SHIMADZU

Application Notes

No. AD-0042

LCMS - 8030

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

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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:

Here is an application note using Shimadzu LCMS-8030, which demonstrates the identification and characterization of trace level impurities down to 0.01% level, using its unique capabilities namely “ultra high speed scanning” and “lowest dwell time”. Characterization of the impurity to such a lower concentration of 0.01% is imperative to pro-actively ensure the quality of the drug substance. We have demonstrated the capability of LCMS-8030 using the drug substance Valsartan, a well known Angiotensin II (AT-II) receptor antagonists. Valsartan has excellent anti-hypertensive activity with very good safety profile. It is considered as a non-peptide AT-II antagonist [(1)]. Though the several method of synthesis of this drug is available, the drug industry continues to develop cost effective process while ensuring the quality of the product by maintaining the process related impurities well below the specifications. However, it is often difficult to generate potential toxicity data of the possible impurities present. Therefore, it is always desirable to ensure that these impurities are below 0.1%, implementing the ICH guidelines. Sometimes, there are probabilities that these impurities may appear slightly greater than 0.1% which requires identification and characterization using modern mass spectrometric technique.

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 were shown in Table 1.

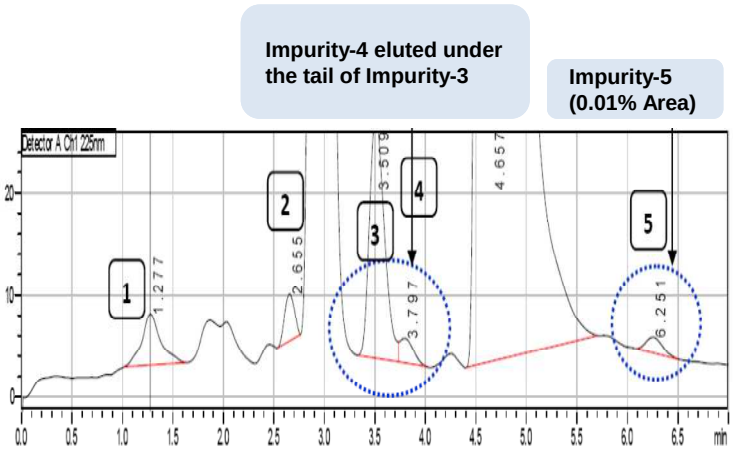

Fig. 1: UV Chromatogram of Valsartan and its impurities

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).

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These impurities were characterized using the following instrumental conditions:

Instrument

HPLC : Shimadzu UFLC XR
MS : LCMS-8030 Triple Quadrupole System

Analysis condition

Column	: Shim-pack XR ODS (75 x 3.0 mm, 2.1 µm)		
Mobile phase	: Formic acid: Water: ACN (0.05:50:50)		
Flow rate	: 300 µL/min	DL temp	250 ^{0}C
Column temp	: 30 ^{0}C	Heat block	400 ^{0}C
Wavelength	: 225 nm	Drying gas	5 L/min
Interface	: ESI	Nebulizing gas	3 L/min
Interface volt	: 4.5 kV	Scan range	50 to1000 amu

Enough care has been taken such that the higher concentration of principal peak did not affect the mass spectrometric parameters during data acquisition of entire run.

Results and Discussions:

Valsartan

The active ingredient compound, valsartan has been characterized. The details of which will be discussed in part II of this application note. This is because part II describes the characterization of impurity that appears at the tail of the valsartan peak (Impurity-5).

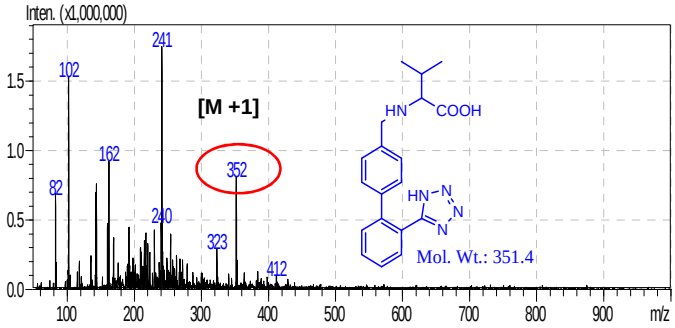
Impurity-1

The observed concentration of impurity-1 was 0.03% only with respect to valsartan peak (by UV area ratio). This impurity was expected to form by the presence of coupled product of bi-phenyl and valine methyl ester in the final reaction. The observed precursor ion of the molecule in the positive mode was at m/z 352 [M+1], confirmed in the simultaneous negative mode analysis as m/z 350 [M-1], due to very fast polarity switching time (15 msec). The more comprehensive way to identify a compound is to monitor or characterize the fragment ions of the compound of interest. The 'Product Ion Scan Mode' has enabled us to select the precursor ion of m/z 352 in +ve ionization mode which has led to major product ions having m/z 207, m/z 190, m/z 180, m/z 167 and m/z 153. The mass spectrum of impurity-1 (Fig: 2) showed three possible ways of protonated forms of the precursor ion (m/z 352). The precursor with protonation at the carboxyl group, amide nitrogen and tetrazole nitrogen reduced to ions of m/z 207. The ion of m/z 207 further fragmented into the ions of m/z 190, m/z 180, m/z 167 and m/z 153.

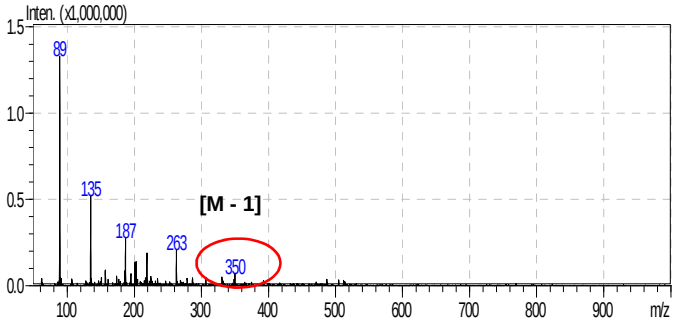
The π bond cleavage between the tetrazole nitrogen atoms due to protonation in 1H tetrazole or 2H tetrazole, which will likely to cause the ring opening via the cleavage of the bond between N1 – N2[2]. This may probably open the ring and cause a rearrangement to close the ring between N1 and N4. In addition to this, the cleavage occured at amino linkage of valine. The ring cleavage followed by rearrangement of tetrazole group and loss of valine group due to amino cleavage led to the formation of ion at m/z 207 (-$C_5H_{10}N_3O_2$). The three member ring, the m/z 207 undergo a ring expansion with an elimination of methyl radical to result in m/z 191[3]. The loss of valine group due to amino bond cleavage along with loss of –HN_3 via tetrazole ring cleavage probably explained the formation of the ion at m/z 180

.The formation of the characteristic ion at m/z 167 could be due to further loss of –CH_2N_2 (rearranged tetrazole group) of m/z 207 due to possible inductive effect. This ion further lost a –CH_2 group to form a stable ion at m/z 153.

MS scan in +ve ionization mode



MS scan in -ve ionization mode



MS/MS of m/z 352 in +ve ionization mode

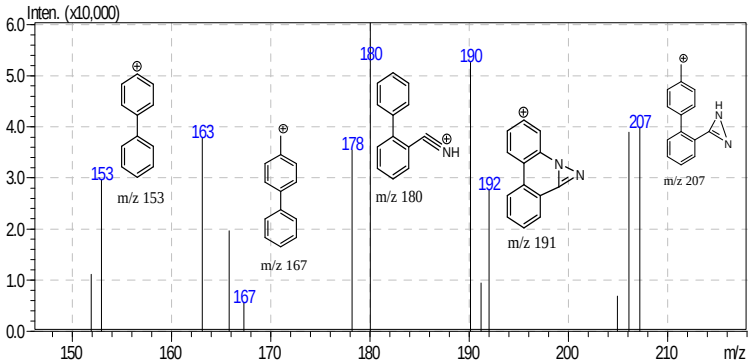
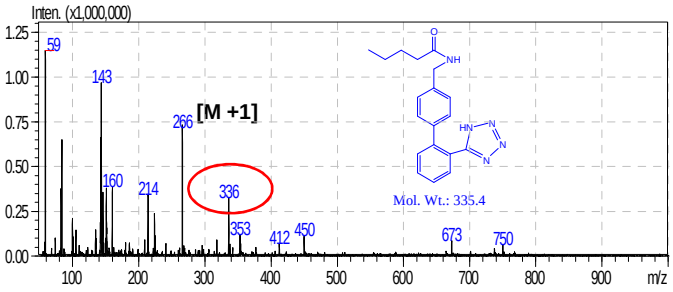


Fig. 2: MS and MS/MS mass spectra of Impurity-1

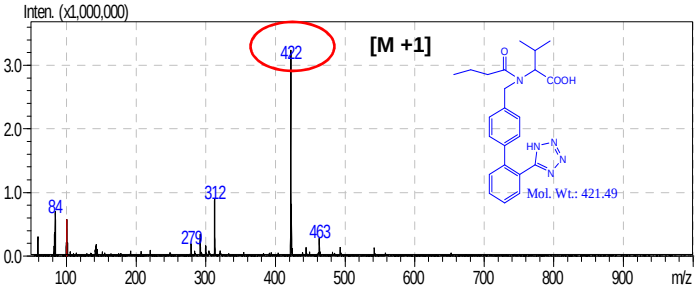
Impurity-2

The observed concentration of impurity-2 was only 0.04% with respect to valsartan peak (by UV area ratio). The probable structure of this impurity was predicted as shown in Fig. 3. It has already been reported as degraded impurity in a valsartan drug product analysis (Surabhi Metha, et al. 2010)[2]. The observed precursor ion of the impurity in the positive mode was at m/z 336 [M+1], confirmed in the simultaneous negative mode analysis as m/z 334 [M-1]. The major product ions were at m/z 235, m/z 207, m/z 190, m/z 180, m/z 167 and m/z 153, similar to the mass spectrum of impurity-1. Therefore, the fragmentation pathway of the major ions at m/z 207, m/z 190, m/z 180, m/z 167 and m/z 153 could probably be the same as that of impurity-1. The proposed fragmentation pathway for the ion at m/z 235 was understood as follows: The loss of valeroyl side chain moiety with amino group (-$C_5H_{11}NO$) led to the formation of a stable ion at m/z 235 due to inductive effect.

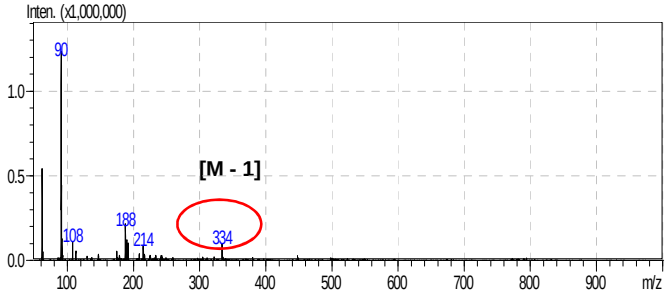
MS scan in +ve ionization mode



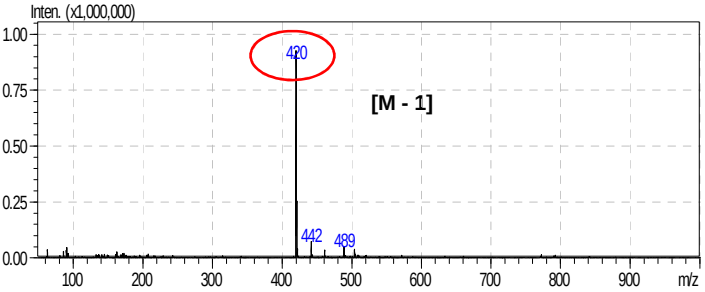
MS scan in +ve ionization mode



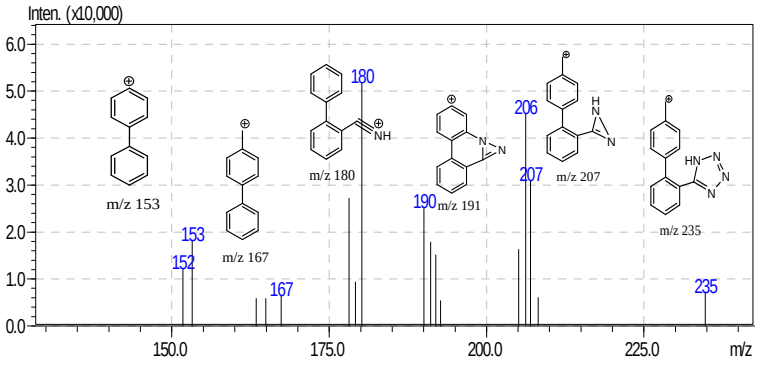
MS scan in -ve ionization mode



MS scan in -ve ionization mode



MS/MS of m/z 336 in +ve ionization mode



MS/MS of m/z 422 scan in +ve ionization mode

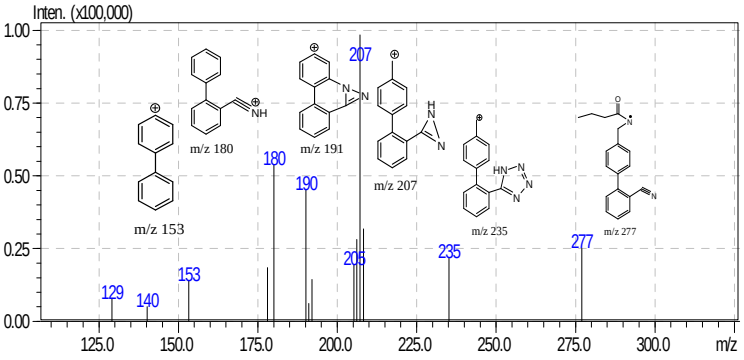


Fig. 3: MS and MS/MS mass spectra Impurity-2

Fig. 4: MS and MS/MS mass spectra of Impurity-3

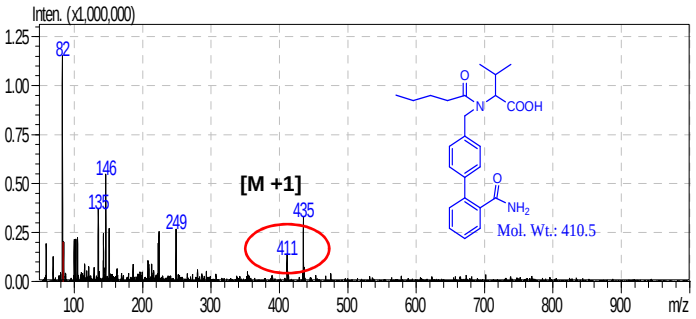
Impurity-3

The observed concentration of impurity-3 was 0.17% with respect to valsartan peak (by UV area ratio). This is a known impurity as referred in USP. The structure of this impurity was shown in Fig. 4. Though it is a known impurity, we have explained the mass spectral pattern to confirm the presence of this impurity in our synthetic process. The observed precursor ion of this impurity in the positive mode was at m/z 422 [M+1], confirmed in the simultaneous negative mode analysis as m/z 420 [M-1]. The major product ions were at m/z 277, m/z 235, m/z 207, m/z 190, m/z 180, and m/z 153, similar to the mass spectrum of impurity-1 & 2. Therefore, the fragmentation pathway of the major ions at m/z 235, m/z 207, m/z 180 and m/z 153 could probably be the same as that of impurity-1 & 2. The proposed fragmentation pathway for the ion at m/z 277 is discussed below. This USP impurity forms due to the interaction of trace level of butyroyl chloride which may be present as related substance in one of the reagents, namely valeroyl chloride. Therefore, the characteristic loss of the function group valine and the rearrangement of tetrazole group (as explained earlier) forming cyanide group at the biphenyl ring led to the presence of the radical at m/z 277.

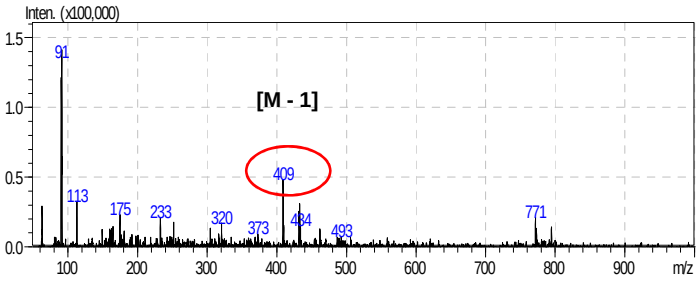
Impurity-4

The observed concentration of impurity-4 was 0.02% with respect to Valsartan peak (by UV area ratio). This impurity was expected to be formed when the trace level of unreacted cyano compound was present in the final reaction. The probable structure of this impurity was derived based on interpretation of MS/MS data generated at this very low concentration. The MS and MS/MS spectra were as shown in Fig. 5. The observed precursor ion of the impurity in the positive mode was at m/z 411 [M+1], confirmed in the simultaneous negative mode analysis as m/z 409 [M-1]. The major product ion was at m/z 210. The fragmentation pathway for this ion was quite simple to explain. The loss of valine and valeroyl side chain at amino bond led to the formation of the ion at m/z 210 due to inductive effect.

MS scan in +ve ionization mode



MS scan in -ve ionization mode



MS/MS of m/z 411 in +ve ionization mode

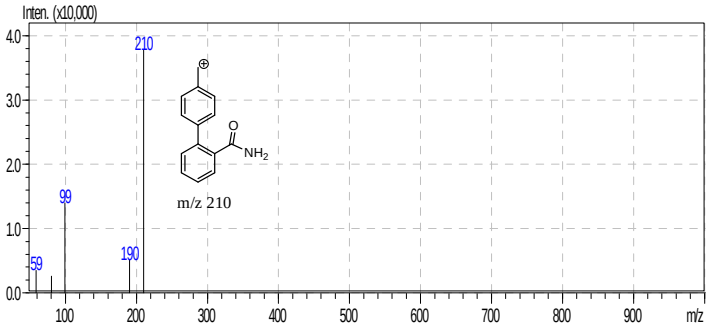


Fig. 5: MS and MS/MS mass spectra of Impurity-4

Conclusion:

The guidelines provided by regulatory authorities such as USFDA, PMDA , EMA, ICH, WHO, TGA, MHRA and others specify that the related substances, degraded products or impurities have to be completely characterized if their concentrations are greater than 0.10%. Till date, no regulatory authorities have ever set the guidelines to characterize the impurities if they are below 0.10%. This is probably due to the limitation of the current technology to characterize the impurities below this concentration on as-is basis. Now due to advancements happened in the technology of mass spectrometry in enhancing the scanning rate with minimum dwell time, we have demonstrated for the first time that trace level impurities down to 0.01% can be fully characterized using Shimadzu triple quad LCMS-8030 system.

Abbreviations:

USFDA : US Food and Drug Administration
PMDA : Pharmaceuticals and medicinal devices agency
EMA : European Medicines Agency
ICH : International Conference on Harmonization
WHO : World Health Organization
TGA : Therapeutic Good Administration
MHRA : Medicinal and Healthcare Products Regulatory Agency

References:

1. Samir Ghosh, A. Sanjeev Kumar and G.N. Mehta, Beilstein Journal of Organic Chemistry, 6, No.27, 2010
2. Wei Liu, Yanling Guo, Cong Han, Xiaonan Huang, Life Science Journal, 5, 2, 2008, 2
3. Romina M.Bianchini, Patricia M. Castellano, Teodoro S. Kaufman, J Pharma. And Biomed. Analysis, 56, 2011, 16

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