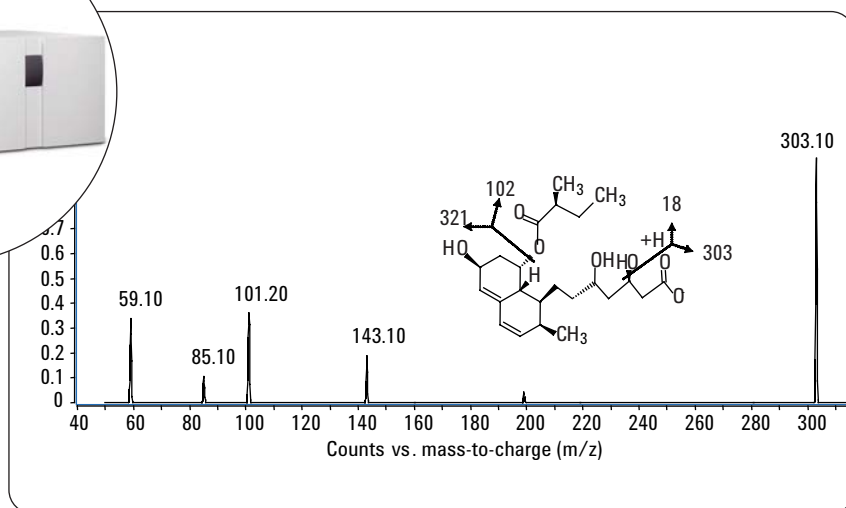
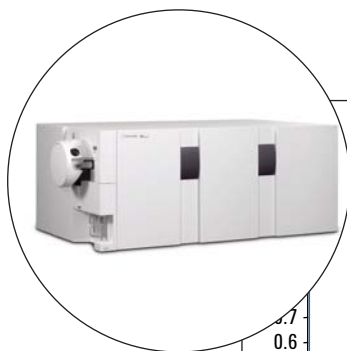


Assessment of in-process impurities in pravastatin using the Agilent 6410 Triple Quadrupole LC/MS in negative mode

Application Note

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Abstract

In-process impurities from pravastatin were separated using an Agilent ZORBAX Eclipse XDB-C18 column with 1.8 μm particle size and were determined using the Agilent 6410 Triple Quadrupole LC/MS system. Neutral and precursor ion scans were performed at specific time segments during the LC/MS run. At the elution time of the main peak, the diverter valve was switched to waste, thereby eliminating interference from the main drug peak during analysis. Both neutral loss and precursor ion scans were used to determine possible unknown impurities.

Agilent Equipment

- 6410 Triple Quadrupole LC/MS system
- 1200 Series Rapid Resolution LC system
- ZORBAX Eclipse XDB-C18 column
- MassHunter Workstation software

Application Area

- Pharmaceutical impurity analysis



Agilent Technologies

Introduction

During manufacturing of active pharmaceutical ingredients (APIs), related impurities are monitored using HPLC methods to check that they are within United States Food and Drug Administration (FDA) specifications. When out-of-specification (OOS) results are detected, MS and MS/MS are carried out to confirm and identify the impurity.

In cases where the impurity is a new one and known standards are not available, common practice is to accumulate the impurity using preparative LC¹ and to perform NMR and other spectroscopic studies, including MS/MS. Generally, ion trap or time-of-flight (TOF) mass spectrometers are used to characterize impurities². The unique ability of triple quadrupole (QQQ) instruments to do precursor ion scans is advantageous in impurity profiling, where unknown impurities are determined. In addition, neutral loss scans enable determination of molecular connectivity within the impurity and serve to screen components having common substructures³.

In this study, Agilent's 6410 Triple Quadrupole LC/MS system was used to screen for impurities present in an in-process sample of pravastatin sodium active pharmaceutical ingredient. The pravastatin peak was fragmented in negative mode and possible fragmentation sites were assigned⁴. Using neutral loss and precursor ion scans in negative mode, known and unknown peaks were determined.

Experimental

The following were used for the analyses:

- Column: Agilent ZORBAX Eclipse XDB-C18, 4.6 x 50 mm, 1.8 μ m (compatible with operation at 600 bar)
- Agilent 1200 Series Rapid Resolution LC (RRLC) system, including:
 - Agilent 1200 Series binary pump SL with degasser
 - Agilent 1200 Series high performance autosampler SL with thermostat
 - Agilent 1200 Series thermostated column compartment
 - Agilent 1200 Series diode-array detector (DAD) SL
- Agilent 6410 Triple Quadrupole LC/MS system with an electrospray source
- Agilent MassHunter Workstation software – Acquisition and Qualitative Analysis B.01.03
- In-process pravastatin samples were obtained from a local company

To be compatible with mass spectrometric analysis, the LC gradient method was modified from the United States Pharmacopeia (USP) method assay. The original USP method is 30 minutes long and utilizes 3.5 μ m columns. The time is reduced to 20 minutes by the use of 1.8 μ m columns. Further reduction in time is possible for fast and ultrafast analyses. Separations were accomplished using the Agilent 1200 Series Rapid Resolution LC (RRLC), where the same RRLC modules can be used for LC, fast LC, and ultrafast LC. The system can handle submicron particles and pressure as high as 600 bar.

To facilitate the determination of low-abundance impurities, the time segment programming in Agilent MassHunter Workstation software was used. Time segment programming allows specific regions of interest during the LC

Parameters	Detail
Wavelength for DAD	238 nm, bandwidth 4 nm, reference OFF
Diluent	50:50 methanol:water
Sample concentration/preparation	0.5 mg/mL in diluent
Injection volume	7 μ L
Needle wash	Flush port, 3 sec using diluent
Sample temperature	4 °C
Column temperature	25 °C
Mobile phase	Buffer A: 90 % 10 mM ammonium acetate and 10 % acetonitrile Buffer B: 90 % acetonitrile and 10 % 10 mM ammonium acetate
Gradient	Time % Buffer B
	0 0
	1 0
	7 23
	10 63
	17 63
	17.1 0
	20 0
Post-time	OFF
Flow	0.8 mL/min

Table 1
HPLC method.

run to be switched to the mass spectrometer. Therefore, higher amounts of relatively pure drug samples can be injected into the mass spectrometer because the main peak is diverted to waste (table 3). Time regions in which solutions are diverted to the MS can be further divided into subsegments to perform different types of scans in different regions.

Parameters	Detail
Mode	Negative electrospray (ESI)
Nebulizer	50 psig
Drying gas flow	10 L/min
Drying gas temp.	300 °C
Capillary voltage	3000 V

Table 2
MS method.

Time	Diverter valve
0.0	Waste
5.0	MS
7.0	Waste
7.8	MS
8.6	Waste

Table 3
Time segment programming for pravastatin impurity analysis.

Results and discussion

USP reports six known impurities for pravastatin⁵ and specifies a limit of not more than 0.1 % of any other impurity and not more than 0.6 % of total impurities found. Table 4 shows the list of known impurities as per USP, and their molecular weights. Note that lactone and compactin impurities appear as sodiated peaks in positive mode.

An LC/MS/MS method was developed to baseline-separate and characterize most of the impurities from pravastatin. Figure 1 shows the total ion chromatogram (TIC) in negative mode, and extracted ion chromatograms of pravastatin including co-eluting 3 α -hydroxyisocompactin (m/z 423.5), pentanoyl impurity (m/z 437.5), and 3''-hydroxypravastatin (m/z 439.5).

Neutral loss scans

Figure 2 shows the negative ion fragmentation of pravastatin (m/z 423), with m/z 321 and 303 as major product ions.

Fragmentation sites were assigned based on literature references^{4,6}.

A loss of 101.2 shows that the side chain of the pravastatin (m/z 102) is lost during collision. To deter-

mine related impurities having the same loss of 102, a neutral loss scan for m/z 102 was performed on the entire chromatogram. As shown in figure 3, the neutral loss scan revealed the presence at about 5.6 minutes of another new impurity of m/z 471.1.

Out of the six known impurities of pravastatin, the pentanoyl impurity is known to have a side chain with m/z 116 rather than m/z 102. Therefore, to determine any other related impurities having the same mass, a neutral loss scan for m/z 116 was performed. As shown in figure 4, the pentanoyl impurity at a retention time of about 8.5 minutes was the only one to have the characteristic side chain of m/z 116.

Impurities of pravastatin reported in USP	Molecular weight in the mode of highest intensity
3''-Hydroxypravastatin	439.5 (negative mode)
6'-Epipravastatin	423.5 (negative mode)
3 α -Hydroxyisocompactin	423.5 (negative mode)
Pentanoyl impurity	437.5 (negative mode)
Pravastatin lactone	407.5 (positive mode)
Compactin	391.5 (positive mode)

Table 4
Impurities reported in USP and their molecular weights in mode of highest intensity.

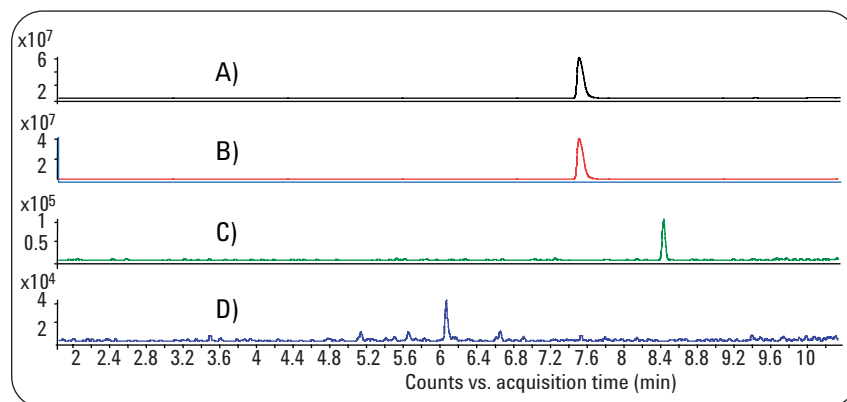


Figure 1
Extracted ion chromatogram of m/z 423.5 (B), 437.5 (C), and 439.5 (D), obtained from the total ion chromatogram (A).

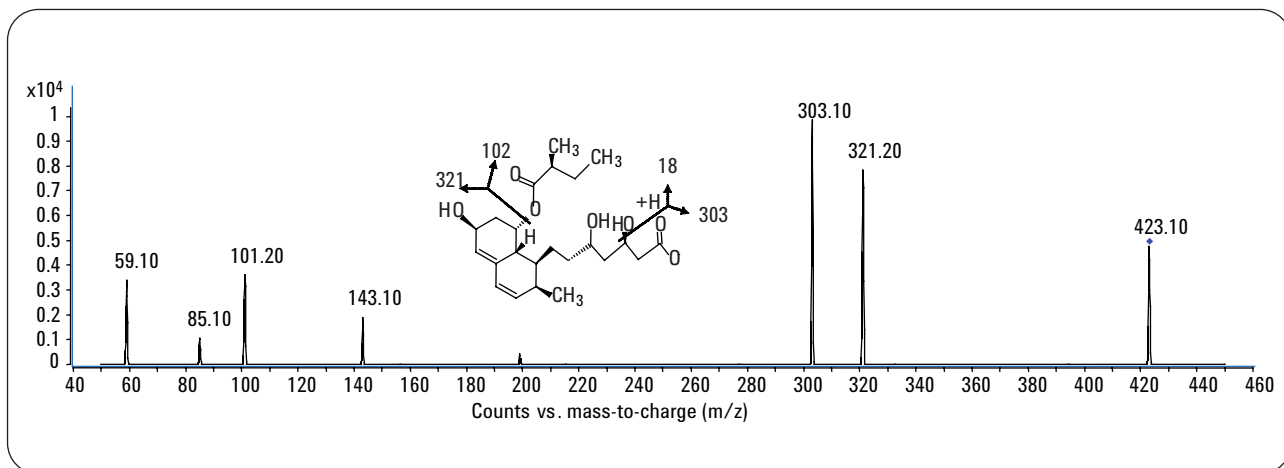


Figure 2
Negative ion fragmentation pattern of pravastatin. Insert shows fragmentation sites of pravastatin.

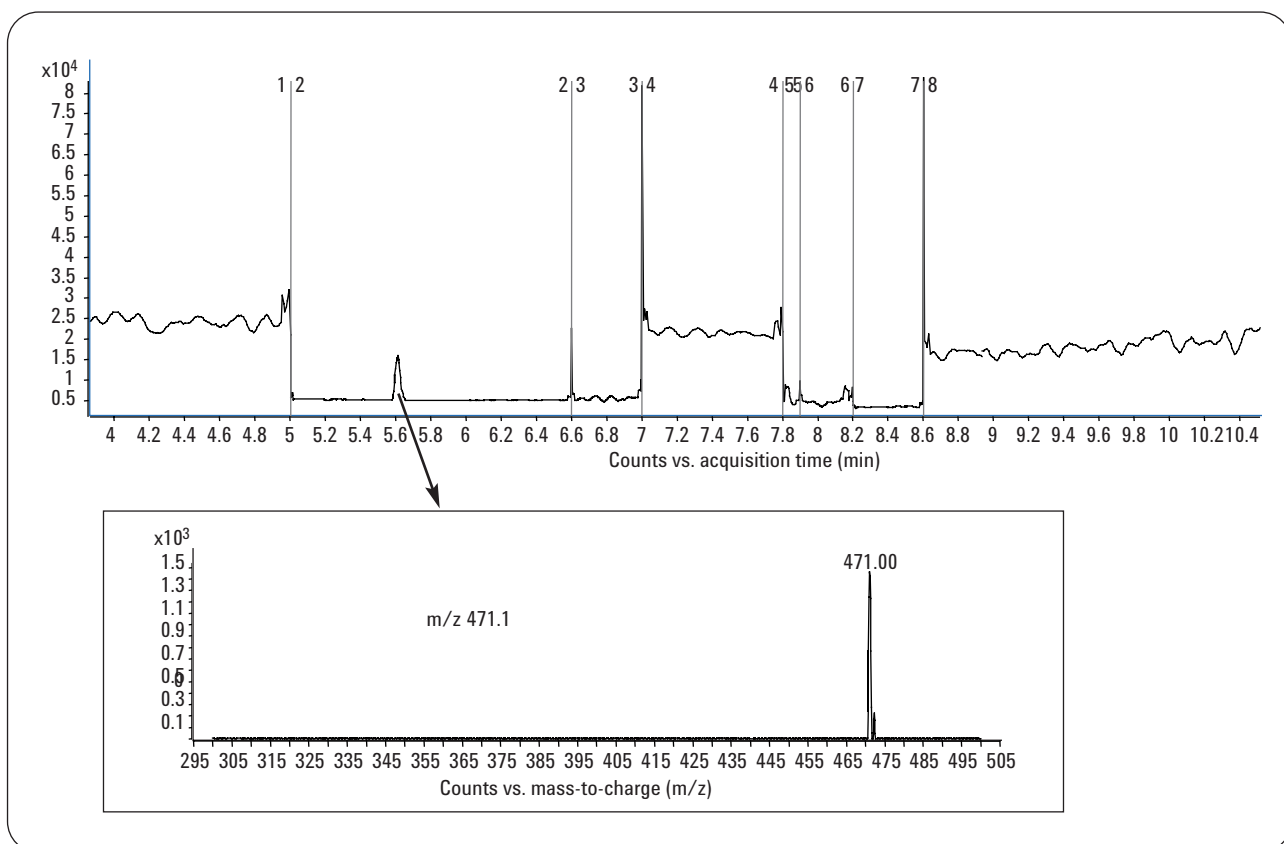


Figure 3
Neutral loss scan of 102 in all time segments shows a new peak with m/z 471.1 at a retention time of 5.6 minutes.

Precursor ion scans

The fragmentation pattern of pravastatin (figure 2) also reveals core sub-structures of m/z 321 and 303. Precursor ion scans programmed at these m/z values can reveal impurities that have the same core structures. Precursor ion scans of m/z 321 or m/z 303 reveal the relative locations of

possible 3α -hydroxyisocompactin and pentanoyl impurities (data not shown). The potential 3α -hydroxyisocompactin impurity is of low intensity and is eluted very close to the main peak.

Similar to utilizing the core structures of APIs to look for new impurities, the core structures of impuri-

ties can also be used to look for similar impurities with the help of precursor ion scans. Fragmentation of the possible $3''$ -hydroxyl impurity (m/z 439) reveals core structures as m/z 337 (because of the loss of the side chain of 102) and m/z 319 (due to loss of a water molecule). Precursor ion scans performed on m/z 319

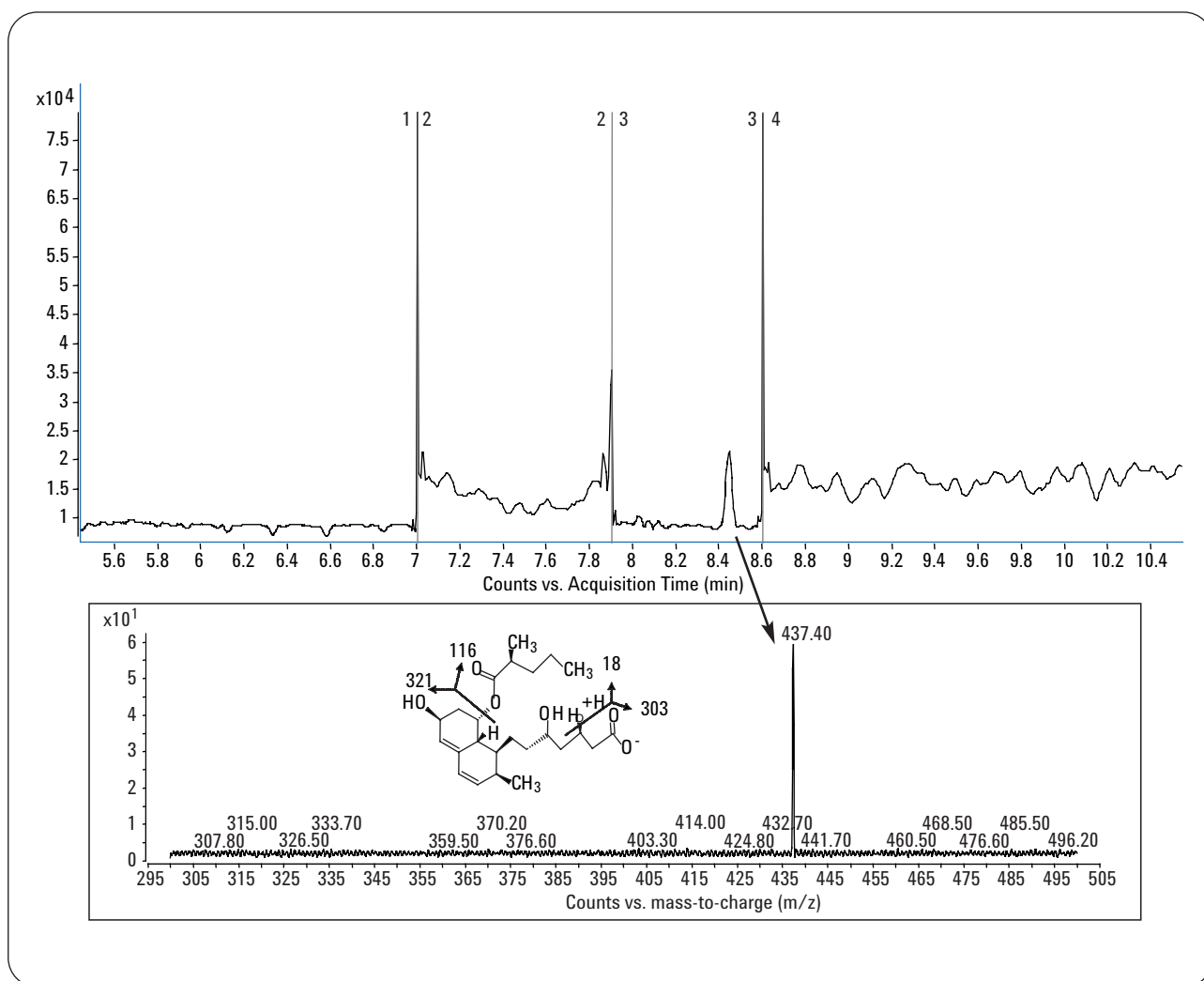


Figure 4
Neutral loss scan of 116 at retention times between 8.4 and 8.6 minutes. Insert shows the proposed fragmentation sites for the pentanoyl impurity (m/z 437).

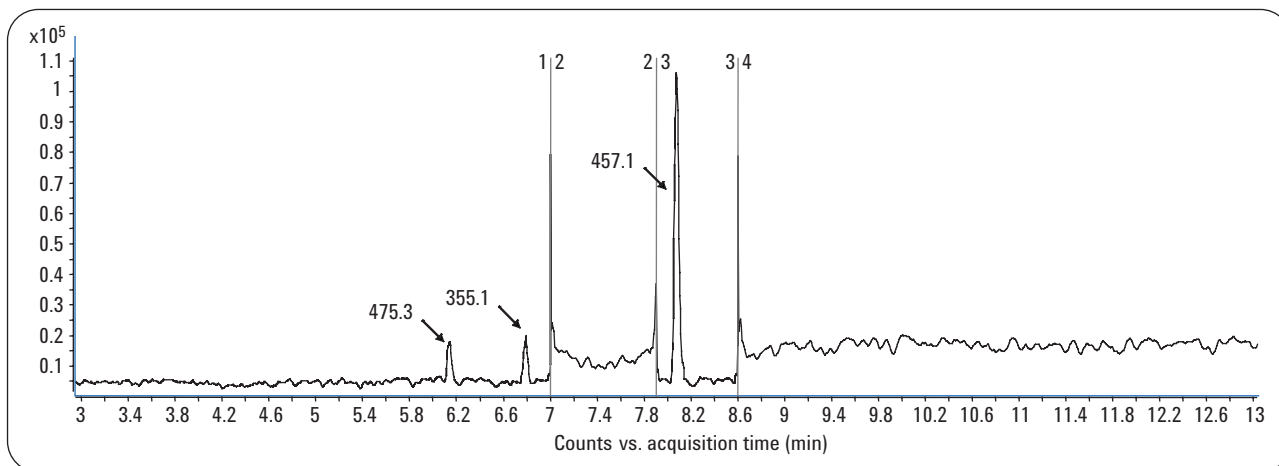


Figure 5
Precursor ion scans of m/z 319 show adduct impurities at m/z 475.3 and 355.1, and a new impurity at m/z 457.1.

reveal low amounts of various adduct impurities at different retention times and one new major impurity with m/z 457.1 at about 8.1 minutes (figure 5).

Conclusion

The Agilent 6410 Triple Quadrupole LC/MS system was used to assess known and unknown impurities using the substructure information from the fragmentation pattern of pravastatin and its impurities. Using its features of precursor ion scan and neutral loss scan, new impurities were discovered.

Time segment programming in MassHunter software facilitated injection of higher amounts of sample while not allowing major peaks to saturate the detector. This helps in characterizing the desired impurities from relatively pure samples in a single LC/MS/MS run.

During in-process monitoring of impurities at the drug discovery or manufacturing phases, fragmentation patterns of unknown impurities are compared against the database of fragments formed

from analogs and the API^{7,8}. This technique helps in determining the origin of an impurity. In the present study, the same technique of determining fragmentation patterns of the API and its impurity enabled us to use product ion and neutral loss scans to look for other related impurities having the same substructure. This study shows the importance of triple-quadrupole MS in discovering and monitoring impurities which otherwise may have been missed.

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