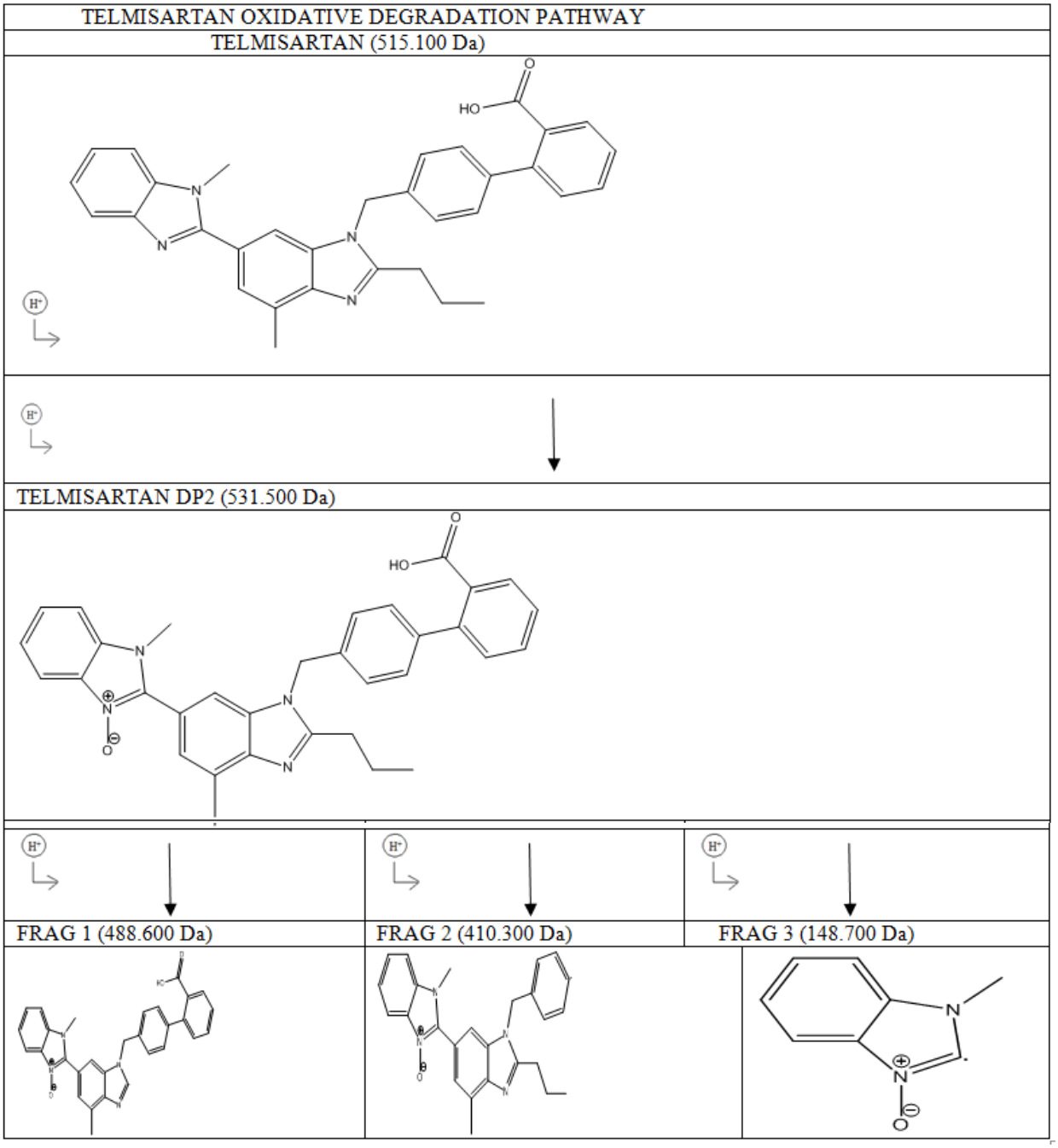
TELMISARTAN ACID DEGRADATION PATHWAY
TELMISARTAN (515.100 Da)



ESI-MS/MS Spectra and Fragmentation pattern of oxidative Degradation solution of Telmisartan :

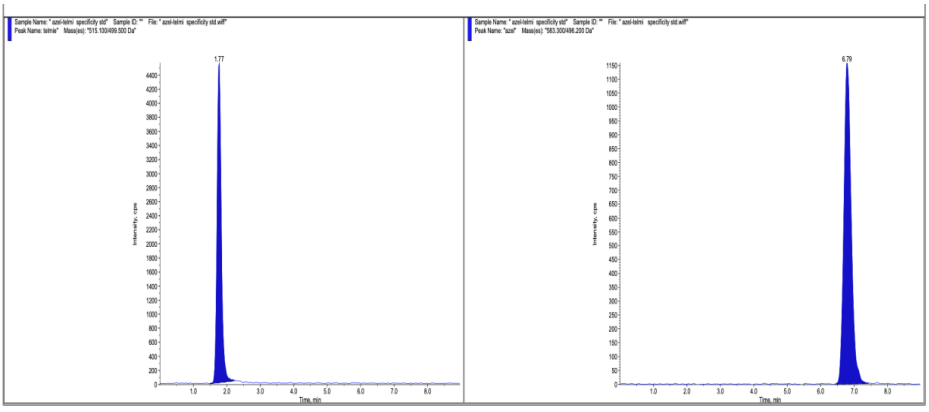


Telmisartan oxidative Degradation Pathway

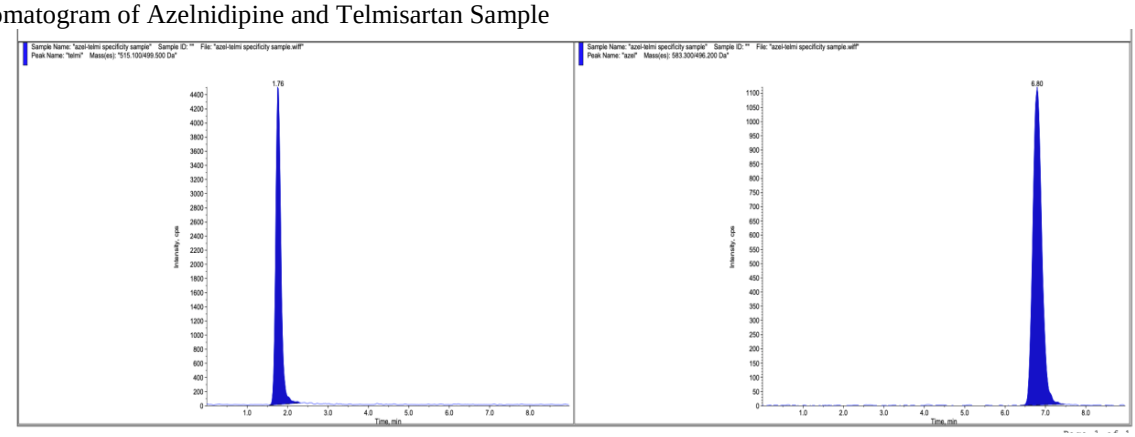


METHOD VALIDATION:

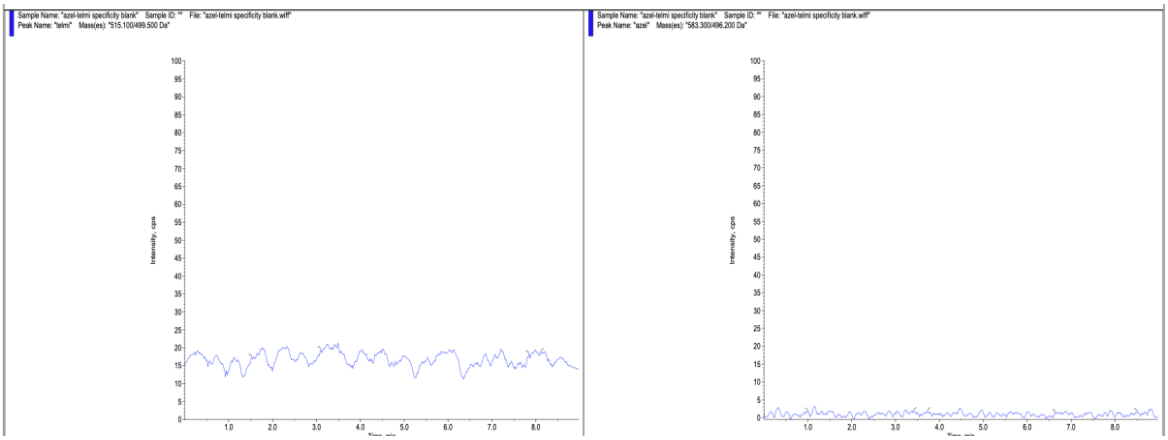
Chromatogram of Azelnidipine and Telmisartan Standard



Chromatogram of Azelnidipine and Telmisartan Sample



Chromatogram of Azelnidipine and Telmisartan Blank



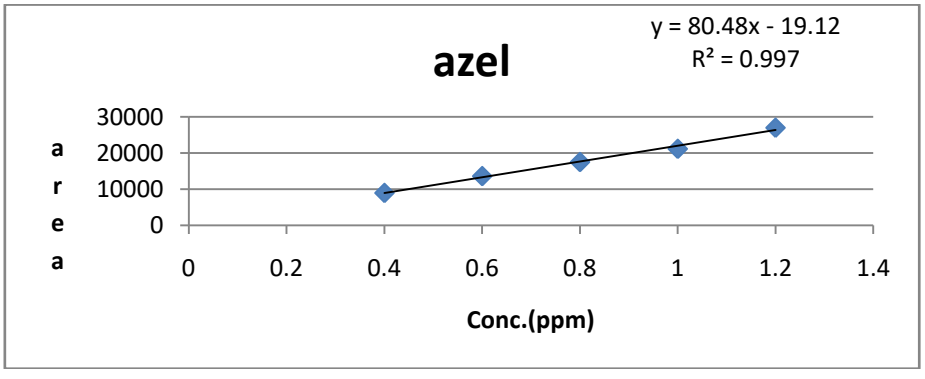
LINEARITY AND RANGE

The linearity for Azelnidipine and Telmisartan were assessed by analysis of standard solution in range of 0.4-1.2µg/ml and 2.0-6.0Azelnidipine and Telmisartan respectively Linearity Data for Azelnidipine Linearity Data for Telmisartan

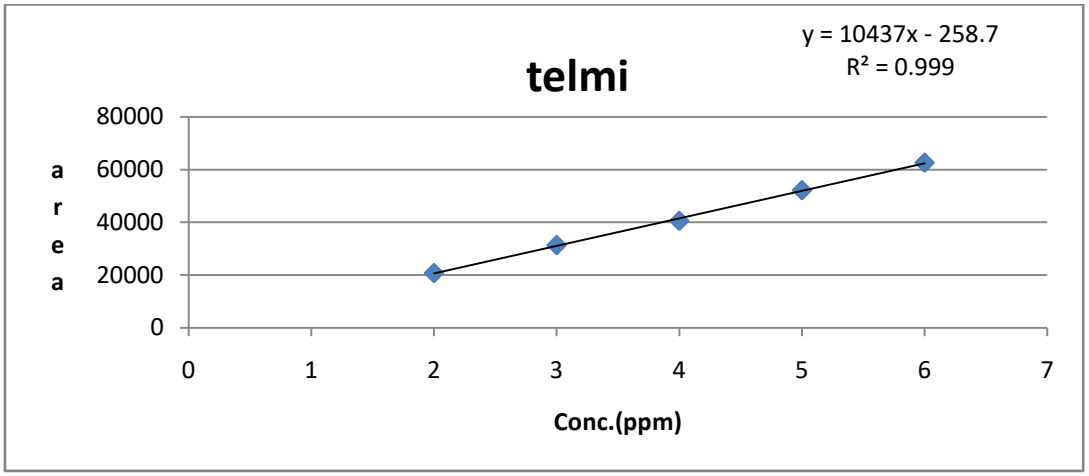
Sr.No	Concentration(µg/ml)	Area
1	0.4	8971.255
2	0.6	13615.669
3	0.8	17492.481
4	1	21135.508
5	1.2	26952.778

Sr.No	Concentration(µg/ml)	Area
1	2	20793.73
2	3	31367.782
3	4	40545.609
4	5	52159.000
5	6	62584.091

Calibration Curve of Azelnidipine(0.4-1.2µg/ml)



Calibration Curve of Telmisartan (2.0-6.0µg/ml)



Precision Repeatability

The data for repeatability of peak area measurement for Azelnidipine and Telmisartan, based on six measurements of same solution of Azelnidipine and Telmisartan are depicted in table 6.12 and 6.13 respectively. The %RSD for Azelnidipine and Telmisartan was found to be 1.665 and 1.331 respectively.

Repeatability Data for Azelnidipine

Azelnidipine				
Sr. No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	0.8	17631.481 16987.361 17558.314 17086.364 16979.364 17194.613	17239.583±287.055	1.665

Repeatability Data for Telmisartan

Telmisartan				
Sr. No.	**Conc (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	4.0	40982.609 41635.981 40972.364 41581.647 41559.341 40227.728	41159.945±547.654	1.331

INTRADAY PRECISION:

Intraday precision data for Estimation of Azelnidipine

Sr. No.	**Azelnidipine**		
	Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	0.4	8824.823±34.168	0.387

2	0.8	18875.773±224.927	1.192
3	1.2	27121.654±493.404	1.819

Intraday precision data for Estimation of Telmisartan

Sr. No.	Telmisartan		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	2.0	21944.325±142.306	0.648
2	4.0	40068.320±587.417	1.466
3	6.0	62413.596±872.219	1.397

INTERDAY PRECISION

Interday Precision data for Estimation of Azelnidipine

Sr. No.	Azelnidipine		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	0.4	9808.267 ±153.216	1.562
2	0.8	17937.276 ± 292.735	1.632
3	1.2	28831.413 ±296.552	1.029

Interday Precision data for Estimation of Telmisartan

Sr. No.	Telmisartan		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	2.0	23192.645 ±290.440	1.252
2	4.0	41891.307 ± 284.864	0.680
3	6.0	63147.586 ±298.983	0.473

Accuracy:

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 6.18 and 6.19 respectively. Percentage recovery for Azelnidipine and Telmisartan was 99.757-100.761% and 100.086-100.583 respectively.

Lod And Loq:

- LOD of Azelnidipine and Telmisartan was found to be 0.097 µg/ml and 0.194 µg/ml respectively.
- LOQ of Azelnidipine and Telmisartan was found to be 0.295 µg/ml and 0.587 µg/ml respectively.

Robustness:

The Robustness effect of changes on Flow Rate ±2, ph ±2, Mobile Phase ±2 was found within acceptance criteria.

ANALYSIS OF MARKETED FORMULATION BY DEVELOPED METHOD

Analysis of Marketed Formulation

Tablet	Label claim		Assay (% of label claim*) Mean ± S. D.	
	AzelnidipineTelmisartan	Telmisartan	% AzelnidipineTelmisartan	% Telmisartan
Azelikem T 5/10	8mg	40mg	100.475±1.585	99.851± 1.228

The assay results were comparable to labeled value of each drug in combined dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

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