CHAPTER-6
IDENTIFICATION, AND CHARACTERISATION OF
DEGRADATION IMPURITY IN VALSARTAN TABLETS
6.1. Introduction

Valsartan is an orally active specific angiotensin II blocker effective in lowering blood pressure in hypertensive patients[1,4]. Valsartan is a non-peptide, it is chemically described as (S)-N-(1-oxopentyl)-N-[(2′-(1H-tetrazol-5-yl)][1,1′-biphenyl]-4-yl|methyl]-L-valine. Its molecular formula is C_{24}H_{29}N_{5}O_{3}, its molecular weight is 435.5. Structural formula (1) is as follows.

![Structural formula](image)

(1)

6.2. Literature and Aim of the present work

Valsartan drug substance is official in USP, EP, and IP. [5-8]. Drug product is official in IP, not official in USP and BP. Literature search reveal that there are few analytical methods available for determination and estimation of valsartan from pharmaceutical formulations[9-10]. But there has been no reported method for identification and characterization of thermal or humidity related degradation product.

In this chapter the method for dentification and characterization of unknown impurity formed in valsartan tablets at accelerated stability conditions (12) is described. The various process related impurities and degradation products are (2-5)
L-Alanine Valsartan (2)  

Butyryl valsartan (3)  

Valsartan Methyl Ester (4)  

Valsartan benzyl ester (5)
6.3. **Experimental**

6.3.1. **Materials And Equipment**

Materials used were Sodium dihydrogen ortho phosphate, Acetonitrile, orthophosphoric acid of HPLC grade were used.

Equipments used for these studies were same that are used in the experiments of chapter -2. Column used was Zorbax SB Phenyl 250* 4.6 mm size of particles present in this column is 0.5μ

6.3.2. **Chromatography**

6.3.2.1. **Mobile phase preparation**

Mobile phase A: pH 3.6 phosphate buffer: 3.12 grams of Sodium dihydrogen orthophosphate was dissolved in 1000 ml of water, orthophosphoric acid was added to adjust the pH to 3.6 and filtered.

Mobile phase B: Degassed Acetonitrile.

6.3.2.2. **Conditions for HPLC**

Flow rate: 1.5 ml/minute
Detection: UV detection 225 nm
Column oven temperature: 30 °C
Run time: 55 minutes
Pump mode: Gradient.
<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Mobile Phase A (v/v)</th>
<th>Mobile Phase B (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{0.01}</td>
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<td>27</td>
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<tr>
<td>T_{05}</td>
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<td>27</td>
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<tr>
<td>T_{55}</td>
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<td>27</td>
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</table>
6.3.3.0. LC-MS-MS Analysis

6.3.3.1. Preparation of mobile phase

Mobile Phase A: pH 4.2 Acetate Buffer: 0.77 g of Ammonium acetate was dissolved in 1 litre of water, acetic acid was used to adjust pH to 4.2. This solution was filtered using Millipore vacuum pump.

Mobile Phase B: Methanol

6.3.3.2. Diluent

For diluent Milli Q water was used.

6.3.3.3. Preparation of sample solution

Sample equivalent to 50mg of valsartan was weighed and transferred into a 50ml volumetric flask, 30ml of diluent was added and sonicated for 20 minutes to dissolve. The solution was made up to 50 ml with diluent and filtered.

6.3.3.4. LC-MS/MS Conditions

The sample solution was subjected to LC-MS analysis using the mobile phase as described under section 6.3.3.1. Detection at wavelength of 225nm and flow rate was 1.0 ml/min. Runtime was 60 min. Gradient elution was used.
One unknown impurity with a mass of 351[(MH)⁺] was detected in addition to 435 [(MH)⁺](Valsartan) with other fragments in the laboratory batch sample.

6.5. Results and discussion

6.5.1. Detection of Impurity

Valsartan tablets are available in the strengths of 40mg, 80mg, 160mg and 320mg. In our routine analysis of valsartan tablets initial samples we did not get any unknown impurity more than the threshold limits (Fig-6.1) but during the accelerated stability 6M 40°C 75 % RH samples showed one unknown impurity (RRT: 0.19)(Fig-6.2) more than identification threshold. An attempt was made to identify and characterize this thermal degradation impurity. To find out that this impurity is getting due to diluent or placebo or API degradation, we have injected diluent (Fig-6.3), 6M stability placebo we did not get any unknown. So it was confirmed API only degrading during formulation stability giving this unknown impurity.
Fig-6.1. Valsartan tablets initial sample

Fig-6.2. Valsartan tablets 6months stability sample

Fig-6.3. Valsartan tablets diluent

Fig-6.4. LC Chromatogram of Valsartan tablets 6M placebo

To check the resolution of valsartan tablets samples and all known impurities spiked with degradation product were analyzed (Fig. 6.5 & 6.6) All the impurities and degradation product were completely separated from valsartan peak. The rrt of degradations impurity is 0.19 when compared to the main peak.

Fig-6.5. valsartan spiked with degradation impurity

Fig-6.6. valsartan tablets spiked with degradation impurity and all known impurities

After detection of the impurity the same sample was subjected to LC-MS analysis. According to fragments obtained in LC –MS analysis the degradation path way was proposed.

6.5.2. Proposed Degradation path way for Degradation Product

Degradation of valsartan in presence of heat and moisture causes the cleavage of pentamido linkage giving rise to
degradation product. The proposed degradation pathway is shown in Scheme 6.2
6.5.2 Synthesis of degradation impurity

The methodology for the synthesis of Des valeryl valsartan (10) is shown in Scheme. 6.1. 2-Triphenylmethyl-5-[4’-(Bromomethyl)]-Biphenyl-2-yl|Tetrazole (6) on condensation with L-Valine methyl ester (7) in the presence of N-N di isopropyl ethyl amine and dimethyl formaamide at 40°C temperature gave N-[[2’-(1-Triphenylmethyltetrazol-5-yl) Biphenyl-4-yl|methyl]-[L]-valine methyl ester (8). This was treated with hydrochloric acid at room temperature to obtain N-[[2’-(1H-Tetrazol-5-YL) Biphenyl-4-yl|Methyl]-[L]-Valine methyl ester (9). This compound (9) on hydrolysis with aqueous sodium hydroxide at room temperature to obtain the final compound N-[[2’-(1H-Tetrazol-5-YL)(1,1’Biphenyl)-4-yl]Methyl]-[L]-Valine(Desvaleryl Valsartan or Degradation impurity ) (10)
The synthesized impurity structure was elucidated by MASS, NMR, IR Spectral data.

6.5.3. Elucidation of structure of degradation impurity

The mass spectrum of this impurity (Fig 6.6) (RRT about 0.19) exhibited molecular ion peak at m/z, 352 amu [(MH)+] in +ve ion mode confirmed the molecular weight of this degradation impurity is 352. This molecular weight is less than the 123 units less than the valsartan mass 435. This indicates that the degradation impurity can potentially be a cleavage product of valsartan. Further 1H spectra of degradation impurity showed the signals of protons δ(ppm); 0.92(m,6H, a & a'),
2.01 (m, 1H, b,), 3.04 (d, 1H, c), 3.76 & 3.94 (ABq, 2H, d), 7.09 (dd, 2H, e & e'), 7.32 (dd, 2H, f & f), 7.52 (m, 2H, g & h), 7.64 (m, 2H, i & j). FTIR spectra of the impurity shows the following characteristic absorption bands (cm\(^{-1}\)), 3032 (Aromatic CH stretch) 3419 (NH stretch), 2969, 2879 (Aliphatic CH stretch) 1622 (C=O stretch), 1567 (C=C stretch) 1244 (C=N stretch), 1066 (C-N stretch). The data indicates that, the degradation impurity as N-[[2'-(1H-Tetrazol-5-yl)-(1,1'-Biphenyl)-4-yl]Methyl]-(L)-Valine, Hydrochloride.

Degradation impurity with assignment of protons (11)
6.6. Conclusion

An unknown degradation impurity was observed in accelerated stability samples of valsartan tablets when the analysis was run by the gradient HPLC method. This degradation impurity was identified and successfully characterized. The proposed structure of the degradant is N-\[2’-(1H-Tetrazol-5-yl)-(1,1’-Biphenyl)-4-yl\]methyl)-(L)-Valine. This may have been formed due to cleavage of the valsartan in presence of heat and moisture.