THE RAPID IDENTIFICATION OF THE IMPURITIES OF SIMVASTATIN USING UPLC® / Q-TOF™ TECHNOLOGY AND AN INTELLIGENT DATA MINING APPROACH

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INTRODUCTION

It is a regulatory submission requirement to fully characterize the impurities of the active pharmaceutical to ensure that sufficient toxicological coverage is obtained during the safety evaluation process [1,2]. The FDA require the following:

Pharmaceuticals dosed at levels greater than 1 g/day[3]:
Impurities > 0.1% of the API must be reported
> 0.5% must be identified
≥ 0.2% must be quantified
For dosing levels up to 1 g/day:
Impurities > 0.05% must be reported
> 1% or 5 µg must be identified
≥ 0.5% or more must be quantified

Liquid chromatography coupled to mass spectrometry is the primary technique used for the identification of impurities [4-5]. This normally requires more than one analytical run to obtain the necessary precursor (full scan MS) and product ion information (targeted MS/MS) required to identify the chemical structure of the impurities. This combined with the long analytical run times makes this a time consuming process.

Bateman et al. described a new approach to this problem of acquiring MS and MS/MS data using alternating high-low collision energy acquisition using 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].

Here we describe the use of sub-2 µm 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 (20 mg tablet). The combination of UV-MS data obtained from the forced degradation studies, exact mass data, and MS^E (high/low collision energies) was used to provided a rapid comprehensive approach to the detection and identification of the impurities and allows for the facile structural elucidation of an unknown impurity.

We also describe how the use of Principal Components Analysis (PCA) of the LC/MS data allowed for the simple comparison of the impurities from different manufacturers' batches of Simvastatin tablets.

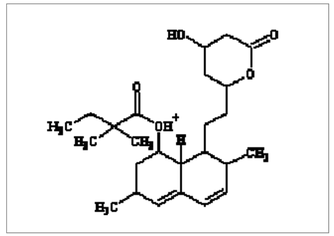


Figure 1. Structure of Simvastatin

METHODS

UPLC/MS/MS Analysis

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:	Waters® ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C18
Dimensions:	100 x 2.1 mm, 1.7 µm
Mobile Phase:	A1: 15 mM 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 µL
Detection:	ACQUITY UPLC PDA @ 238 nm

MS Conditions

Instrument:	Waters SYNAPT™ MS
Software:	MassLynx™ 4.1

Tune Page Parameters

Source:	ESi (Positive ion mode)
Capillary:	3.2 V
Sample Cone:	35 for reference 60 for analyte
Extraction Cone:	4.5 V
Desolvation Temp:	350 °C
Source Temp:	120 °C
Cone Flow:	0.0 L/Hr
Desolvation Flow:	800 L/Hr

Tof Settings

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

MS^E Settings

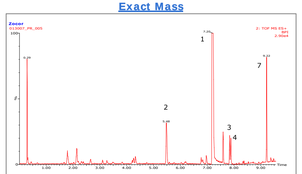
Low collision energy:	5 eV
High collision energy:	25 eV

RESULTS

The sub-2 µm particle LC operated at elevated flow rates allowed for the complete separation of all of the impurities in just 10 minutes (Figure 2). By using the approach of alternating collision energies, it was possible to obtain the precursor intact ion information and the fragment ion information under exact mass conditions in one analytical run. Despite the narrow LC peaks the high data acquisition rate of the mass spectrometer allowed for the collection of high quality accurate mass data. The common fragment ions produce in high collision energy data was used to identify all drug related impurities (Figure 3). The fragment ion information and accurate mass data was used to confirm the identities of the impurities (Figure 4).

RESULTS

Exact Mass



Compound	[M+H]	Result	ppm/mDa
1) Simvastatin	419.2797	419.2787	2.4/1
2) Simvastaitn Acid	437.2903	437.2889	3.2/1.4
3) Simvastatin Acetate	461.2903	461.2902	0.2/0.1
4) Anhydrosimvastatin	401.2692	401.2683	2.2/0.9
5) Lovastatin	405.2641	N/A	N/A
6) Anhydrosimvastatin acid	405.2641	N/A	N/A
7) Simvastatin Dimer	837.5517	837.5539	2.6/2.2

Figure 2. UPLC/MS separation of Simvastatin impurities

MS^E and the Identification of Common Fragments

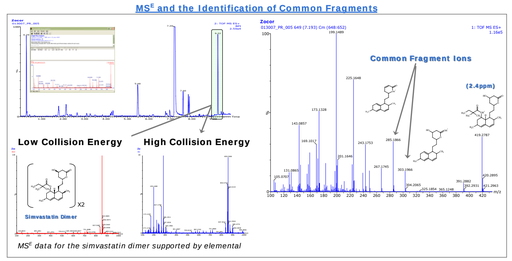


Figure 3. UPLC/MS identification of the dimer impurity of Simvastatin

Unknown Impurity Determination

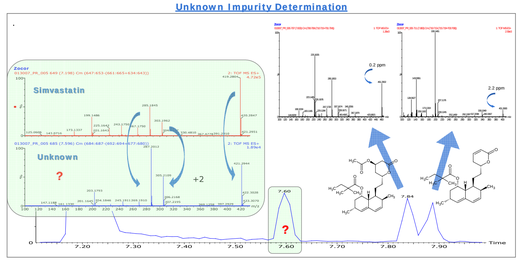


Figure 4. UPLC/MS data for unknown impurity

DISCUSSION

The data derived from the identification of the known impurities and the information provided in the fragmentation pathway allowed for a preliminary structure for the new-unknown impurity peak to be postulated. The MS fragmentation data showed a +2 mass units shift in the mass fragments of the impurity The data below illustrates that it is most likely simvastatin related (Figure 5).

Chemical Structure Proposals

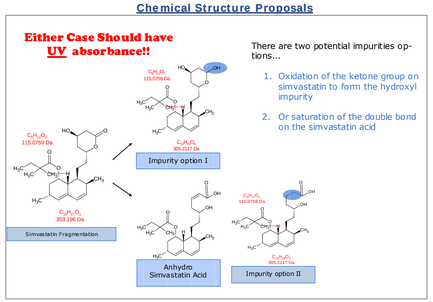


Figure 5. Potential structures for unknown impurity

However the LC/UV information obtained from the forced degradation studies showed there was no UV signal at the retention time corresponding unknown MS peak. The earlier proposed structures would have shown absorption in the UV region based on the conjugated bonds within the ring structure. Below is a new proposed structure that would be more representative of the data observed (Figure 6).

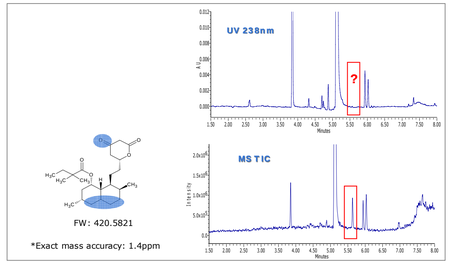


Figure 6. UPLC/UV and MS chromatogram and new proposed structure.

The comparison of batches of pharmaceutical products can be a complicated and time consuming. The ability to rapidly screen and compare batches would yield information on changes in the chemical process or identify counterfeit products. The UPLC/MS method was used to screen 4 batches of Simvastatin tablets from different manufacturers, the resulting data was analyzed by PCA data reduction and the resulting scores and loading plots are displayed in Figure 7. The loadings plot revealed that the different samples of tablets were separated in the scores plot due to the relative concentration ratios of the Simvastatin impurities.

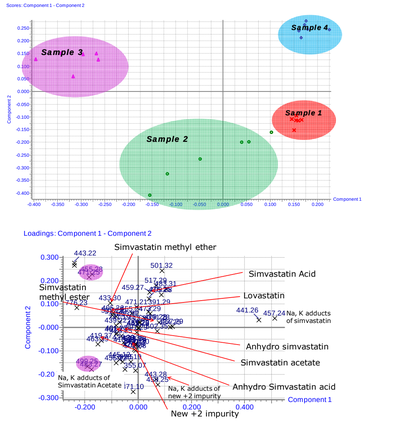


Figure 7. PCA scores and loadings plot for analysis of four manufacturers' batches of Simvastatin tablets

CONCLUSION

- 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 hyphenated quadrupole TOF MS instrumentation ensure the correct peak assignments based on the MS spectra.
- The application of MS/MS and exact mass using MS^E allows for the facile identification of the impurities of interest.
- The use of PCA data analysis allowed for the facile separation of different batches of Simvastatin tablets based on their impurity profile.

References

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