[APPLICATION NOTE]

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IDENTIFICATION OF THE IMPURITIES OF SIMVASTATIN USING EXACT MASS UPLC/MS

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INTRODUCTION

The rapid detection and characterization of the impurities of drug manufacturing or synthesis process play a central role in drug development, during development scale-up or during primary manufacturing. These impurities may arise from unwanted side reactions, reactions with the solvents/catalysts, from unreacted intermediates, or resulting from reactions of impurities in the original starting materials.

It is necessary to fully characterize and identify all of the impurities in the final product as these may be either toxic or pharmacologically active. Also, from a patent protection point of view, it is necessary to understand these side reactions as they are diagnostic of the manufacturing process. The failure to detect and identify all impurities in a timely manner can result in significant, costly delays. These delays may result in the late shipment of a productions batch, hold up in production due to the time taken in raw material acceptance, or may result in delaying the drug development process during the manufacture of bulk material for safety and formulation testing. All could be extremely costly. Therefore it is necessary to have an analytical solution that allows rapid, accurate identification of any impurities in a batch of material.

The process of impurity identification is often carried out by a combination of liquid chromatography and mass spectrometry. The need to resolve and detect many components requires a high resolution LC methodology that often results in time-consuming analysis times with traditional chromatography systems. In order to characterize these impurities, it is often necessary to perform several analytical runs to obtain the necessary MS and MS/MS data.

The ability to obtain this information in one analytical run increases efficiency; however, data-directed MS/MS often results in the loss of data for closely eluting peaks.

Figure 1. The ACQUITY UPLC system with the Q-Tof Premier mass spectrometer.

UltraPerformance LC® (UPLC®) combined with Q-Tof Premier™ exact mass MS, operating with alternating low- and high-collision energies (MS^E), has been successfully employed for the identification of drug and endogenous metabolites. The rapid switching of the collision cell energy produces both precursor and product ions of all of the analytes in the sample while maintaining a sufficient number of data points across the peak for reliable quantification. This MS capability, when combined with the high resolution generated by the UPLC system, allows for rapid and unbiased analysis.

EXPERIMENTAL

LC Conditions

LC system:	Waters ACQUITY UPLC system
Column:	ACQUITY UPLC BEH C_{18} column 2.1 x 100 mm, 1.7 µm
Column temp.:	30 °C
Flow rate:	600 µL/min
Mobile phase A:	15 mM Ammonium acetate, pH 4.5
Mobile phase B:	Acetonitrile
Gradient:	50 to 95% B/7 min
Injection volume:	2 µL, PLUNO mode

MS Conditions

MS system:	Waters Q-Tof Premier mass spectrometer
Ionization mode:	ESI positive / ESI negative, V-mode
Capillary voltage:	3200 V
Cone voltage:	35 V
Desolvation temp.:	450 °C
Desolvation gas:	800 L/Hr
Source temp.:	120 °C
Acquisition range:	100 to 1000 m/z
Collision energies:	High 25 V, Low 5 V

This approach of UPLC/TOF-MS was applied to the analysis of the impurities of simvastatin tablets from four different manufactures. Simvastatin is a hypolipidemic drug belonging to the class of pharmaceuticals called statins. It is used to control hypercholesterolemia (elevated cholesterol levels) and to prevent cardiovascular disease. Simvastatin is a synthetic derivate of a fermentation product of *Aspergillus terreus*.

In this study, the 10 tablets from each manufacturer were dissolved in a solution of 0.3% acetic 80:20 acetonitrile/water to produce a 0.1 mg/mL solution. This was filtered and stored refrigerated prior to analysis. The resulting LC/MS data was analyzed using the exact mass tools within MassLynx™ software.

RESULTS

The high resolving power of the ACQUITY UPLC system allowed for the complete resolution of all of the impurities in the simvastatin samples, Figure 2. The main peak in the chromatogram eluted with a retention time of 3.12 minutes. The low-collision energy MS spectra of this peak showed that the exact mass of the major peak was 419.2797, giving an elemental composition of $C_{25}H_{39}O_5$, with a mass error of 1.0 ppm. This elemental composition corresponded to the parent compound simvastatin, Figure 3.

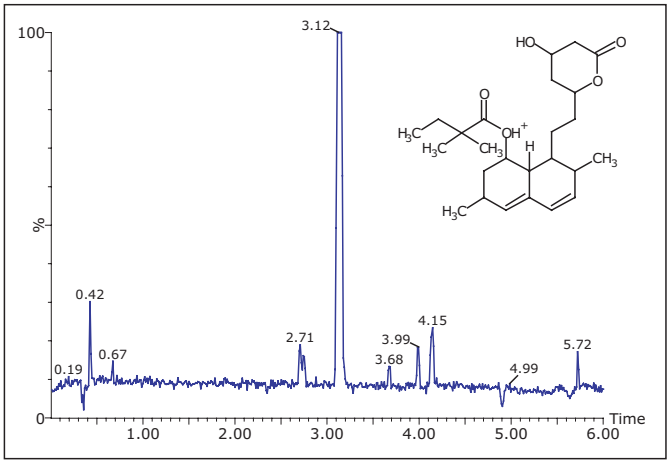


Figure 2. Impurities of simvastatin.

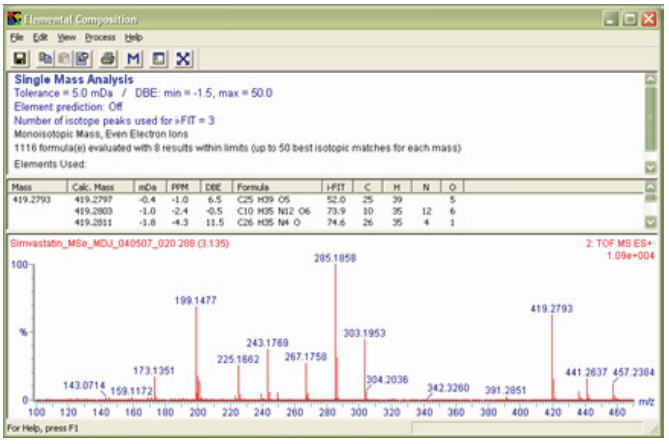
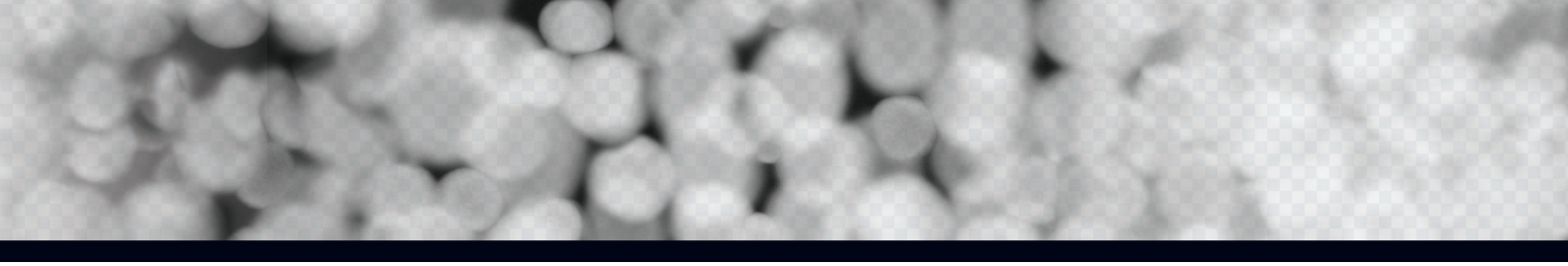


Figure 3. Elemental composition analysis of simvastatin.



The MS spectra revealed diagnostic fragment ions with the masses of 285.1858 and 303.1953. These fragment ions were easily rationalized by the loss of the butyl-ketone group from the two-ring system. Using this information, it was possible to filter the data using the Accurate Mass Filter tool in MassLynx to highlight all of the components in the sample related to simvastatin, Figure 4.

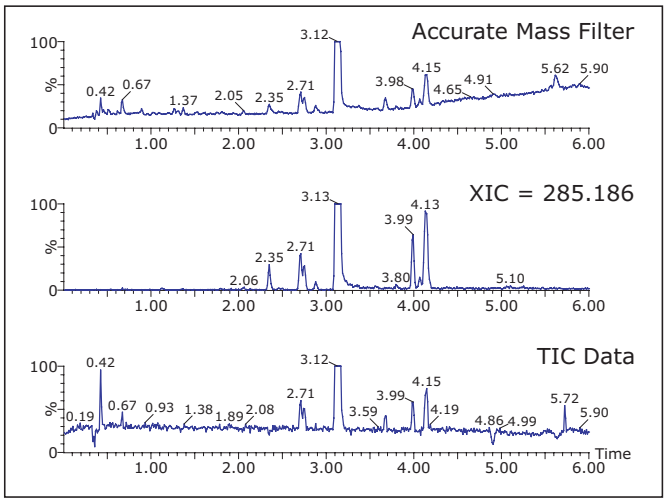


Figure 4. Highlighted components in the sample related to simvastatin.

Accurate mass filter

The accurate mass filter processes the data, removing all of the peaks from the data that do not contain peaks with a mass fraction within the preset limits +20 mDa and -15 mDa of 0.2797. We can see that when compared to the total ion chromatogram (TIC) data the filtered data signal reveals more information about related components in the sample, allowing for faster data review.

Impurity identification

The use of the low- and high-collision energy data allowed for the complete identification of the fragment ion and thus the structure of the impurities.

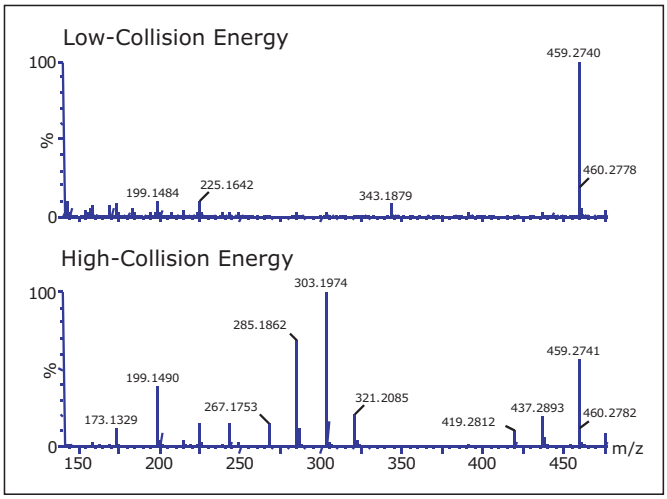


Figure 5. Low- and high-collision energy spectra.

The data in Figure 5 shows the low- and high-collision energy data from the peak eluting at 2.34 minutes. The two spectra illustrated how the low-collision energy data shows the precursor ion while the high-collision energy data give rise to the product ion spectrum.

The accurate mass data 449.2740 produced an elemental composition of $C_{27}H_{39}O_6$, which corresponds to the acid impurity of simvastatin, with a mass error of 1.3 ppm. The fragment ion pattern generated by the high-collision energy data confirmed that this peak was the acid impurity of simvastatin, Figure 6.

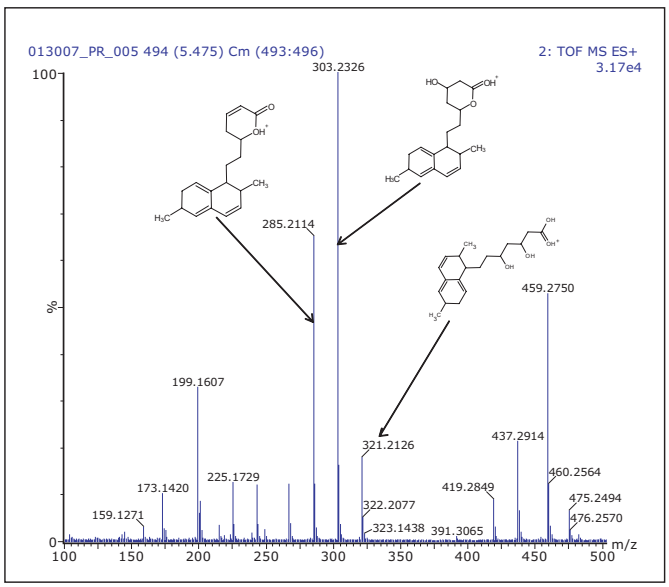


Figure 6. Assigned high-energy spectra of simvastatin acid.

The use of alternating collision energies to generate the fragment ions allows all of the ions to be produced under exact mass conditions, for easy interpretation of the fragmentation pattern. The peak eluting at 4.13 minutes gave rise to a spectrum with a peak of mass 401.2706, resulting in an elemental composition of $C_{25}H_{37}O_4$ with a mass error of 3.5 ppm, Figure 7.

Using this exact mass data and the fragmentation allowed this impurity peak to be assigned as the anhydro impurity of simvastatin. Using the exact mass data and the exact mass fragment ion information, it was possible to identify all of the components in the sample – with the exception of the peak eluting at 3.68 minutes.

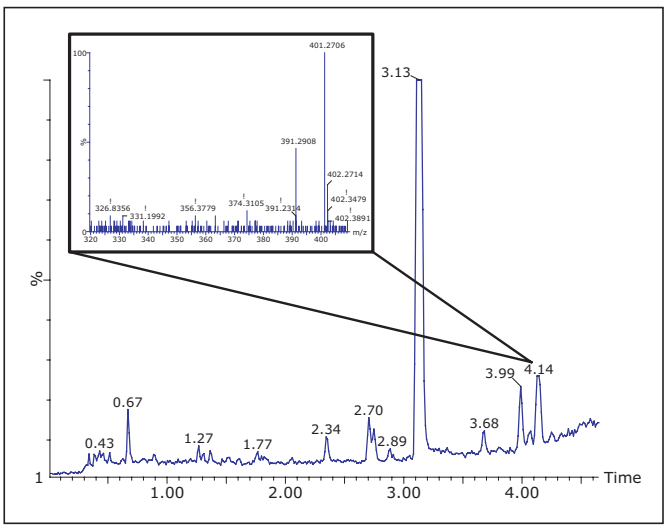


Figure 7. Chromatogram and specta of anhydro simvastatin.

Unknown impurity

The unknown impurity of simvastatin, eluting at 3.7 minutes, gave rise to a parent ion with the mass of 421.2942, Figure 8, producing an elemental composition of $C_{25}H_{41}O_5$. This value is two mass units greater than that of simvastatin.

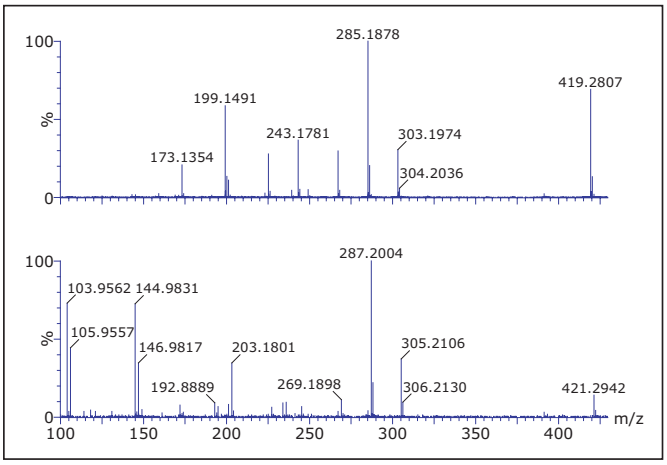


Figure 8: Spectra of simvastatin and the unknown impurity.

A careful review of the spectra showed that the fragment ions were also increased by two mass units to give m/z = 287 and 305. This impurity could be rationalized by the reduction of a double bond in the top cyclic ring in the anhydro-simvastatin acid impurity, by the oxidation of the ketone in the top ring in the parent simvastatin to form a hydroxyl group, or by the saturation of the fused double-ring system and formation of two ketones on the top-ring system.

The impurity identity was confirmed by the analysis of the sample in negative ion mode. When the sample was analyzed in negative ion mode, the simvastatin acid impurity increased in intensity, whereas all the other peaks showed reduced intensity – thus suggesting that the new peak was not the reduced version of the anhydro-simvastatin acid, but was either the oxidized ketone form of simvastatin or the saturated ring system form. The absence of a UV chromaphore for this impurity confirmed its identity as the saturated ring compound.

CONCLUSION

Impurity profiling and identification is an essential part of the drug development and formulation process. The sensitivity and flexibility of exact mass time-of-flight mass spectrometry with alternating collision cell energies, combined with the high resolving power of the ACQUITY UPLC system, allows for the rapid profiling and identification of impurities.

By using the approach of alternating collision cell energies in the Q-Tof Premier, it was possible to obtain both the precursor intact parent mass ion information and the fragment ion information under exact mass conditions. As this approach does not require the pre-selection of ions to obtain the MS/MS information, all the required data for structural elucidation can be obtained in one analytical run. The Accurate Mass Filter tool within MassLynx software provided a rapid means of simplifying the data set and highlighting related components in the data set.

The analysis of simvastatin impurities were detected and identified in one simple 7.0-minute analysis by using this approach. The unbiased collection of the data ensured that all the peaks in the sample were detected with a sufficient number of points across the peaks for accurate semi-quantitative analysis of the data. The exact mass data generated by the Q-Tof Premier, less than 3 ppm in V-mode, allowed for the easy identification of an unreported impurity of simvastatin, without the need for time-consuming re-analysis of the data.

The combination of the increased resolution and throughput of ACQUITY UPLC, with the ability of the Q-Tof Premier to collect precursor and product ion data in one analytical run, removes the need for multiple long analytical runs to acquire the required data to identify potential impurities. The data analysis and visualization tools within MassLynx software simplify the data analysis process. These factors combine to dramatically reduce the time to profile samples and identify impurities. Reducing the time required to characterize samples then allows for faster drug development, raw material acceptance, or product release.

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