Identification & Confirmation of Structurally Related Degradation Products of Simvastatin

Power of QTRAP® Systems for Identification and Confirmation of Degradation Products

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Qualitative and quantitative determination of structurally related impurities have become an essential part of the product development process within the pharmaceutical industry. It is necessary to fully characterize and identify structurally similar impurities in final products because they may be toxic or pharmacologically active.

Chemical stability of drug molecules is of prime importance as it can affect the safety and efficacy of the drug products. Forced degradation is a process which involves degradation of drug products at conditions more severe than typical, which accelerates the generation of structurally related degradation products, thereby providing crucial information on the stability of the molecule. The process of impurity identification is often carried out by a combination of liquid chromatography (LC) with UV/PDA detection along with mass spectrometry (MS) to achieve resolution, specificity and sensitivity.

Described here is a robust strategy on QTRAP® systems for simultaneous identification, characterization and quantification of structurally similar impurities from a forced degradation study of Simvastatin.

Key Feature of QTRAP® Systems for Impurity Profiling

- Single hybrid platform for qualitative and quantitative analysis
- Data dependent workflows with multiple survey scan options for both untargeted (EMS -full scan MS) and targeted detection (Precursor ion and Neutral loss scans) of potential impurities. Detection triggers acquisition of a full scan MS/MS (EPI) with very high sensitivity.
- Easy to use structure relation establishment software tools for MS/MS fragmentation interpretation and unknown compound identification using PeakView® Software for data review
- Report results for individual and total impurity levels in % peak areas from UV-PDA data.

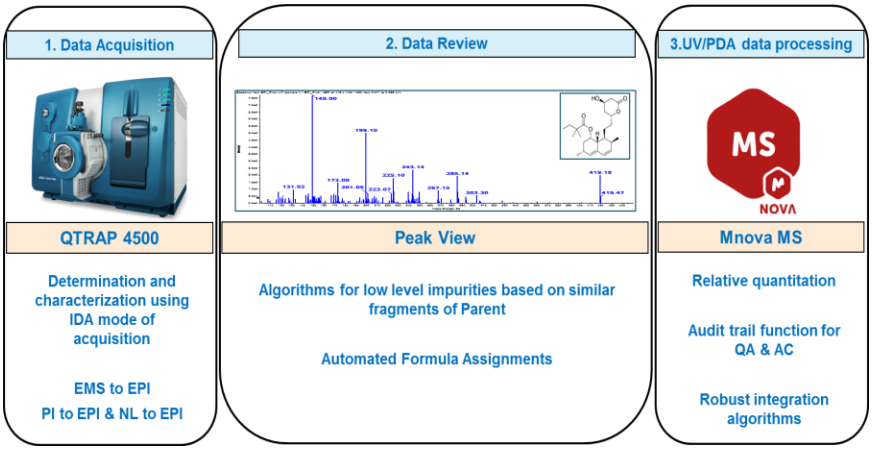

Figure 1. Impurity Profiling Workflow. Data Acquisition using QTRAP® 4500 system, both untargeted survey scans (EMS) and targeted survey scans (Precursor ion PI and Neutral loss NL scans) were used to trigger EPI (full scan MS/MS) data for identification. Data processing was performed using PeakView® Software to identify the components. Finally, UV/PDA data was processed using Mnova.

Methods

Sample Preparation: Simvastatin (10 mg x10 Nos) tablets were dissolved in a solution of 0.3 % acetic acid and 80:20 acetonitrile/water (diluent) to produce a 0.1 mg/ mL solution. The solution was subjected for forced degradation by adding 0.01 N HCl and incubated for 2 hours at room temperature. The solution was neutralized by addition of Ammonium bicarbonate solution and diluted to 0.025 mg/mL with diluent.

Chromatography: Sample analysis was performed by using Shimadzu XR Prominence system equipped with UV detector. 5 mM ammonium formate pH 4.5 as mobile phase A and Acetonitrile gradient was used as mobile phase B on Phenomenex Kinetex C18, 2.1 X 100 mm, 2.6 µm column. The flow rate was 0.6 mL/min and the column heated at 40ºC. Shimadzu UV detector was set at 238 nm.

Mass Spectrometry: QTRAP® 4500 system was operated in positive electrospray mode using a Turbo V™ Source. The data acquisition method consisted of an Enhanced Mass (EMS) survey scan (m/z 100 to 1000) followed by Enhanced Product Ion (EPI) dependent scans (m/z 100 to 1000). The EMS scan data was used for identification of impurities which then triggered the acquisition of EPI data (full scan ion trap MS/MS) for confirmation and structure elucidation of related and unknown impurities. In addition, precursor ion scans (PI) and neutral loss scans (NL) were also used as survey scans to detect structurally related compounds. In all data dependent experiments, Dynamic Background Subtraction (DBS) was used to reduce acquisition of background MS/MS.

Data Processing: Data was processed using PeakView® Software 2.2. For the UV data processing Mnova has been used, which has the capability of visualization of UV/PDA data, its integration, background subtraction, display of mass spectrum, peak purity and generating reports. Processing can be done in batch mode.

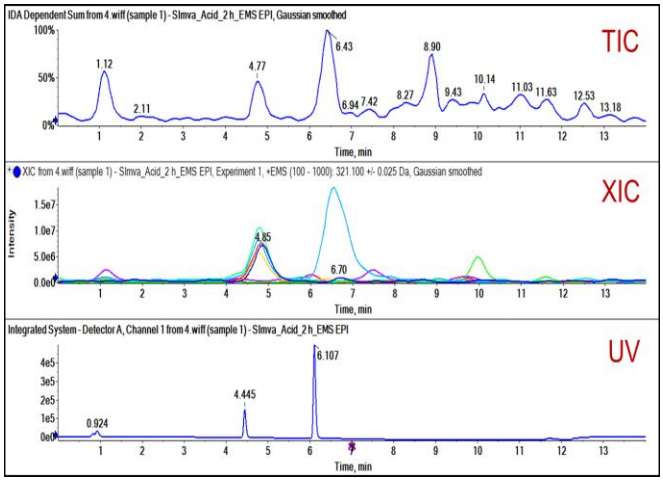

Figure 3. Profiles of Simvastatin Sample. Total Ion chromatogram (TIC), extracted ion chromatogram (XIC) of major degradation products and UV profile of Simvastatin (Acid hydrolyzed) sample.

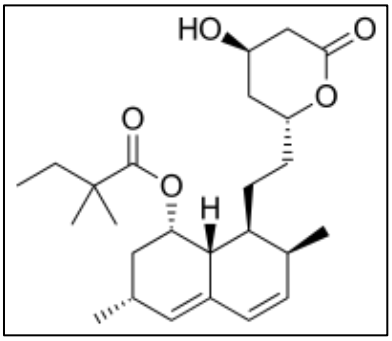

Figure 2. Simvastatin Structure. Simvastatin a hypolipidemic drug belonging to the class of pharmaceuticals called statins, is chemically designated as [(1S, 3R, 7R, 8S, 8aR)-8-[2-[(2R, 4R)-4-hydroxy-6-oxo-oxan-2-y1l] ethyl]-3,7-dimethyl-1, 2,3,7,8,8a-hexahydronaphthalen-1-y1] 2,2-dimethylbutanoate. It is used for the treatment of hypercholesterolemia. Following conversion of this lactone prodrug to its hydroxyl acid form, the compound is a potent competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis.

Table 1. List of Simvastatin Impurities Observed in EMS Spectra.

Compound Name	Observed m/z
Simvastatin Lactone Diol	*321.21*
Anhydro Simvastatin	*401.27*
Lovastatin	*405.26*
Simvastatin	*419.26*
Simvastatin Acid	*437.29*
Simvastatin Acetate	*461.29*
Simvastatin Dimer (Ammoniated)	*854.70*
Unknown-1	*464.34*
Unknown-2	*482.40*

Detection of Major Simvastatin Impurities

The present study was carried out to demonstrate the unique workflows of the QTRAP mass spectrometer for impurity and degradant analysis. The MS data combined with the UV data is key for confident detection, identification and quantitation (Figure 3). Analysis of the MS data provides a list of major impurities found due to the forced degradation process (Table 1).

Confirmation of the Major Impurities from the Non-Targeted Experiment

Analysis of the UV data enables the quantitation of the relative % of the Simvastatin impurities. Figure 5 (top) illustrates the analysis of the UV data by Mnova MS Software which computes the relative % to the main product based on area. The MS spectra extracted at the retention times of the major UV peaks (0.91, 4.46 and 6.9 mins) is shown (bottom). The presence of other MS peaks eluting at the same RT of the Simvastatin (RT: 6.118) suggests co-eluting impurities, which was confirmed by comparing the MS/MS pattern of simvastatin & the coeluting impurity (Figure 4). The comparison suggests these are many similar product ions present in the coeluting impurity in comparison to simvastatin product ions. Simvastatin [M+H]+ is seen in the spectrum (m/z – 419 annotated with arrow) along with other intense peaks at m/z 441 [M+Na]+ and 457.18 [M+K]+. The spectrum of the impurities eluting at RT 0.91 and 4.46 are also shown in Figure 5.

The structure of Simvastatin was imported into the fragmentation prediction tool of PeakView software. PeakView automatically compares the acquire MS/MS spectrum with a theoretical predicted fragmentation pattern to identify potential fragment structures. (Figure 6). The major observed fragments were 303, 285 & 199 which corresponds to the loss of the ester chain from simvastatin and subsequent water losses.

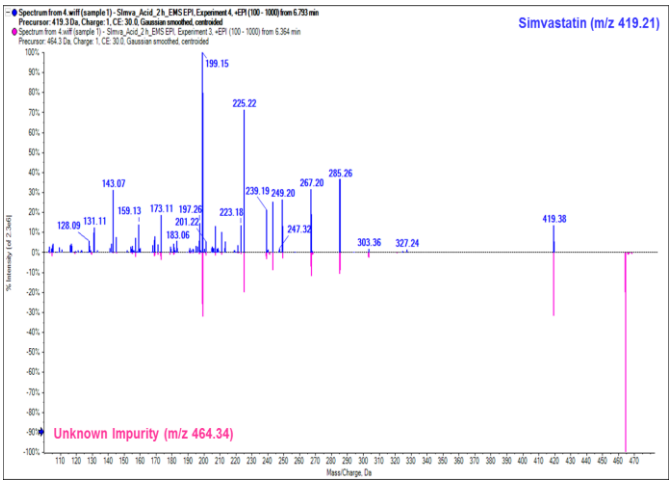

Figure 4. Comparison of MS/MS Simvastatin (m/z 419.21) to Coeluting Impurity (464.34). MS/MS matching shows majority of fragment ions are the same, suggesting a structurally related degradation product.

Using the same structural identification workflow, the Simvastatin lactone diol impurity, m/z 321, was confirmed (Figure 7).

Thus, this workflow provides the advantage of not only identifying the masses of the degradation products but also generates the fragmentation pattern of the identified molecules in a single LC-MS run which can be used for the further confirmation of any structural similarity.

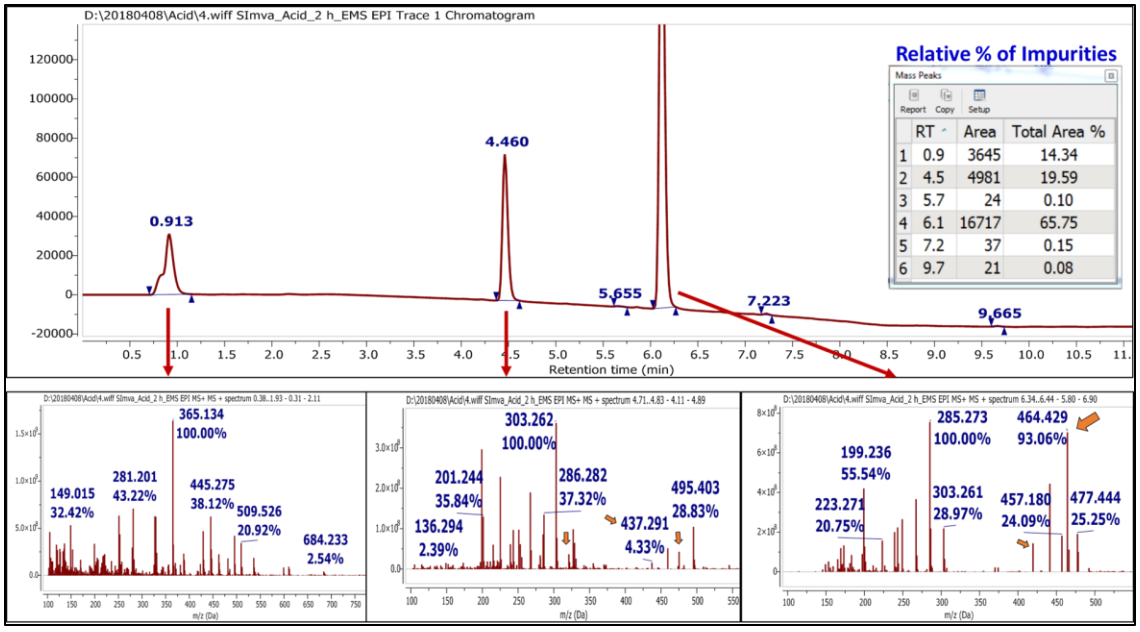

Figure 5. UV Data Processing by Using Mnova MS Software. UV signals from the major components are shown (top) and the relative % based on UV area is computed (inset). (bottom) The MS spectra extracted at the retention times of the major UV peaks (0.91, 4.46 and 6.19 mins) are shown.

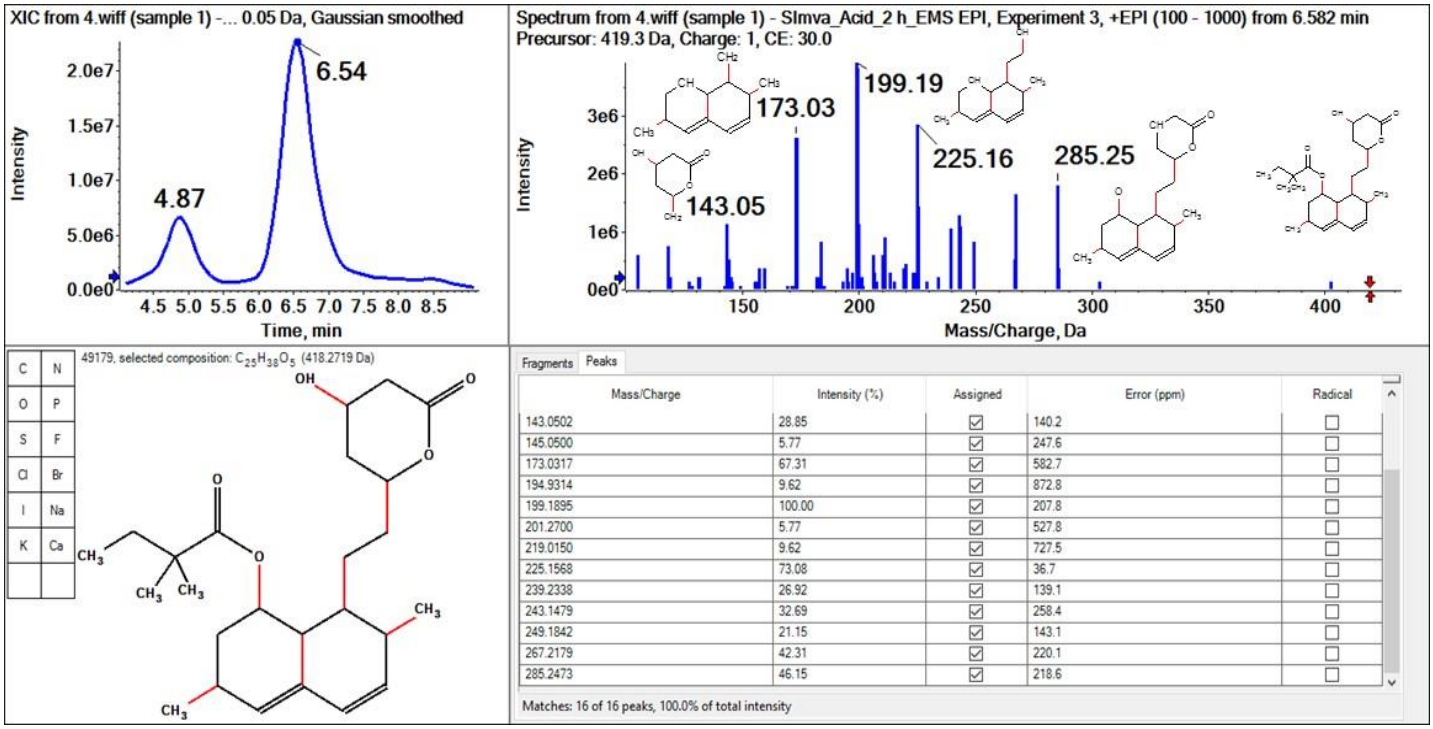

Figure 6. Identification of Common MS/MS Fragments from Simvastatin. The high-quality MS/MS spectrum of simvastatin generated using the EMS to EPI workflow was compared with the theoretical product ion spectra generated using simvastatin's mol file in the PeakView software and compared for similarity check.

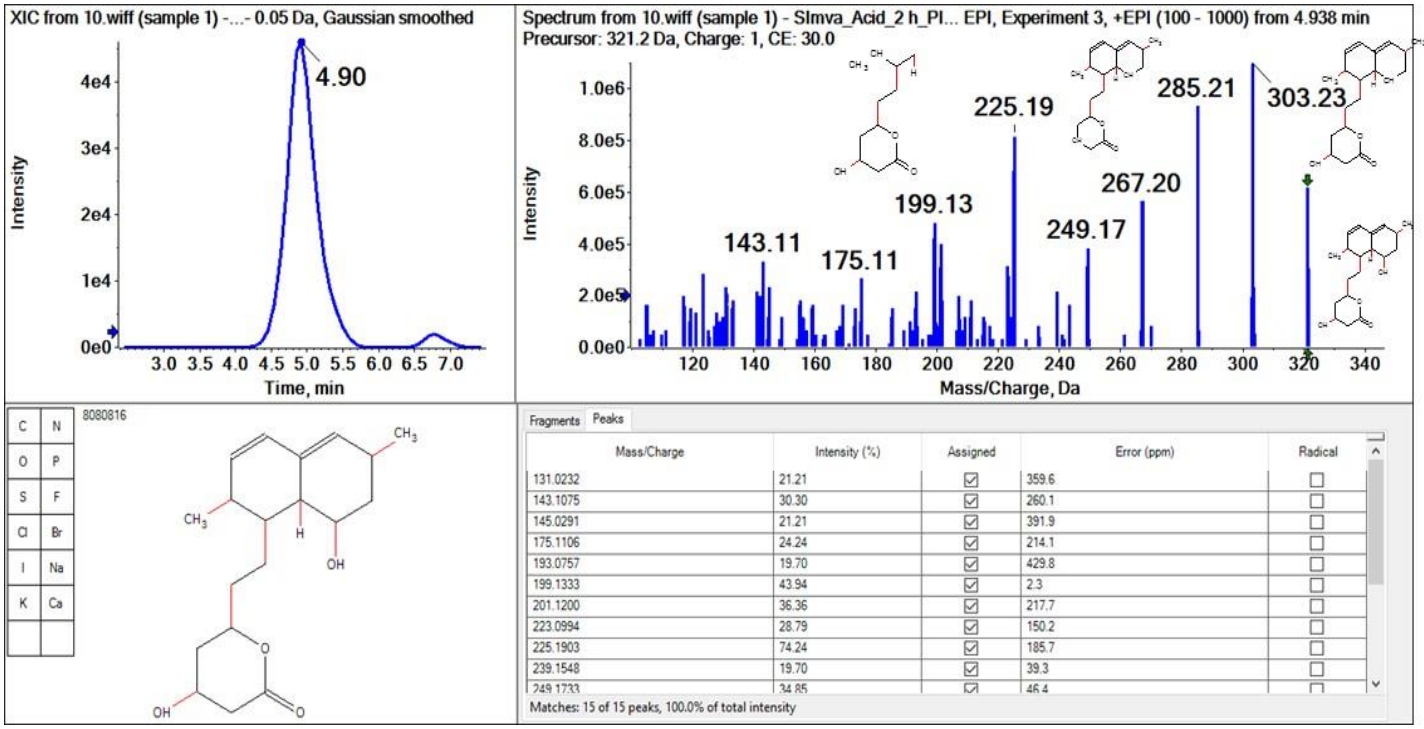

Figure 7. MS/MS Confirmation of Structurally Related Known Impurity (Simvastatin Lactone Diol). The high-quality MS/MS spectrum of simvastatin lactone Diol generated using the EMS to EPI workflow was used compared with the theoretical product ion spectra generated using mol file in PeakView software and compared for similarity check.

Targeting Specific Impurity Types with PI and NL Experiments

One advantage of using the QTRAP system for this application is the potential to run very selective triple quadrupole scans like precursor ion/neutral loss scans to not only identify the molecule but also for the simultaneous generation of product ion spectra, which can be used for structural confirmation or identification of structural similarity with a parent molecule. Here the unique configuration of the QTRAP can be leveraged, specifically the ability of the instrument to switch between triple quadrupole mode and ion trap mode in < 1msec (TripleTrap™ Scanning). This allows for very powerful, novel workflows to be constructed for the identification of structurally related molecules present in a sample.

The precursor ion and neutral loss triggered MS/MS experiments used for analysis of the force degraded samples of simvastatin further confirmed the degradation products identified from EMS to EPI. Figure 8 shows the PI to EPI spectrum. Even though the m/z 437 is observed in EMS to EPI scan at a very low intensity level the selective scan functions like PI to EPI and NL to EPI could further confirm this identification clearly suggests the presence of m/z 437. Figure 9 shows how Mnova integrates UV data with both the PI and the NL based acquisitions. Figure 10 shows the comparison of fragmentation pattern on m/z 437 vs Simvastatin fragmentation.

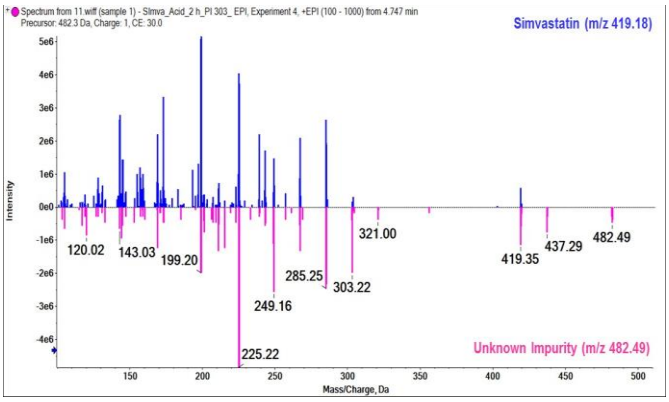

Figure 8. Comparison of MS/MS Simvastatin to Impurity Detected from Precursor Ion Scan Experiment. MS/MS matching shows many similar fragment ions are the same, again suggesting a structurally related degradation product.

Hence these selective workflows can provide vital information on the low-level degradation products.

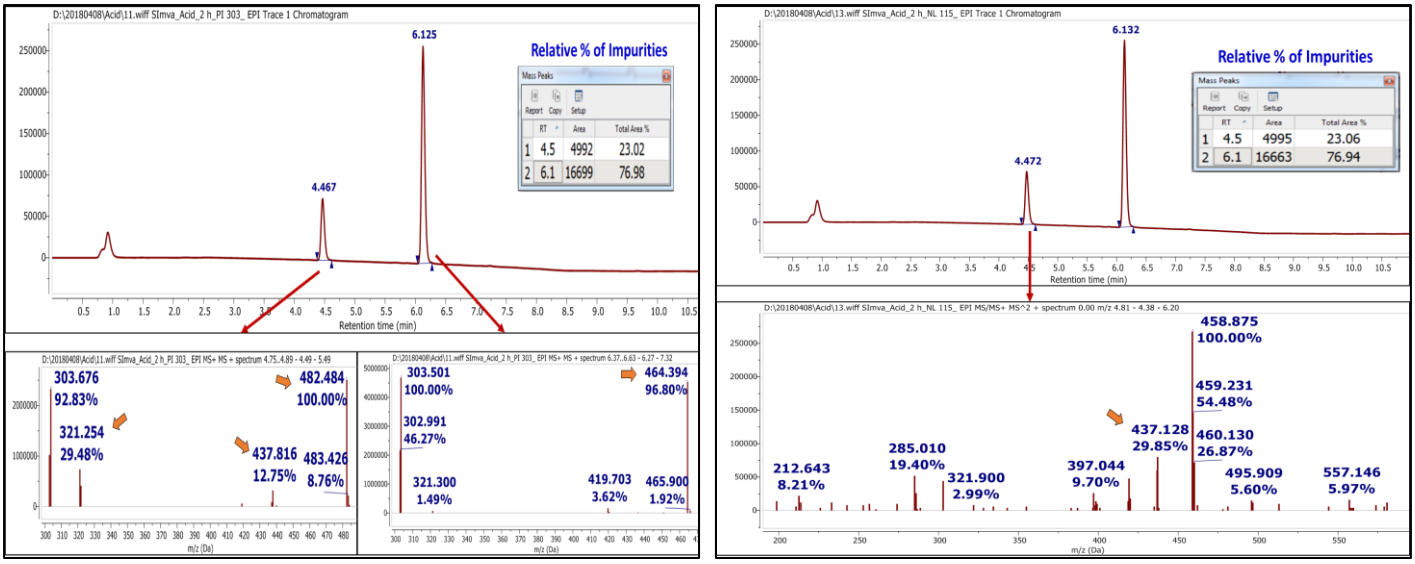

Figure 9. UV Data Processing for the Targeted Experiments. (Left) UV data for the precursor ion scan experiment using a target fragment ion of m/z 303. (Right) UV data for the neutral loss scan experiment using a target neutral loss of mass 115.990. Targeted scans such as these will specifically drive the acquisition of MS/MS on compounds that have related structural elements.

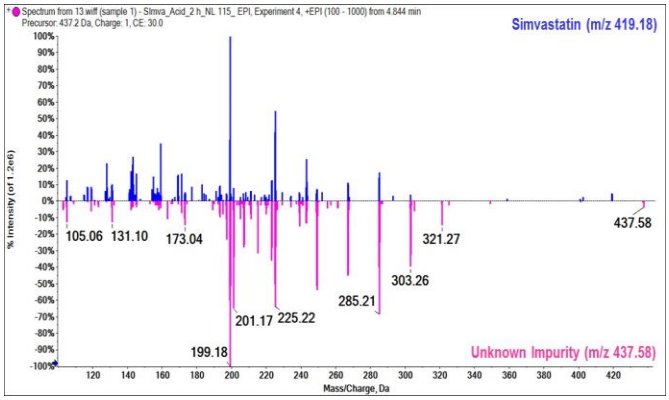

Figure 10. Comparison of MS/MS Simvastatin to Impurity Detected from Neutral Loss Scan Experiment. MS/MS matching shows many similar fragment ions are the same, confirming detection of Simvastatin Acid.

Conclusions

This study demonstrates the potential of QTRAP 4500 system for degradation/impurity analysis with high sensitivity and fast scanning enabled detection of degradation products. Selective scan modes such as Precursor Ion scan to Enhanced Product Ion scan and Neutral Loss scan to Enhanced Product Ion scan were effective in detecting the degradation product at low levels. These modes demonstrated high level of selectivity and sensitivity for low level degradation product identification

Information Dependent Acquisition workflows allowed generation of both MS and MS/MS data in a single run with a high level of sensitivity which helped in identification and confirmation of the degradation products in a single LC MS run. Further the MS/MS data helped provide structural similarity confirmation.

MNova software can support UV/PDA data analysis in tandem with LC/MS/MS by associating MS and MS/MS data with UV/PDA chromatographic peaks.

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