

STRUCTURAL ELUCIDATION OF AN UNKNOWN IMPURITY PRESENT IN A SIMVASTATIN IMPURITY PROFILE UTILIZING SUB 2 μ m POROUS PARTICLE LC COMBINED WITH OA-TOF, MS/MS, AND MS^E

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Michael Jones, Emma Marsden-Edwards, Robert Plumb, Peter Alden, Paul Rainville, and Jose Castro-Perez
Waters Corporation, 34 Maple Street, Milford, Massachusetts, 01757, USA

INTRODUCTION

During the drug development process it is a regulatory requirement to fully characterize the impurity profile of the active pharmaceutical to ensure that sufficient toxicological coverage is obtained during the safety evaluation process [1,2]. The FDA guidelines for impurity analysis states that for pharmaceuticals dosed at levels greater than 1g/day any impurity greater than 0.1% of the API must be reported, those greater than 0.5% must be identified and any greater than 0.2% must be quantified [3]. For compounds dosed at a level up to 1g/day impurities that are present at levels in excess of 0.05% must be reported, those present at levels greater than 1% or 5 μ g must be identified and those present at 0.5% must be quantified.

Mass spectrometry is the primary technique used for the identification of impurities, with quadrupole, tandem quadrupole and ion trap mass spectrometer being particularly popular for the task of impurity analysis [4-5]. The analytical procedure usually required more than one analytical run to obtain the precursor and product ion information required to identify the chemical structure of the impurities. The first analytical run is carried out in full scan mode to obtain parent ion information, followed by a second analytical run in MS/MS mode to obtain fragment ion information. With the long analytical run times used in impurity analysis this can be a time consuming process. This process was streamlined even further by Bateman et-al in 2002 with the development of the use of alternating high-low collision energy in a hybrid quadrupole TOF instrument to provide both precursor and product ion information in one analytical run [6]. This approach was utilized for the analysis of drug metabolites in urine and *in vitro* samples providing rapid accurate identification of drug metabolites by generating accurate mass precursor and product ion data in one analytical run [7-8].

In this paper we describe the use of sub 2 μ m porous particle LC coupled with hybrid quadrupole time of flight mass spectrometry for profiling and identification of the impurities of the cholesterol-lowering medicine Simvastatin (20mg tablet). The combination of UV-MS data obtained from the forced degradation studies, exact mass data, and MS^E (high/low collision energies) will show how this technique provides a rapid comprehensive approach to the detection and identification of the impurities of the statin and allows for the facile structural elucidation of an unknown impurity.

EXPERIMENTAL

Materials:

United States Pharmacopoeia Simvastatin RS (Rockville, MD);

Reagents: Acetonitrile Optima; Fisher Scientific (Fairlawn, NJ); Lot#050580. Ammonium Acetate and acetic acid; Sigma-Aldrich (St. Louis, MO); .

UPLC Conditions

Instrument: ACQUITY UPLC[®]
Column: ACQUITY UPLC[®] BEH C₁₈
Dimensions: 100 x 2.1mm, 1.7 μ m
Mobile Phase: A1: 15mM Ammonium acetate pH 4.5
B1: Acetonitrile
Gradient: 25-50% B over 6 minutes
50-95% B from 6-9 minutes
Flow Rate: 0.80 mL/min
Temperature: 65°C
Injection Volume: 3 μ L; PLUNO injection mode
Detection: ACQUITY PDA @ 238 nm

MS Conditions

Instrument: Waters[®] Q-ToF Premier[™]
Software: Masslynx[™] 4.1

Tune Page Parameters:

Source: ES+
Capillary (V): 3.2
Sample Cone (V): 35 for reference
60 for analyte

Extraction Cone (V): 4.5
Desolvation Temp (°C): 350.0
Source Temp (°C): 120.0
Cone Flow (L/Hr): 0.0
Desolvation Flow (L/Hr): 800.0

ToF Settings

Acquisition Range: 100 - 800Da
Scan Time: 0.095s
Interscan delay: 0.005s
Lock mass: 300pg/ μ L leucine/enkephalin @ 30 μ L/min

MS^E settings

Low collision energy: 5eV
High collision energy: 25eV

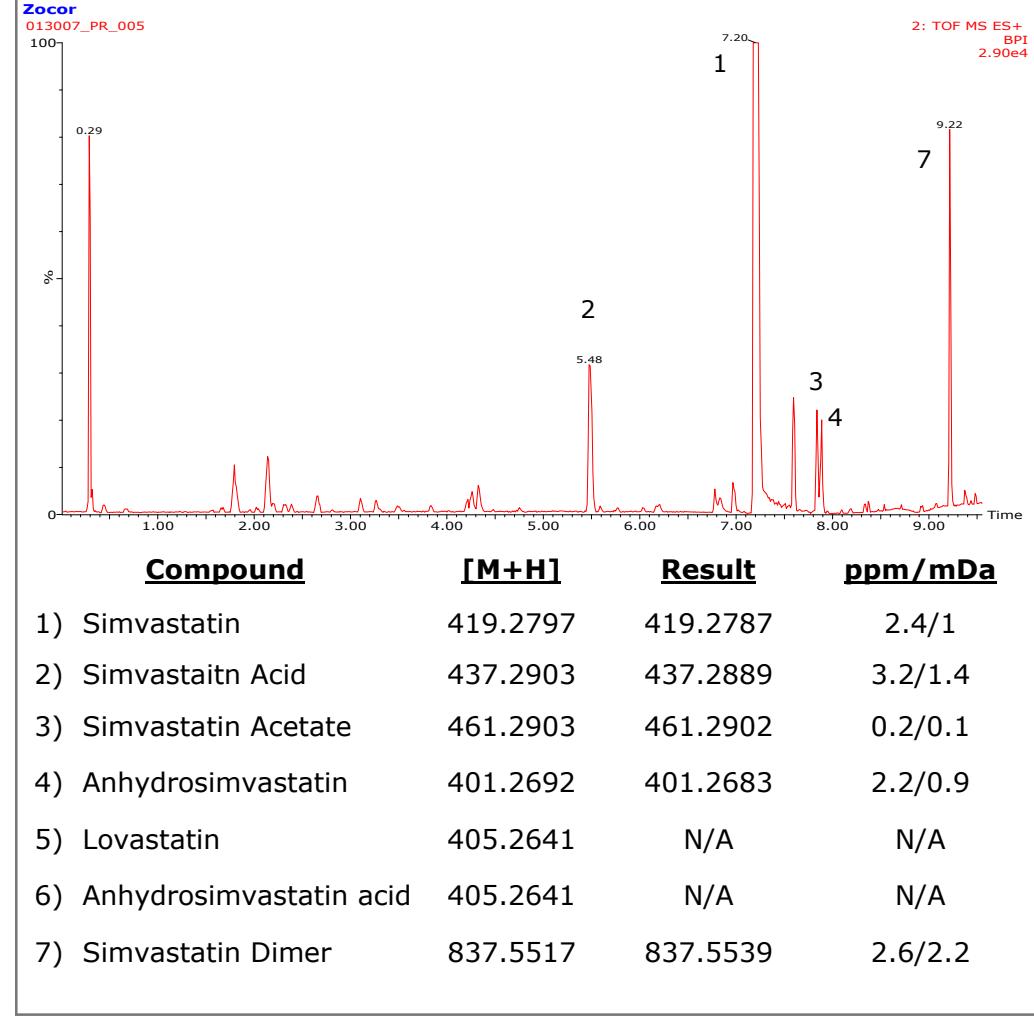
CONCLUSIONS

- Impurity profiling requires a high resolution high sensitivity analytical technique to ensure the detection and characterization of the samples
- New modern high resolution LC systems enable the rapid complete profiling of impurity samples.
- Combining these system with quadrupole MS instrumentation ensure the correct peak assignments based on the MS spectra
- The application of MS/MS and exact mass using MSE allows for the facile identification of the impurities of interest
- Beneficial to have orthogonal data sets such as UV(*and/or* NMR) in conjunction with MS to best confirm structural identity for unknown peaks

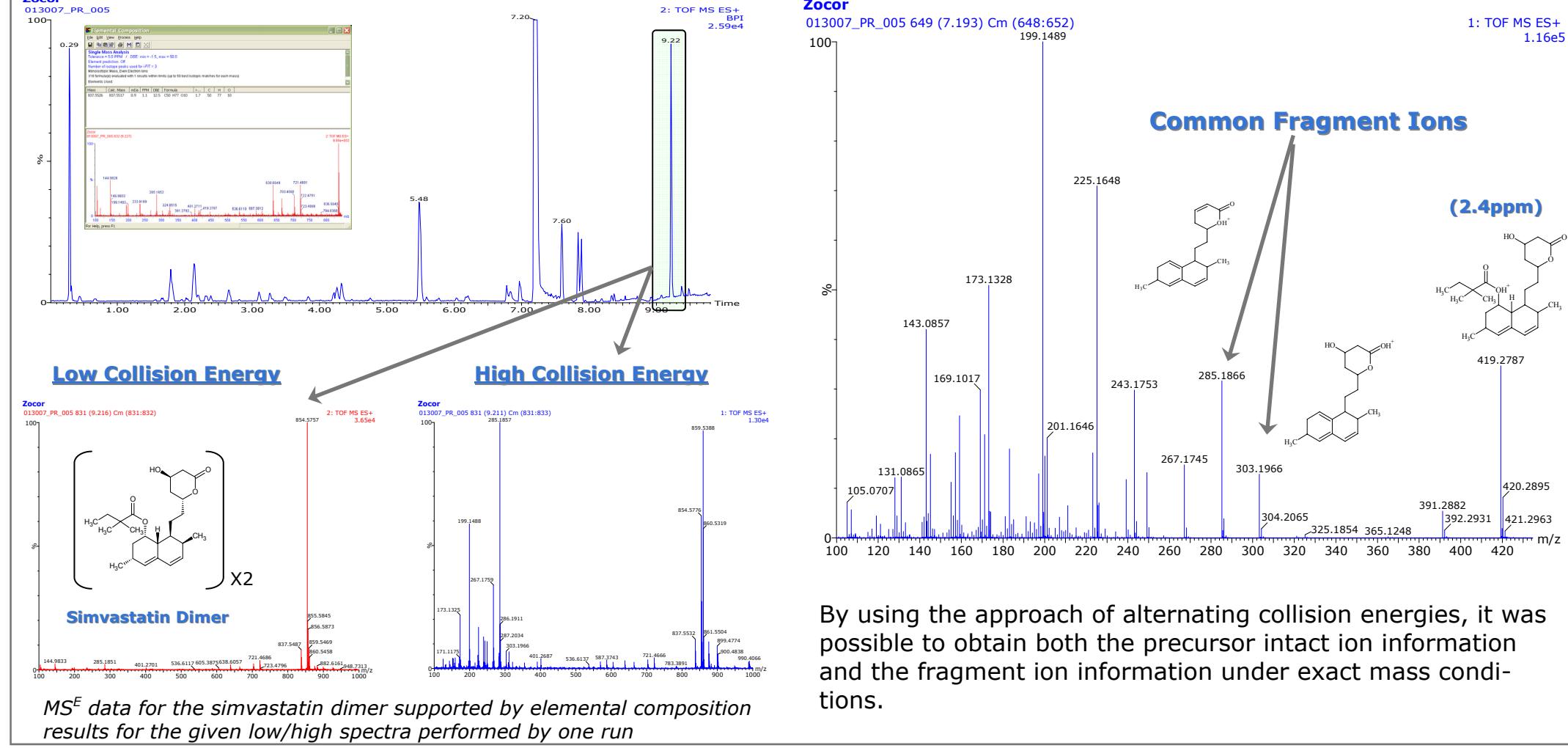
References

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Exact Mass



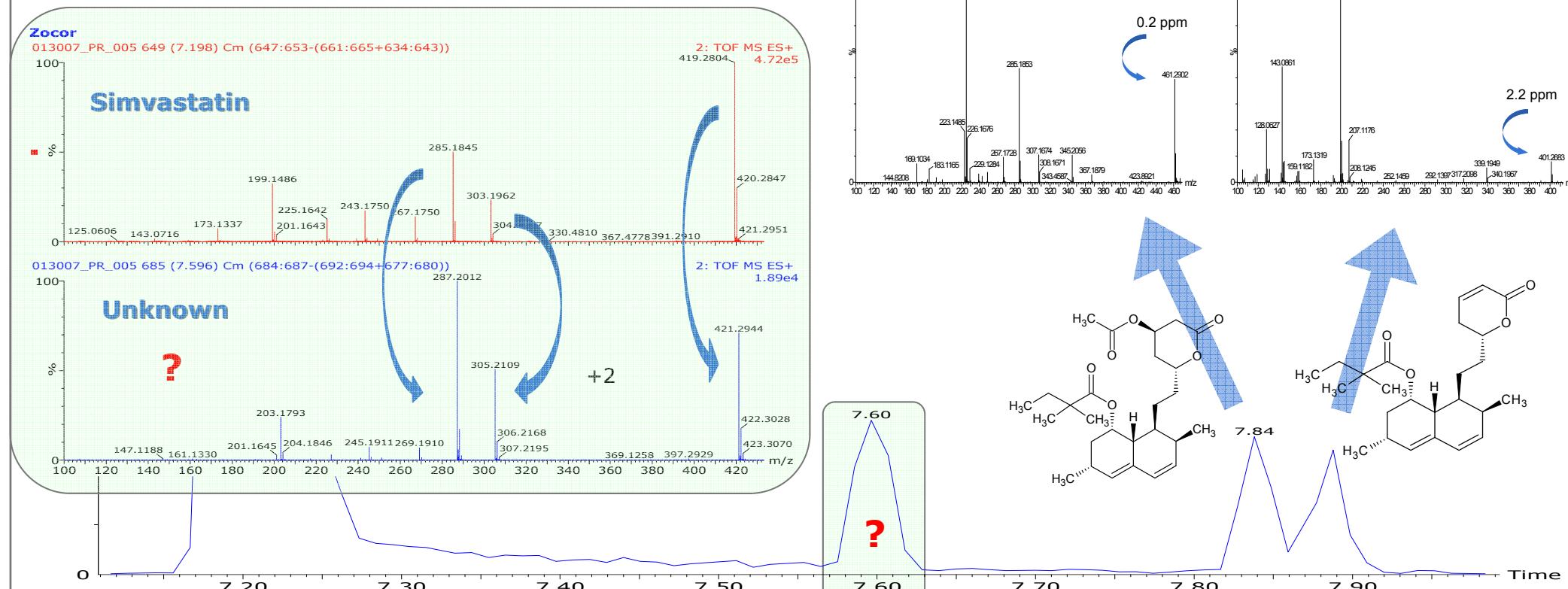
MS^E and the Identification of Common Fragments



By using the approach of alternating collision energies, it was possible to obtain both the precursor intact ion information and the fragment ion information under exact mass conditions.

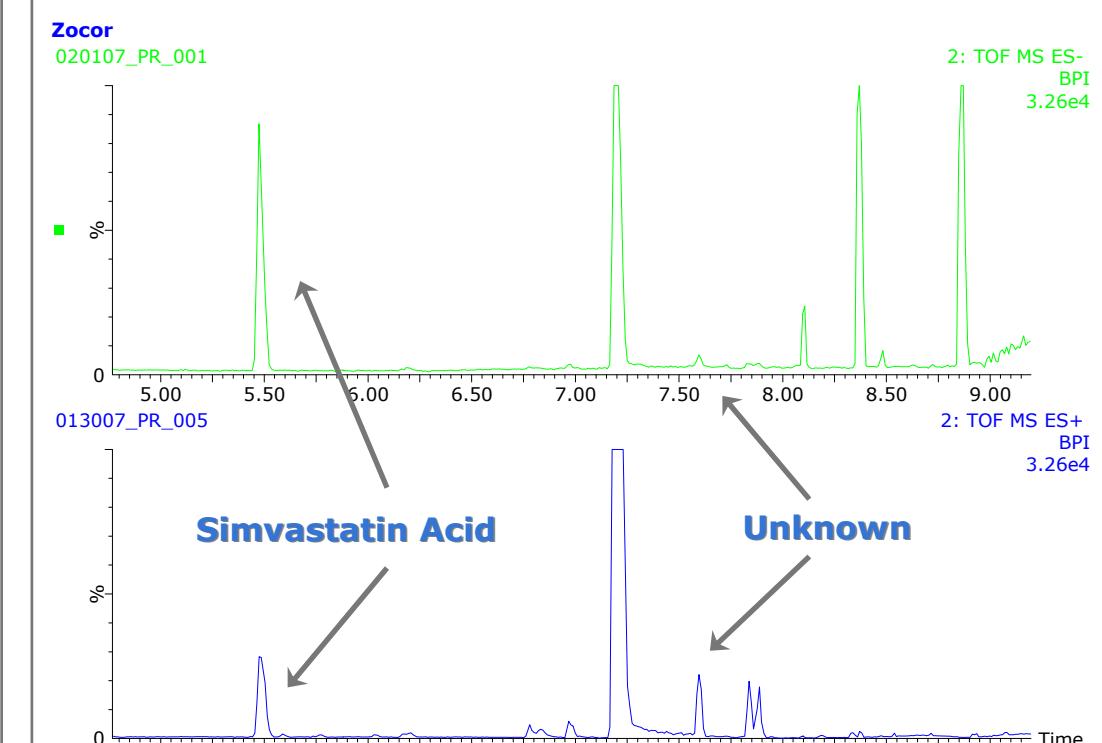
Unknown Impurity Determination

Confirmation of the known impurities and identification of common fragment ions allowed for fragmenting confidence in the data set and preliminary structural assumptions for the unknown impurity peak. The data below illustrates that it is most likely simvastatin related.

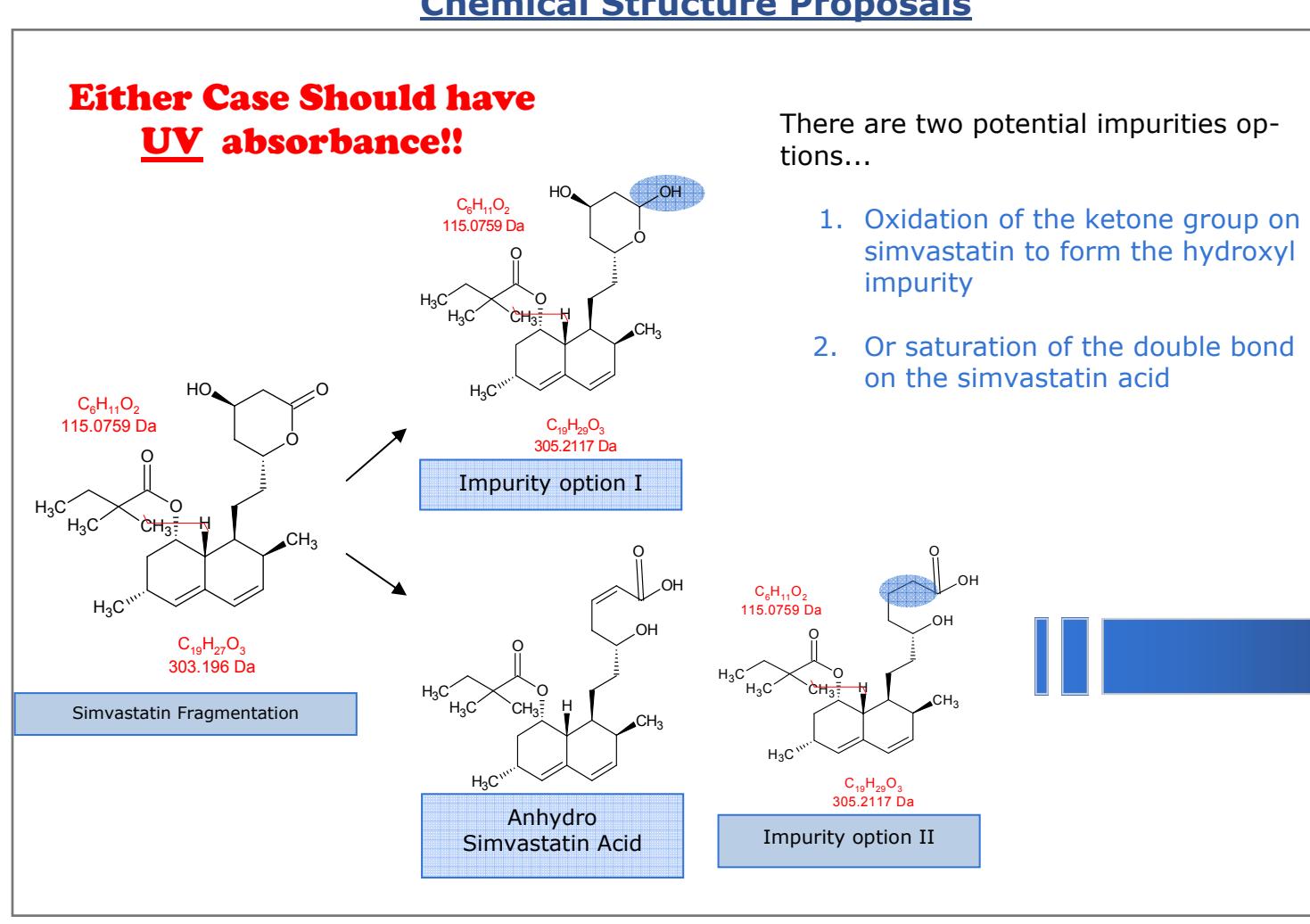


Pos/Neg Results

The analysis of the sample in negative ion ESI should result in an increased signal for an acid containing compound. A Hydroxyl containing compound should show a comparatively reduced signal in negative ion mode. Negative ion data confirmed the impurity as the hydroxylated form.

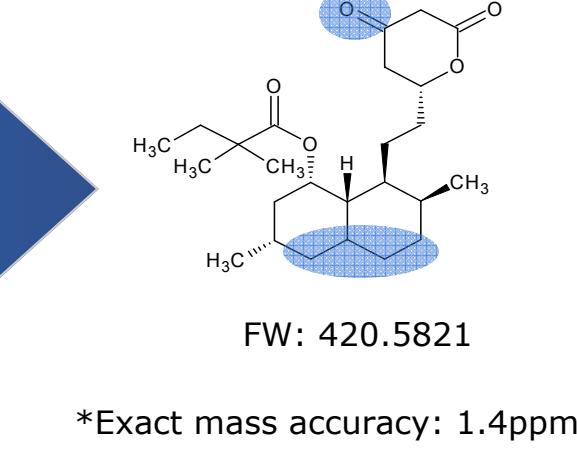


Chemical Structure Proposals



There are two potential impurities options...

- Oxidation of the ketone group on simvastatin to form the hydroxyl impurity
- Or saturation of the double bond on the simvastatin acid



...Looking back at the Forced Degradation study

