**Application News**

**No. LC-15-ADI-036**

**Ultra fast Purification Liquid Chromatography**

Isolation and identification of Atorvastatin degradation impurities by UFPLC

**Introduction:**

Atorvastatin is an antilipemic drug belonging to the statins class, whose reference drug is Pfizer’s Lipitor® (shown in Figure 1). It is used to reduce the levels of lipoproteins rich in cholesterol and reduce the risk of coronary artery disease. The drug in question is commonly sought after by pharmaceutical industries that produce generic drugs, due to the fact that the drug has a high value price, it is consumed globally, and its patent expired in late 2010. Atorvastatin has been found to degrade under acid and basic conditions.

Prominence UFPLC, Ultra Fast Preparative and Purification Liquid Chromatograph (Shown in Figure 2.), enables fast recovery of highly purified target compounds from complex samples such as organic synthesis reaction mixtures and natural products. Preparative LC (Prep LC) is a widely used technique in many research developments and manufacturing applications, including the synthesis of new drug compounds and the discovery of active components in natural products. It is mostly used to collect large amounts of unknown compounds in foods and drugs for subsequent structural analysis.

**Features**

i. **Comprehensive Automation of Preparative LC, Concentration, Purification, Elution, Collection and Powderization Only in 1.5 Hours**
   - Dedicated automation software to assist chemists in preparative procedure through collection
   - The time of evaporation can be reduced by up to 90% because of collection with organic solvent

ii. **High Purity as a Free Base**
   - Removal of counter ions derived from preparative mobile phase
   - De-salting and conversion to free base with ammonia/water

iii. **Small Footprint and Low-initial-cost**
   - Your lab space can be kept with high functionality by small footprint
   - Available in two standard configurations to match your requirements
     - Standard system with one trapping column
     - Advanced system with five trapping columns

**Experimental:**

Acid Degradation

200 mg of Atorvastatin API sample was dissolved in 10 mL of methanol and added 10 mL of 0.1 N hydrochloric acid and kept at 80°C for 1 hr. After degradation, added a few mL of methanol to dissolve residue and diluted to 10 mL. This solution was used for analysis on UFPLC for fraction collection. 10 µL of the solution was taken and diluted with 1mL of acetonitrile/water (1:1) to make 200 mg/L and then injected into HPLC

**Analytical Conditions**

| Mobile phase A | 0.1% TFA in water |
| Mobile phase B | Acetonitrile |
| Gradient program | (0.01/ 40, 10.00/50, 15.00/70, 20.00/90, 25.00/90, 30.00/40, 35.00/40) (Time in mins /%) |
| Column | Shim-pack GIS C-18 (250X10mm, 5µ) |
| Flow Rate | 5.0 ml/min |
| Wavelength | 245 nm |

**Results and Discussion**

Automation of preparative LC, concentration, purification, elution and collection controlled by dedicated automation software (see page 3) assists chemists in clearly identifying the peaks which are trapped and collected in specific color code. 1D chromatogram is shown in Figure 3 and corresponding area percentages are given in Table 1:

<table>
<thead>
<tr>
<th>Peak#</th>
<th>Name</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Atorvastatin</td>
<td>4.421</td>
<td>14932410</td>
<td>27.214</td>
</tr>
<tr>
<td>2</td>
<td>Impurity H</td>
<td>5.449</td>
<td>17169678</td>
<td>31.292</td>
</tr>
<tr>
<td>3</td>
<td>Unknown imp</td>
<td>6.032</td>
<td>22767800</td>
<td>41.494</td>
</tr>
</tbody>
</table>

The UFPLC system is capable of trapping maximum 5 peaks in one injection run on 5 different trap columns. It also rinses the individual trap columns by different rinsing solution to remove salts. It ensures that the compounds are transferred into free bases before they are eluted.
The trap column can retain compounds of different polarity due to its large retention capacity. Additionally, rinsing the column with ammonia/water after trapping allows compounds to be recovered as free bases, which are generally easier to powderize and typically yields greater quality result when used in drug screening and pharmacokinetic studies.

Atorvastatin degradation solution was injected into UFPLC to collect different impurity peak. The fractions were collected as free bases after online rinsing and desalting. The collected fractions of individual peaks were injected on Nexera X2 UHPLC system to check the purity. The individual chromatograms are shown in Figure 4, 5 and 6. The degradation solution was also injected on LCMSMS as shown in Figure 7 to check the m/z of degradation impurities. The collected purified fractions were also injected into LCMS to confirm the m/z of the impurities.

The Prominence UFPLC system utilizes Shimadzu's proprietary purification technology that shortens the time required for fractionation, concentration, purification, and recovery, to about 90 minutes from the conventional eight hours or more (shown in figure 15). The system also enables the recovery of high-purity target compounds. The Prominence UFPLC greatly improves the efficiency of preparative fraction collection and purification workflows in pharmaceutical, food, chemical and other industries as well as research organizations.
Figure 11. TIC of Product ion scan, imp H

Figure 12. MS/MS spectrum of imp H

Figure 13. TIC of Product ion scan, unknown imp

Figure 14. MS/MS spectrum of unknown imp

>>Existing method

30 min

8-12 hours

Synthesis → Fractionation → Powderization (centrifugal dryer or freeze dryer etc) → Approx. 8-12.5 hours

Mobile phase derived components remain

Takes time due to high water content

>>Prominence UFPLC

30 min → 20 min → 10-40 min

Takes only one and half hours

With the dedicated Purification Solution software, the analysis status can be quickly confirmed at a glance using the peak tracking function.
To ensure reliable fractionation and purification of precious samples, the Purification Solution software offers three fractionation modes

**Automatic Fractionation Mode**
In this mode, the software automatically identifies peaks and collects fractions based on parameter settings.

**Manual Fractionation Mode**
In this mode, the mouse pointer is used to fractionate peaks while viewing the window. When the same sample is concentrated by repeated injections, the first fractionation range is saved then the second and subsequent samples are automatically fractionated using the same fractionation range.

**Time-Specified Fractionation Mode**
This mode collects fractions based on the retention times in previously acquired data. It is ideal for routinely performed preparative purification processes.

**Conclusion**

The Prominence UFPLC seamlessly integrates traditional Prep LC with novel fraction trapping for up to five compounds of interest. The instrument is controlled by a dedicated walk-up software designed to empower non-expert users to easily set conditions for chromatographic separation and isolation of target compounds, trapping, purifying, eluting and collecting highly purified compounds in as little as 90 mins. For applications involving the isolation of low concentration targets, repeated injection and collection to the same trapping column to increase the amount of compound trapped on column prior to elution is easily accomplished.

The Prominence UFPLC eliminates some of the problems associated with conventional Prep LC, especially poor purity of collected compounds due to mobile phase additives, which become contaminants in the final collected fraction and inhibit powderization. Shimadzu’s “Shim-pack C2P-H” trap column strongly retains target compounds allowing unwanted organic solvents, water and additives to be flushed away in very quick time.