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Analysis of Atorvastatin as per USP Monograph Utilizing Alliance™ iS HPLC System

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Abstract

The USP monograph of atorvastatin requires adherence to strict criteria for the assay analysis of atorvastatin in terms of peak area/retention time precision, peak tailing, and resolution. Specifically, the monograph requires that the relative standard deviation (RSD) for multiple injections (between three and six) to be less than 0.6% for peak areas and retention time of atorvastatin. In this study, several strategies were implemented to meet the stipulated USP monograph criteria. These measures included using low evaporation caps for mobile phase bottles, utilizing non-preslit septa for vial caps, and maintaining samples at a controlled temperature of 10 °C in the autosampler. These precautions are in conjunction with the Alliance iS HPLC System. Resulted in very consistent peak areas and retention times for both atorvastatin and its related compound. Notably, the %RSD for the peak area and retention time of atorvastatin in six replicate injections was 0.4 and 0.1, respectively.

Benefits

- The utilization of Alliance iS HPLC System allowed for highly consistent peak area and retention time of atorvastatin
- Excellent control of flow rate, sample temperature, and column temperature

- Low evaporation ACQUITY™ APC Reservoir Cap mitigates the risk of evaporation when volatile solvents are used in the mobile phase

Introduction

Atorvastatin, a widely prescribed statin drug, is used to lower cholesterol levels and reduce the risk of cardiovascular diseases.[1,2] To ensure the safety and efficacy of this drug, it is essential to analyze atorvastatin and related compounds using techniques such as High-Performance Liquid Chromatography (HPLC).[3,4] The USP monograph for atorvastatin assay analysis using HPLC requires careful control of all aspects of the method, including mobile phase and sample preparation.[5] The monograph's conditions make it difficult to meet the system suitability criteria. The volatile components in the sample and mobile phase can evaporate during the analysis, causing changes in the peak areas and retention times. This can lead to the method failing system suitability even if the column and instrument are functioning properly. The specific conditions that pose a challenge include the use of a highly volatile sample diluent (1:1:2 acetonitrile: tetrahydrofuran (THF): water), a volatile component in the mobile phase (THF at 12%), and a long analysis time (two hours).

Previously, a thorough investigation was conducted to assess the influence of these conditions on meeting the system suitability criteria.[6] The study revealed that the effects of sample preparation and mobile phase composition can be effectively controlled by implementing two measures. Firstly, instead of utilizing a single vial, multiple vials should be employed for the replicate injections. Secondly, employing low vapor reservoir caps for the mobile phase reservoirs proves beneficial. These measures help mitigate the impact of sample preparation and mobile phase composition, contributing to improved control and reproducibility of the analysis. This study will demonstrate the analysis of atorvastatin according to the USP monograph using the Alliance iS HPLC System. Figure 1 illustrates the chemical structures of atorvastatin and its related compound used in this study.

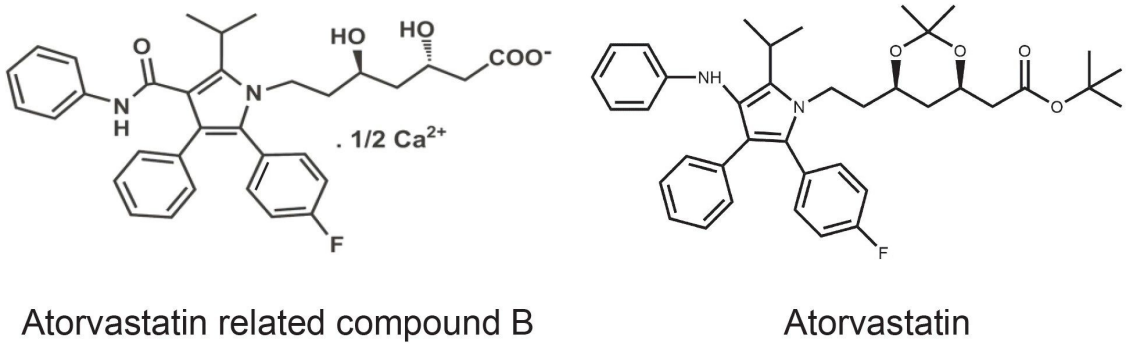

Figure 1. Chemical structures of atorvastatin and its related compound that were used in this study.

Experimental

Materials and Standard Preparations

The sample preparation was carried out according to the USP monograph.[5] The reference standards for atorvastatin and atorvastatin related compound B were both purchased from USP (Rockville, MD). The diluent was prepared by mixing acetonitrile, stabilizer-free tetrahydrofuran, and water in a ratio of 1:1:2. All samples were prepared immediately prior to analysis. The system suitability standard was prepared by dissolving atorvastatin calcium and atorvastatin related compound B in the diluent to a final concentration of 0.05 mg/mL and 0.06 mg/mL, respectively. A specific volume (300 µL) of standards was aliquoted into maximum recovery vials with both preslit and non-preslit screw caps.

LC method conditions:

Column details	ZORBAX™ Rx-C_8, 80Å, 4.6 × 250 mm, 5 µm		
Column temperature	35 °C		
Mobile phase A	Acetonitrile, stabilizer free THF, buffer (21:12:67)		
Mobile phase B	Acetonitrile, stabilizer free THF, buffer (61:12:27)		
Buffer	3.9 g/L Ammonium acetate in water (pH5.0±0.1)		
Flow rate	1.5 mL per minute		
Gradient profile	Time (mins)	% Mobile phase A	% Mobile phase B
	0	100	0
	40	100	0
	70	20	80
	85	0	100
	100	0	100
	105	100	0
	115	100	0
Detector type	TUV Filter: normal data rate: 10 Hz		
Detector wavelength	244 nm		
Injection volume	20 µL		
Autosampler needle wash solvent	Methanol		
Wash solvent	1:1:2 ACN/THF/H_2O		
Run time	115 minutes		
Mobile phase bottle caps:	ACQUITY™ APC reservoir cap (p/n 205001152)		

Data management

Chromatography software	Empower 3.6 Feature Release 5

Results and Discussion

USP Assay Results

The HPLC method for assay analysis of atorvastatin according to the USP monograph requires a very lengthy run time of over two hours and the use of volatile organic solvents for both the mobile phase and the sample diluent. The volatility of these solvents can affect the repeatability of the analysis in two ways. Firstly, the differing

evaporation rates of the mobile phase components can impact the elution or retention time of the analytes. Secondly, organic evaporation from the sample diluent can affect the concentration and solubility of the analytes, both of which can impact system suitability requirements (Table 1). Therefore, it is critical to follow the previously recommended steps to mitigate these evaporation aspects and reduce variability in the method.[6] These steps include using low evaporation caps for solvent bottles and injecting replicate injections from separate vials each capped with a non-preslit cap. The samples were also maintained at a low temperature of 10 °C in the autosampler to control the evaporation of the diluent. Employing these steps, the USP assay for atorvastatin was performed successfully. Specifically, the USP criteria were met for this analysis (Table 1), including peak area and retention time %RSD. Figure 2 displays the overlay chromatogram of the replicate injections. It is worth mentioning here that the Alliance iS HPLC System employs an inline metering device to enhance injection precision. By flushing the flow path during acquisition, this device minimizes the formation of air bubbles.[7]

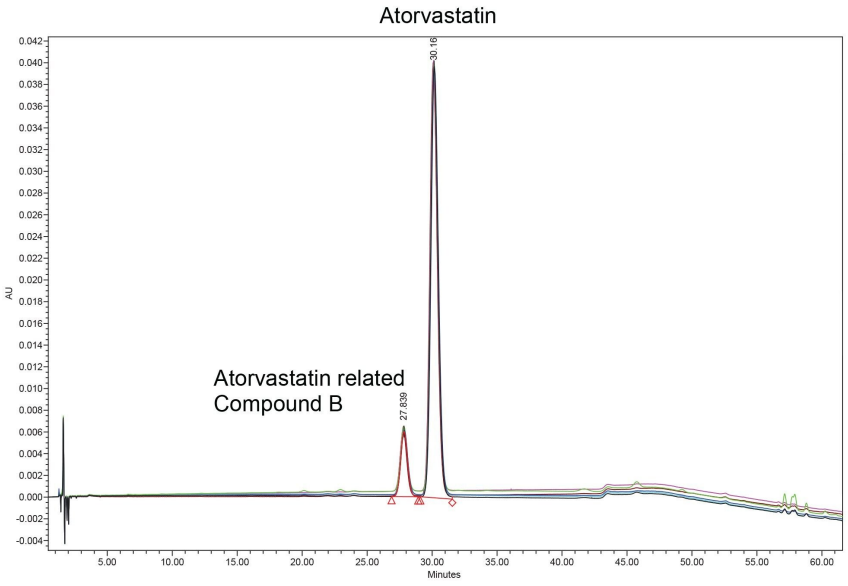

Figure 2. Overlay chromatogram of six replicate injections of the system suitability mixture. Each injection was obtained from a separate vial, with each vial capped using non-pre-slit septa.

Criteria		Results
Resolution	Not less than (NLT) 1.5 between peaks for atorvastatin related compound B and atorvastatin, system suitability solution	2.3
Tailing factor	Not More Than (NMT) 1.6%, standard solution	1.1
Relative standard deviation	NMT 0.6%, Standard solution	0.4% for peak area and 0.1% for retention time

Table 1. System Suitability criteria and actual results generated in this study of the USP assay of atorvastatin calcium.

A Comparison of Non-Preslit Septa versus Preslit Septa

To evaluate the effect of different kinds of vial caps on evaporation, the System Suitability results for replicate injections made from non-preslit vial caps were compared with preslit caps. To do this, six replicate injections were aspired from the same vial that was capped with a non-preslit septa. The results of this experiment showed much larger variability in peak areas compared to separate vials with non-preslit septa. For example, as shown in table 2, the %RSD for the peak area was 1.0 for six replicate injections from the same vial that is capped with a preslit septa. This is much larger deviation from what was observed for the injections that were taken from non-preslit septa. These findings indicate that using separate vials each capped with non pre slit septa is a very useful approach when dealing with volatile sample diluents.

It should be mentioned here that significant retention time shifts were not observed in any of the experiments, which can be attributed to the utilization of low evaporation caps (AQUITY APC) on the mobile phase bottles. These caps are specifically designed to reduce solvent evaporation from the bottles and are equipped with an outlet relief valve to release any pressure accumulation in the mobile phase bottle. Table 2 displays a summary of the findings from this experiment.

Six injections from the same vial (preslit Septa)

Component summary table
Name: Atorvastatin

	Vial	Inj	RT	Area	Height	USP resolution	USP resolution (HH)	USP tailing
1	1:B,8	1	30.21	1597638	39425	2.27	2.30	1.14
2	1:B,8	2	30.23	1619965	39908	2.26	2.30	1.14
3	1:B,8	3	30.27	1627260	40076	2.27	2.30	1.14
4	1:B,8	4	30.29	1629678	40162	2.27	2.30	1.14
5	1:B,8	5	30.32	1635760	40284	2.28	2.31	1.14
6	1:B,8	6	30.39	1642236	40384	2.28	2.31	1.14
Mean			30.28	1625422.869	40040.036	2.3	2.3	1.1
Std. dev.			0.06	15574.292	343.261	0.0	0.0	0.0
% RSD			0.2	1.0	0.9	0.2	0.2	0.2

Six injections from six different vials (non-preslit Septa)

Component summary table
Name: Atorvastatin

	Vial	Inj	RT	Area	Height	USP resolution	USP resolution (HH)	USP tailing
1	1:B,2	1	30.097	1619285	39995	2.25	2.28	1.13
2	1:B,3	1	30.128	1614908	39748	2.25	2.28	1.13
3	1:B,4	1	30.156	1606862	39448	2.25	2.29	1.13
4	1:B,5	1	30.160	1611359	39762	2.25	2.28	1.13
5	1:B,6	1	30.160	1609817	39722	2.25	2.28	1.13
6	1:B,7	1	30.189	1598913	39310	2.25	2.28	1.13
Mean			30.148	1610190.667	39663.925	2.3	2.3	1.1
Std. dev.			0.032	6996.15	245.658	0.0	0.0	0.0
% RSD			0.1	0.4	0.4	0.1	0.1	0.3

Table 2. Data from six replicate injections of an atorvastatin standard. The data in the left side of the table represents six replicate injections of a sample taken from the same vial. The right side of the table shows the data obtained from six replicate injections, each taken from a separate vial with a non-preslit septa.

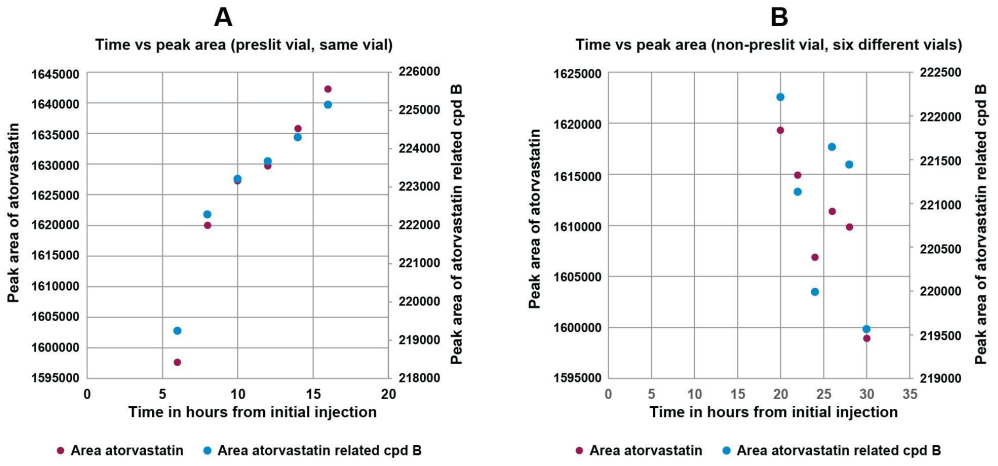

Figure 3. Peak area data for atorvastatin and atorvastatin related compound B over a 30-hour period. A depicts six injections obtained from a single vial, capped with a preslit septa, while B illustrates data from six injections using six different vials, each capped with non-preslit septa.

Figure 3 displays the peak area trends for atorvastatin and its related compound B over a 30-hour analysis period. The data shows that when using preslit septa caps, the peak areas for both analytes gradually increased, likely due to evaporation causing a higher concentration of analytes. In contrast, when using non-preslit vial caps, no clear trend in peak areas was observed, suggesting no evaporation occurred. It should be noted here that the peak area distribution for the six injections from different vials with non-preslit caps exhibited significantly smaller values on the y-axis compared to those observed with the preslit cap.

Conclusion

- These results demonstrate the consistent peak areas, retention times, USP tailing, and resolution that can be obtained for the assay analysis of atorvastatin using Alliance iS HPLC System
- Utilizing Low evaporation ACQUITY APC Reservoir Caps minimizes evaporation of volatile organic solvent in mobile phase allowing for very consistent retention times

- Utilizing multiple vials, each capped with non-preslit septa, proves advantageous for samples dissolved in volatile diluents

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