SHIMADZU

Application Notes

No. AD-0043

LCMS - 8030

The unparalleled high speed scanning reverberates the power of LCMS-8030 towards the characterization of ultra trace impurities in Pharma analysis – Part II

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Objective:

To characterize the related substances / other impurities using Ultra High Speed LC/MS/MS technique, when the level of impurities are below 0.1%.

Introduction:

In the first part of the application note (AD-0042), the characterization of ultra trace level impurities of Valsartan has been discussed. This application note discusses the characterization of the impurity whose concentration is only 0.01% by area. This application note is to demonstrate the power of LCMS-8030 to detect, record and provide significant MS/MS spectrum for characterization, when this kind of impurity appears at the tail of a main compound (here it is Valsartan).

Experimental:

Valsartan was in-house synthesized at Indian Institute of Chromatography and Mass Spectrometry (IICMS), Chennai, India. The purity of the compound was found to be 99.73% (Area normalization method). The data analysis showed one known USP impurity (Impurity-B) of 0.17% and four process related unknown impurities of concentration ranging from 0.01% to 0.04% (Fig. 1). The impurity retentions and their concentrations are shown in Table 1.

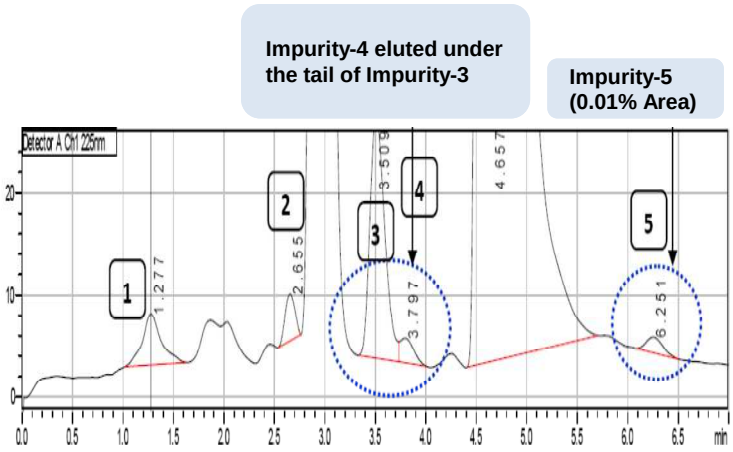


Fig. 1: UV Chromatogram of Valsartan and its impurities (225nm)

Impurities	Retention time (min)	Level of impurities in UV detection (%Area)
Impurity-1	1.277	0.03
Impurity-2	2.655	0.04
Impurity-3	3.509	0.17
Impurity-4	3.797	0.02
Valsartan	4.657	--
Impurity-5	**6.251**	**0.01**

Table 1: The retention time and level of impurities of Valsartan by area normalization method (225nm).

Results and Discussions:

Impurity-5

The observed concentration of impurity-5 is only 0.01% with respect to valsartan peak (by UV area ratio). This impurity is expected to form during the hydrolysis of the methylester in the last step of valsartan crude synthesis. The methyl may attack the nitrogen on the tetrazole acid isostere to form this impurity-5. The observed precursor ion of the impurity-5 in the positive mode is at m/z 450 [M+1], confirmed in the simultaneous negative mode analysis as m/z 448 [M-1] which is 14 mass units more than that of valsartan (Figure 2). Considering the similar structure of impurity-5 (Figure 3) and valsartan, the increase of 14 units suggested that a methyl had replaced the hydrogen on the carboxyl group of valine or the tetrazole ring.

To confirm the above observations, MS/MS analysis is performed (As explained in the part I of this application note). The MS/MS spectrum of valsartan from its precursor ion m/z 436 exhibited a series of product ions in positive ionization mode at m/z 291, m/z 235, m/z 207, m/z 192, m/z 180 and m/z 153. Similarly, the MS/MS spectrum of impurity-5 from its precursor ion m/z 450 exhibited a series of product ion in positive ionization mode at m/z 249 (235+14), m/z 221 (207+14), m/z 206 (192+14), m/z 192 and m/z 180. The pattern of the product ions from the valsartan and impurity-5 indicates that the two compounds have similar structures.

The key step in elucidating the impurity structure is to understand the fragmentation pattern of valsartan and impurity-5. The proposed fragmentation pattern of the valsartan and impurity-5 are presented in Figure 3 & figure 5. The cleavage on the bond of the acyl nitrogen and the bridging methylene group of valsartan have resulted the product ion at m/z 235. The product ion of impurity-5 at m/z 249 suggests that a methyl has replaced the hydrogen on the tetrazole ring rather than the one on the carboxyl group of valine, which is further confirmed by the simultaneous existence of product ions at m/z 192 and m/z 206 (192+14) in the impurity-5. The table 2 illustrates the structural similarity between the product ions of valsartan and impurity-5 with the existence of methyl group in the tetrazole ring.

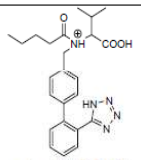
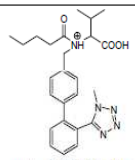
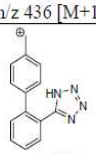
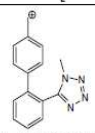
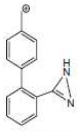
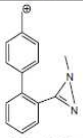
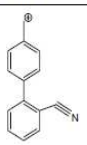
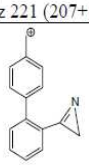
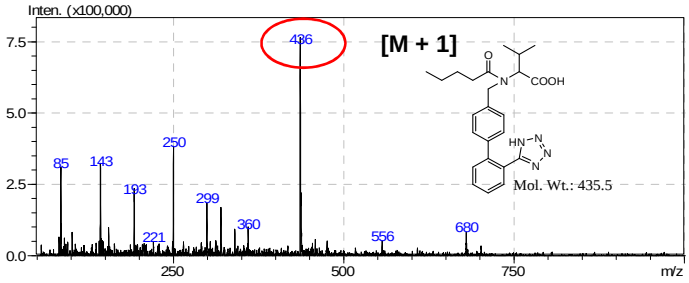
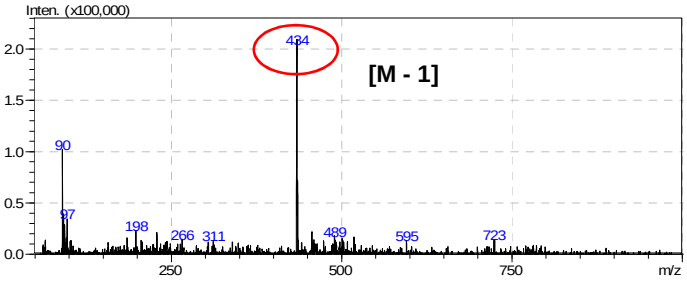
Description	Valsartan	Impurity-5
Precursor ion	 m/z 436 [M+1]	 m/z 450 [M+1]
Product ion	 m/z 235	 m/z 249 (235+14)
	 m/z 207	 m/z 221 (207+14)
	 m/z 192	 m/z 206 (192+14)

Table 2: The characteristic product ions of impurity-5 where the mass is increased by 14 mass units with respect to the product ion of valsartan

MS scan in +ve ionization mode



MS scan in -ve ionization mode



MS/MS of m/z 436 in +ve ionization mode

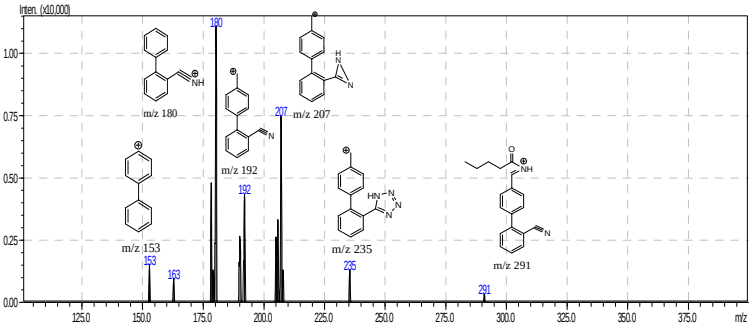


Fig. 2: MS and MS/MS mass spectra of Valsartan

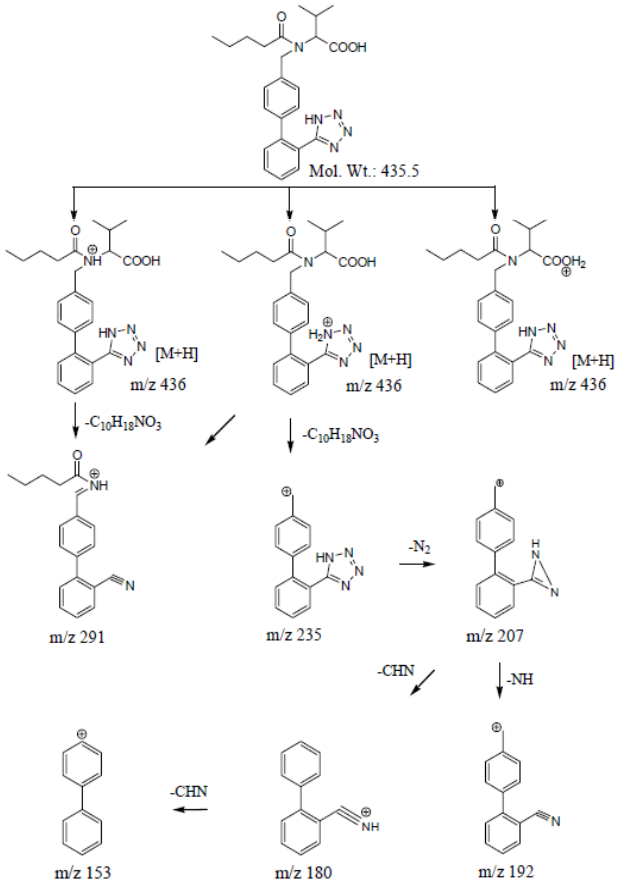
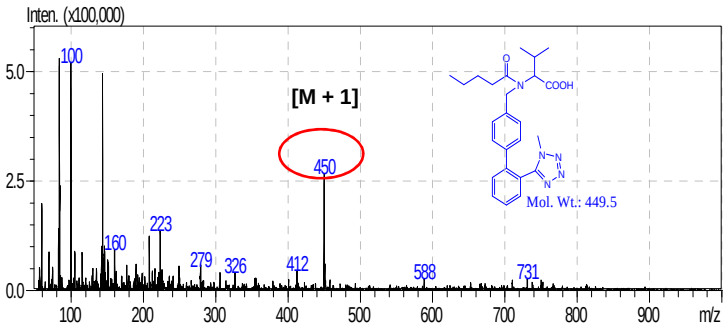
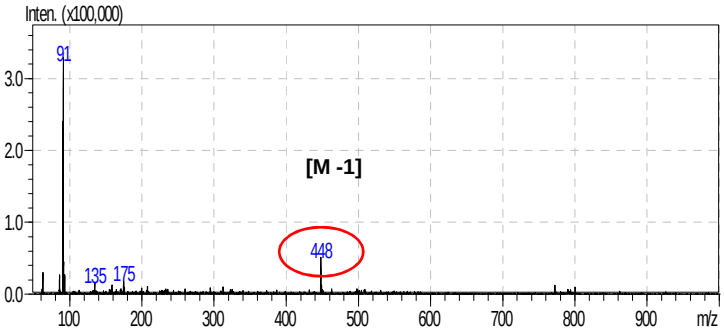


Fig. 3: The proposed fragmentation pathway of Valsartan

MS scan in +ve ionization mode



MS scan in -ve ionization mode



MS/MS of m/z 450 in +ve ionization mode

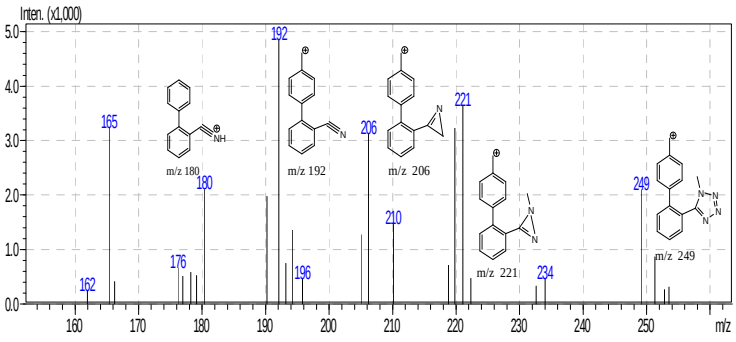


Fig. 4: MS and MS/MS mass spectra of Impurity-5

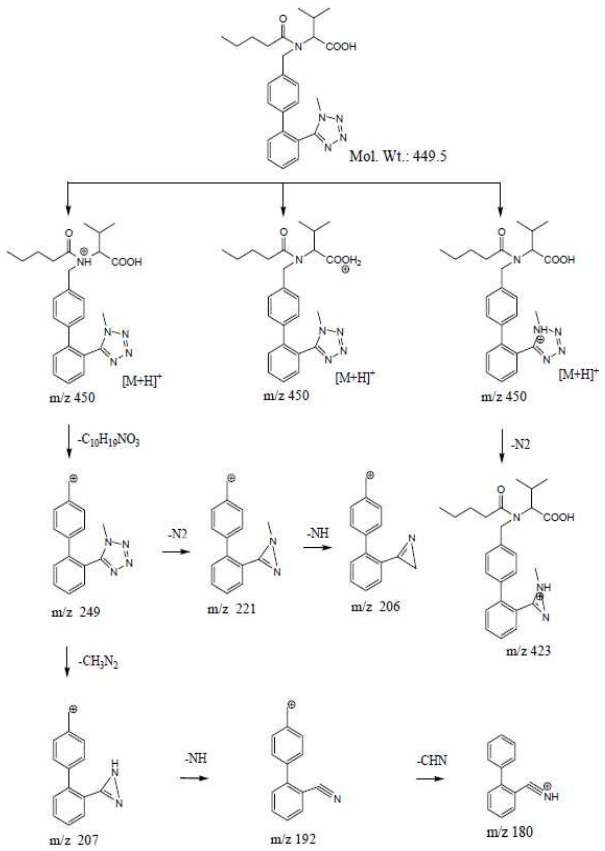


Fig. 5: The proposed fragmentation pathway of impurity-5

Conclusion:

Shimadzu LCMS-8030 triple quadrupole system has successfully demonstrated the power of characterization of these kinds of impurities at a concentration of 0.01%. This is possible because of the unique feature of the system, namely, ultra high speed scanning with least dwell time.

References:

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