SHIMADZU
Excellence in Science

Application News

No. LC-15-ADI-036

UFPLC

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.), which enables fast recovery of highly purified target compounds from complex samples such as organic synthesis reaction mixtures and natural products. Prep LC is a widely used technique in many research development and manufacturing applications, including the synthesis of new drug compounds, the discovery of active components in natural products, and as a mechanism to collect large amounts of unknown compounds in foods and drugs for subsequent structural analysis.

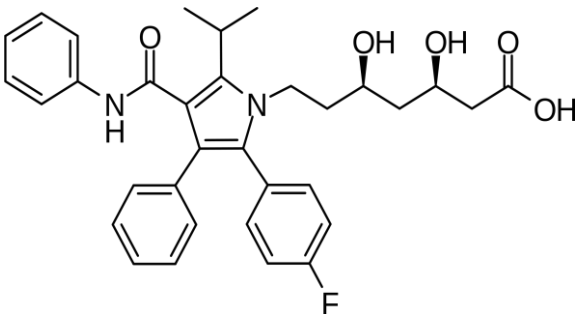


Figure 1. Atorvastatin

Figure 2. UFPLC

❑ 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 prep 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 requirement
  - Standard System with one trapping column
  - Advanced System with five trapping columns

❑ Experimental

Acid Degradation

200 mg of Atorvastatin API sample was dissolved in 10mL of methanol and added 10 mL of 0.1N Hydrochloric acid and kept at 80^0C for 1 hr. After the degradation added few ml of methanol to dissolve residue. This solution was used for analysis on UFPLC for fraction collection. Taken 10µL and diluted with 1mL of Acetonitrile : water (1:1) to make 200 ppm and then injected in HPLC.

Analytical Conditions

Mobile phase A	: 0.1% Trifluoroacetic acid in water
Mobile phase B	: Acetonitrile
Gradient program	: (0.01/ 40, 10.00/50, 15.00/70, 20.00/90, 25.00/90,0.00/40,35.00/40) (Time in mins / % B Conc.)
Column	: ShimPak C-18 (250X10mm, 5µ)
Flow Rate	: 5.0 ml/min
Wavelength	: 245 nm

❑ Preparation for Analysis :

The degradation sample was diluted with methanol to make the clear solution. After dilution the sample concentration was 10 mg/ml. Before UFPLC analysis diluted samples were analyzed on Nexera system to check the extent of degradation. The fast method was developed on Nexera to check the purity of degradation samples and fractions collected by UFPLC.

❑ Results and Discussion :

Automation of Preparative LC, Concentration, Purification, Elution, Collection controlled by dedicated automation software 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 1:Area Percentage

Peak#	Name	Ret. Time	Area	Area%
1	Atorvastatin	4.421	14932410	27.214
2	Impurity H	5.449	17169678	31.292
3	Unknown imp	6.032	22767800	41.494

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 compound is in the form of free base before it elutes. High retention capacity of trap columns can retain compounds of different polarity. Additionally, rinsing the column with an aqueous ammonia solution after trapping allows compounds to be recovered as free bases, which are generally easier to powederize and typically yields greater quality result when used in drug screening and pharmacokinetic studies.

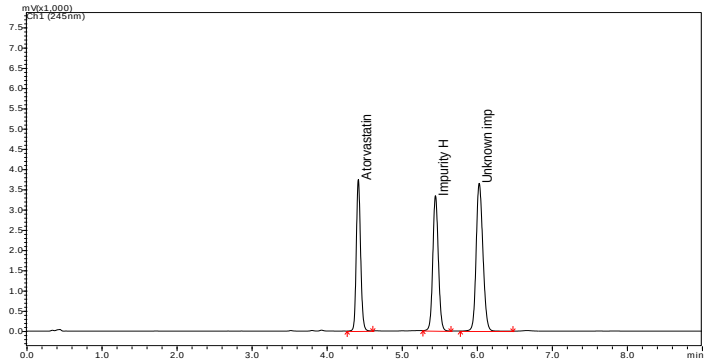


Figure 3. Crude

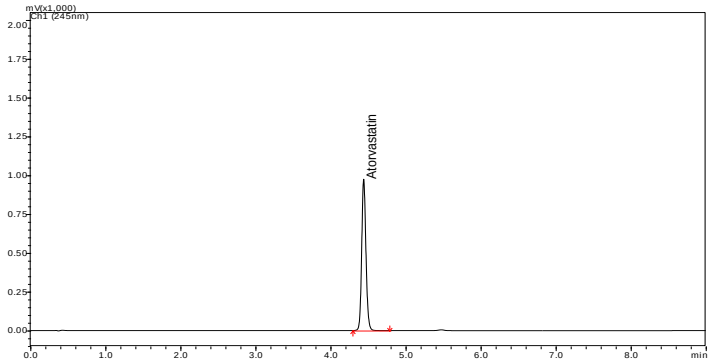


Figure 4. Peak 1

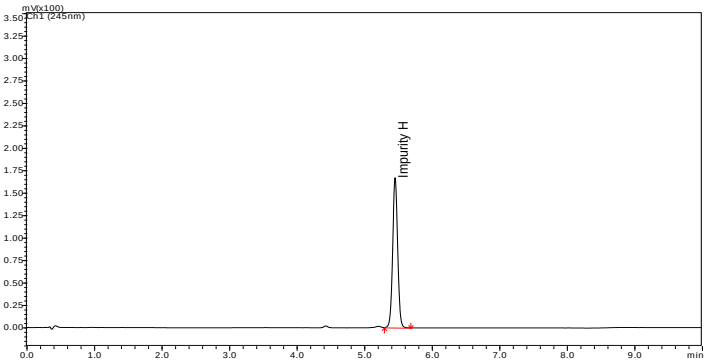


Figure 5. Peak 2

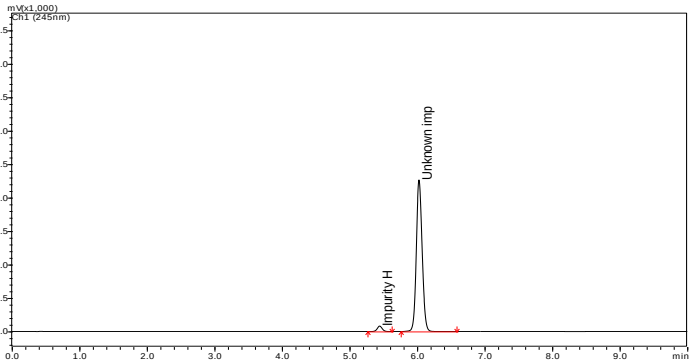


Figure 6. Peak 3

Atorvastatin degradation solution was injected on UFPLC to collect different impurity peak. The fractions were collected as free base after online rinsing and desalting. The collected fractions of individual peaks were injected on Nexera UHPLC system to check the purity. The individual chromatograms are shown in Figure 4,5 & 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 on LCMS to confirm the m/z of the impurities. The individual chromatograms are shown in Figure 8,9 & 10. The two degradation impurities showed m/z of 541.30 and 573.20. These peaks were further subjected to product ion scan to see the structural similarity between Atorvastatin and the degradation impurities (Figure 11 & 13). The fragmentation pattern (Figure 12 & 14) of both the impurities are identical after m/z 318 which indicates the structural similarities between them.

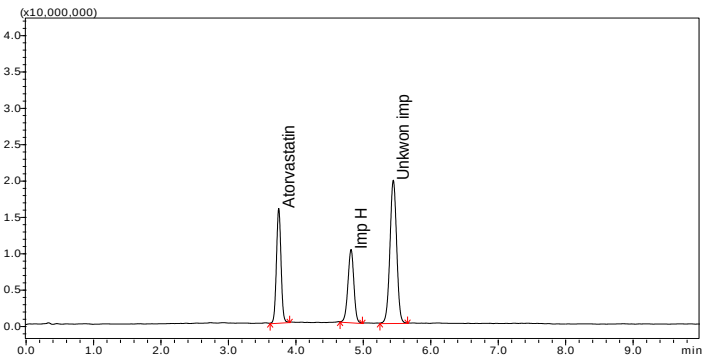


Figure 7. Extracted TIC of crude degradation sample

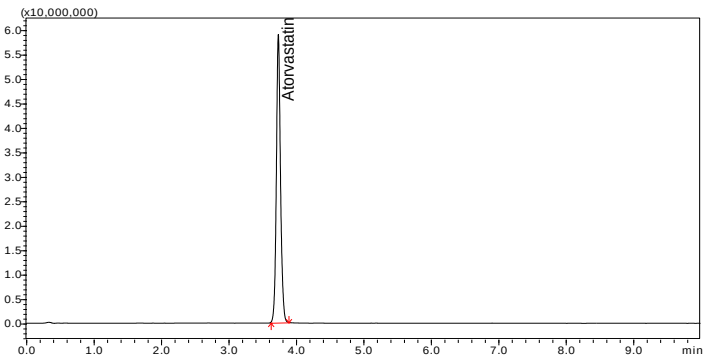


Figure 8. Extracted TIC of Atorvastatin

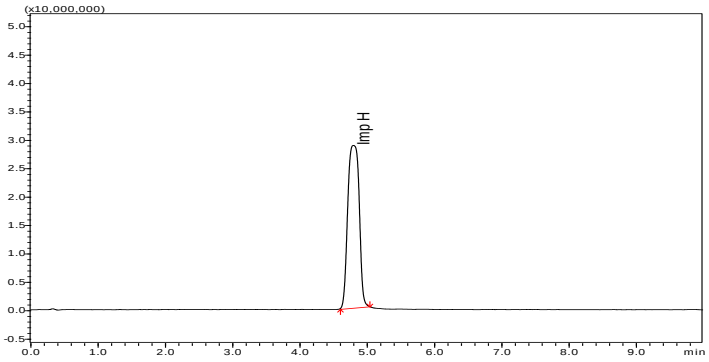


Figure 9. Extracted TIC of imp H

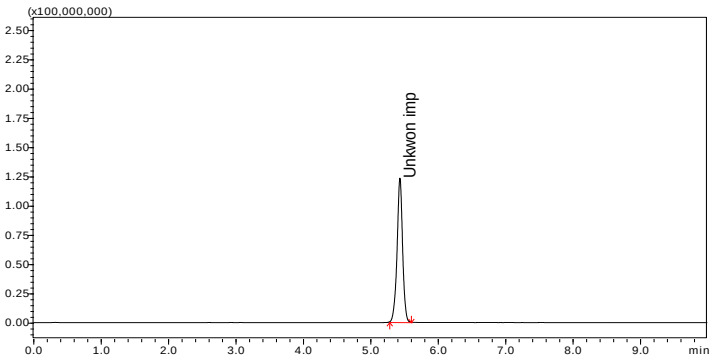


Figure 10. Extracted TIC of Unknown imp

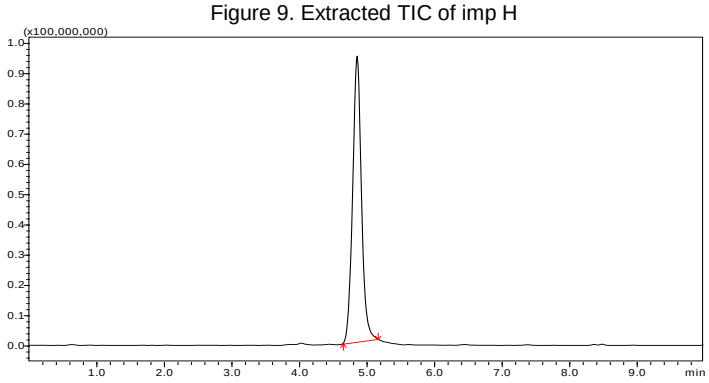


Figure 11. TIC of Product ion scan imp H

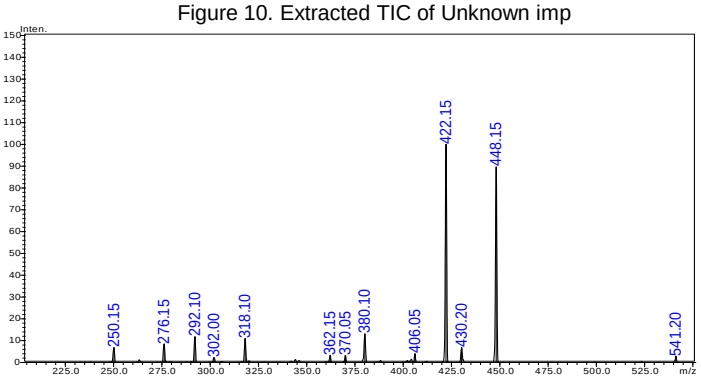


Figure 12. Spectra of Product ion scan imp H

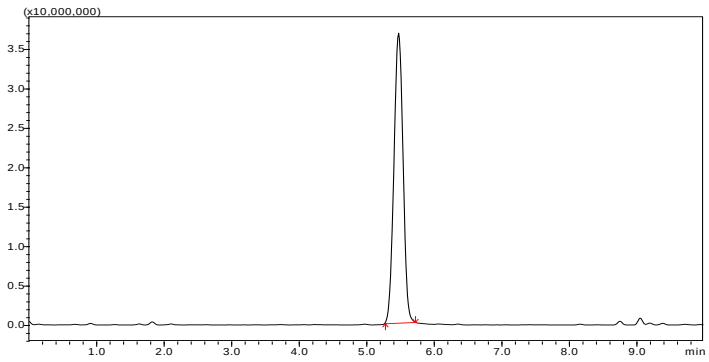


Figure 13. TIC of Product ion scan unknown imp

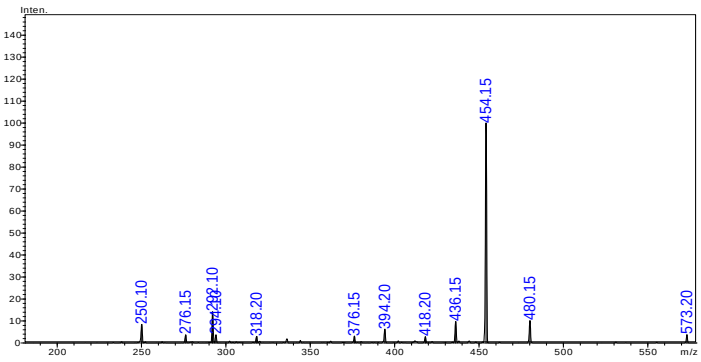


Figure 14. Spectra of Product ion scan unknown imp

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.

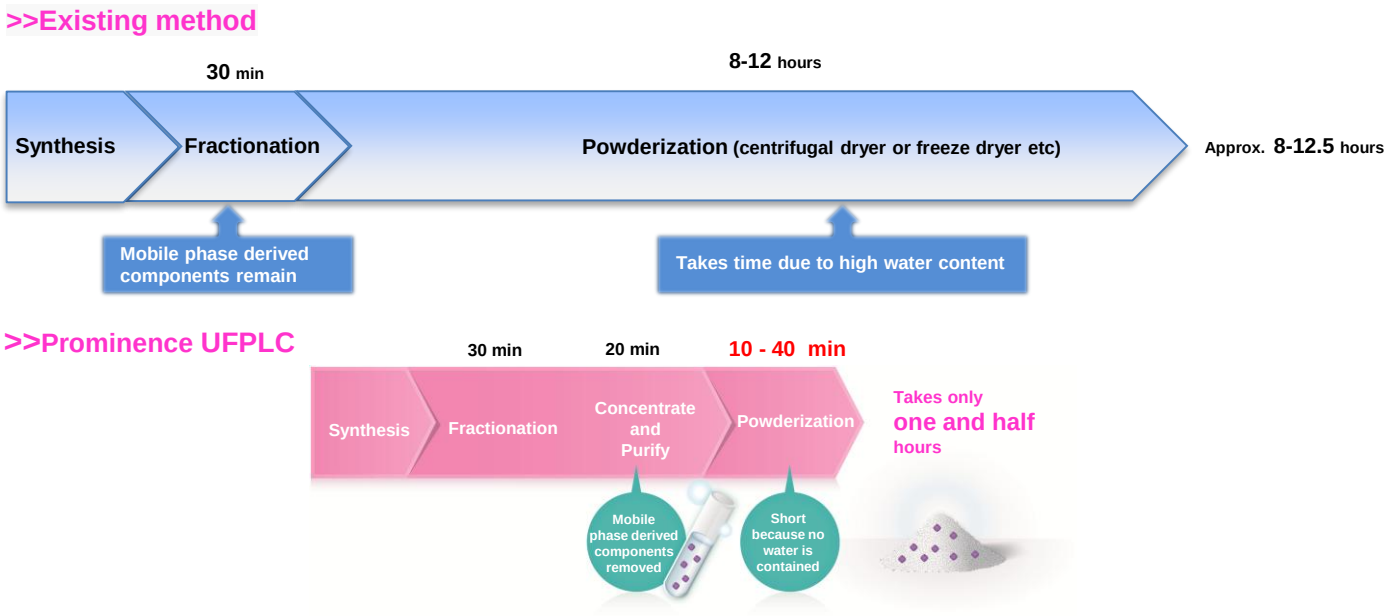


Figure 15. UFPLC purification cycle

With the dedicated Purification Solution software, the analysis status can be quickly confirmed at a glance using the peak tracking function.

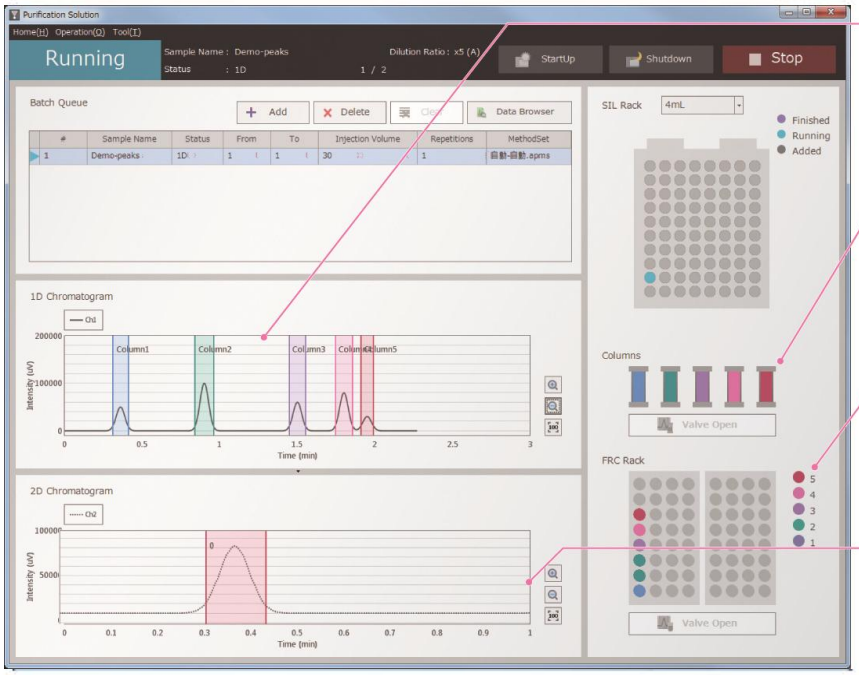
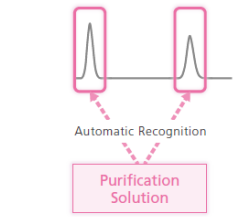
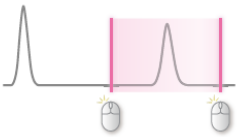


Figure 16. Purification solution

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 and 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 upto 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 90mins. For applications involving the isolation of low concentration targets, replicate 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" trapping column strongly retains target compounds allowing unwanted organic solvents, water and additives to be flushed away in very quick time.

SHIMADZU
Shimadzu Analytical (India) Pvt.Ltd.
1 A/B, Rushabh Chambers, Makwana Road,
Marol, Andheri (East), Mumbai- 400059, India.
Tel: +91 22 29204741 Fax: +91 22 29205679
www.shimadzu.in