APPLICATIONS

Enantiomeric Purity Analysis of the Drug Product Atorvastatin on Lux® Amylose-1 According to the United States Pharmacopeia (USP) Monograph 2263

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In this technical note, we report the enantiomeric separation between Atorvastatin and related compound E (enantiomer of Atorvastatin) using the polysaccharide-based chiral stationary phase Lux Amylose-1 (L51 USP column) according to United States Pharmacopeia monograph 2263.

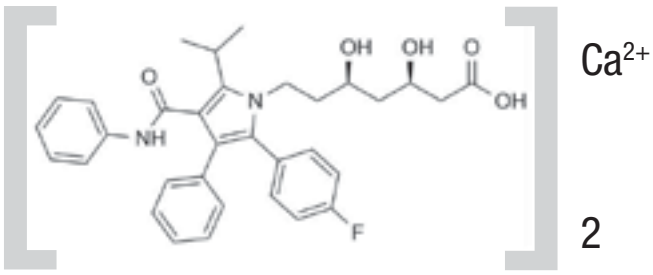
Introduction

The USP establishes methods and provides reference standards for medicines, food ingredients, dietary supplement products, and active pharmaceutical ingredients (API). These methods and standards are used by regulatory agencies and pharmaceutical manufacturers to ensure that APIs and drug products are of the appropriate identity, strength, quality, purity, and consistency. In the case of chiral drugs, such as Atorvastatin (**Figure 1**.), a chiral HPLC method is generally used to assess chiral purity.

Atorvastatin is marketed by Pfizer as a calcium salt under the trade name Lipitor and is a member of the drug class known as statins, used for lowering blood cholesterol. Like all statins, Atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body. Lipitor is arguably the best selling drug in pharmaceutical history. Since it was approved in 1996 total sales exceed USD $125 billion, and the drug has topped the list of best-selling branded pharmaceuticals in the world for nearly a decade. When Pfizer's patent on Lipitor expired on November 30, 2011, generic Atorvastatin became available in the United States, initially manufactured only by generic drug makers Watson Pharmaceuticals and India's Ranbaxy Laboratories.

In this technote, we report the enantiomeric separation between Atorvastatin Calcium (the Active Pharmaceutical Ingredient) depicted in **Figure 1** and Related Compound E (enantiomer of Atorvastatin) using the new Lux Amylose-1 chiral stationary phase (CSP) according to the current USP method.

Figure 1.
Atorvastatin Calcium (3R,5S)



Materials and Methods

Analyses were performed using an Agilent 1200 HPLC (Agilent Technologies Santa Clara, CA United States) consisting of a LC binary pump system interfaced with a diode array detector (DAD), automated auto sampler and thermostated column compartment. The Lux Amylose-1 column used for analysis was obtained from Phenomenex (Torrance, CA, USA). All solvents were purchased from Honeywell (Morristown, NJ, USA) and Sigma-Aldrich. USP Atorvastatin Calcium RS 100mg Cat # 1044516 and USP Atorvastatin Related Compound E 10mg Cat # 12601 reference standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

USP Monograph 2263 for Enantiomeric Purity:

Mobile phase: Hexane, dehydrated alcohol, and trifluoroacetic acid (940:60:1)

System suitability stock solution: 5 mg/mL of USP Atorvastatin Calcium RS and 37.5 mg/mL of USP Atorvastatin Related Compound E RS in methanol. [NOTE—Atorvastatin related compound E is the 3S,5S enantiomer of atorvastatin.]

System suitability solution: Transfer 2.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.

Sample solution: Transfer 10 mg of Atorvastatin Calcium to a 10-mL volumetric flask, dissolve in 2.0 mL of methanol, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.

Chromatographic system
Mode: LC
Detector: UV 244 nm
Column: 4.6-mm x 25-cm; packing L51
Flow rate: 1.0 mL/min
Injection size: 20 µL

System Suitability
Samples: *System suitability solution*
[NOTE—The elution order of the peaks is atorvastatin related compound E followed by atorvastatin.]
Resolution: NLT 2.0 between the peaks for atorvastatin related compound E and atorvastatin

Analysis:
Samples: *Sample solution*
Calculate the percentage of atorvastatin related compound E in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_u/r_T) \times 100$$

r_u = peak response for atorvastatin related compound E

r_T = sum of the responses of the peaks atorvastatin related compound E and atorvastatin

Acceptance criteria: NMT 0.3 % of atorvastatin related compound E

Result and Discussion

The enantiomeric separation between Atorvastatin (the Active Pharmaceutical Ingredient or API) depicted in **Figure 1** and related compound E (the enantiomer of Atorvastatin) is important for the analysis of chiral drug purity. In this technote we report the enantiomeric separation using the new Lux Amylose-1 chiral stationary phase according to the United States Pharmacopeia Monograph 2263. The USP standards were dissolved in the mobile phase and injected on an Agilent HPLC system as described in the material and methods section.

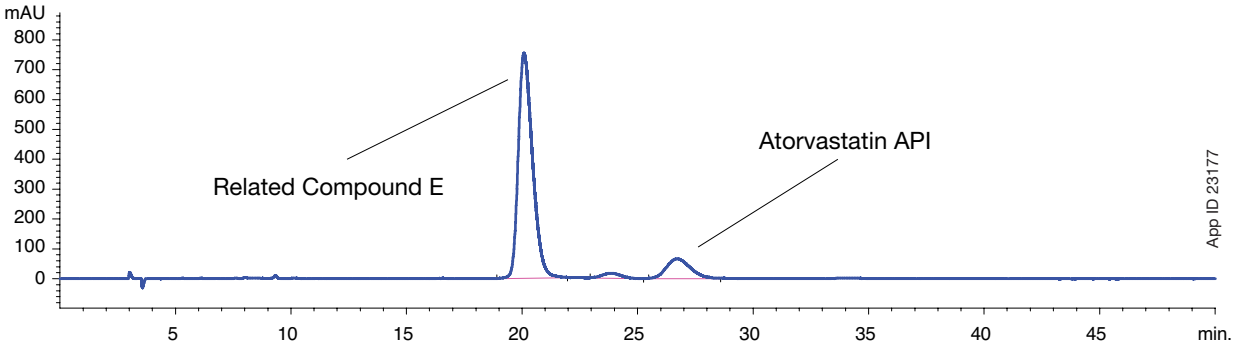
The USP method gives guidelines for order of elution specifically for these compounds of interest. According to the monograph note, the Atorvastatin related compound E should elute first, followed by Atorvastatin. The resolution (Rs) criteria for the system suitability solution between the two enantiomers must be no less than (NLT) 2.0 and the amount of related compound E in the sample solution shall be no more than (NMT) 0.3 % for the drug to meet the chiral purity acceptance criteria.

USP reference standards were first injected on the Lux Amylose-1 column following the USP monograph method. The representative chromatogram for the system suitability solution on the Lux Amylose-1 column is shown in **Figure 2**. The retention times for the compounds E and Atorvastatin were 20.080 minutes and 26.713 minutes, respectively. The resolution obtained with the Lux Amylose-1 column was 3.1, well within the USP method system suitability criteria of no less than 2.0. **Figure 3** displays the chromatogram of Atorvastatin sample solution run on Lux Amylose-1 column. The calculated percentage of compound E is 0.04 %, well below the 0.3 % required by the USP method acceptance criteria.

HPLC Conditions:

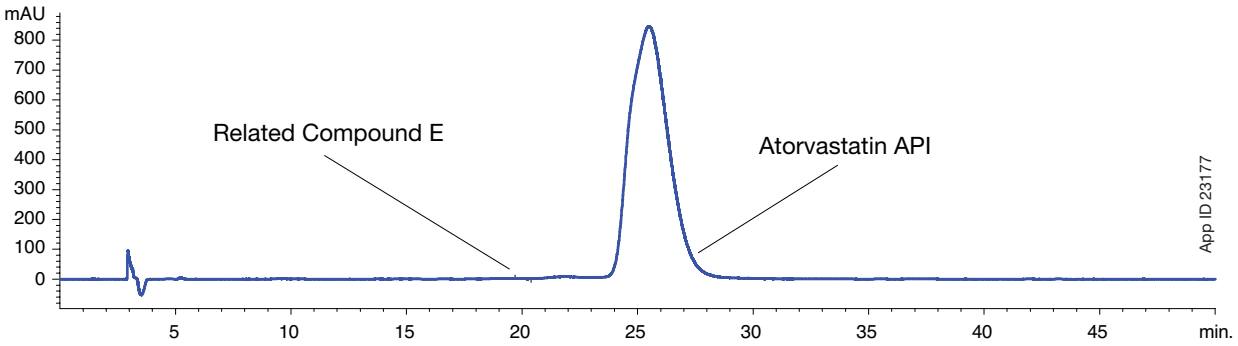
Columns: Lux 5 µm Amylose-1
Dimensions: 250 x 4.6 mm
Part Number: 00G-4732-E0
Mobile Phase: According to USP method
Flow Rate: 1 mL/min
Injection Volumn: 20 µL
Temperature: Ambient
Detection: UV @ 244 nm
Sample: 1. Related Compound E
2. Atorvastatin

Figure 2.
System suitability solution on Lux® 5 µm Amylose-1



Resolution (Rs) of 3.1 meeting USP criteria of NLT 2.0

Figure 3.
Sample solution on Lux® 5 µm Amylose-1



% related compound E = (r_u/r_T) X100 = 23.5/(23.5+58503.2) = 0.04 %
(Acceptance NMT 0.3%).

r_u = peak response for atorvastatin related compound E

r_T = sum of the peak responses for atorvastatin related compound E and atorvastatin

Conclusion

The results shown above demonstrate that the newest L51 USP Column, Lux Amylose-1, can be successfully used to analyze Atorvastatin API according to the USP method. The obtained resolution for system suitability was 3.1, which is well above the required NLT 2.0. The percentage of Atorvastatin related compound E in the Atorvastatin sample analyzed was 0.04 %, well below the acceptance criteria of NMT 0.3 %.

Ordering Information

3 µm Minibore, MidBore™, and Analytical Columns (mm)								SecurityGuard™ Cartridges (mm)	
Phases	**50 x 2.0**	**150 x 2.0**	**150 x 3.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 2.0***	**4 x 3.0***
								/10pk	/10pk
Amylose-1	00B-4729-B0	00F-4729-B0	00F-4729-Y0	00B-4729-E0	00D-4729-E0	00F-4729-E0	00G-4729-E0	AJ0-9337	AJ0-9336
							for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Minibore, Analytical and Semi-Prep Columns (mm)							SecurityGuard Cartridges (mm)		
Phases	**50 x 2.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**250 x 10.0**	**4 x 2.0***	**4 x 3.0***	**10 x 10.0‡**
							/10pk	/10pk	/3pk
Amylose-1	00B-4732-B0	00B-4732-E0	00D-4732-E0	00F-4732-E0	00G-4732-E0	00G-4732-N0	AJ0-9337	AJ0-9336	AJ0-9344
						for ID:	2.0–3.0 mm	3.2–8.0 mm	9.0–16.0 mm

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard Cartridges (mm)	
Phases	**150 x 21.2**	**250 x 21.2**	**250 x 30**	**250 x 50**	**15 x 21.2****	**15 x 30.0♦**
	Inquire	Inquire	Inquire	Inquire	/ea	/ea
Amylose-1	00F-4732-P0-AX	00G-4732-P0-AX	00G-4732-U0-AX	00G-4732-V0-AX	AJ0-9338	AJ0-9339
				for ID:	18–29 mm	30–49 mm

* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282
‡ Semi-Prep SecurityGuard Cartridges require holder, Part No.: AJ0-9281
**HPLC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223
SFC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8617
♦ HPLC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277
SFC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8618

guarantee

If Lux analytical columns (≤ 4,6 mm ID) do not provide at least an equivalent or better chiral separation as compared to a competing column of the same particle size, similar phase and dimensions, send in your comparative data within 45 days for a FULL REFUND.

APPLICATIONS

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