Application Note: 405

Analysis of Simvastatin Tablets by High Speed LC

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Key Words

- Accela™ High Speed LC
- Hypersil GOLD™ Column
- Pharmaceuticals
- Throughput

Goal:

Increase throughput of the USP Method for Simvastatin tenfold by employing high-speed liquid chromatography on a 1.9 µm Hypersil GOLD column. Demonstrate by example how to transfer a conventional HPLC method to high speed format.

Introduction

A 100 mm Hypersil GOLD column containing 1.9 µm diameter particles will deliver the same efficiency as a 250 mm column containing 5 µm particles, in less time. Even novice chromatographers can transfer inefficient older methods to high-speed LC by consulting an easy-to-use online Method Transfer Calculator. This application note shows how to transfer a method to high-speed LC, using the USP Method for Simvastatin as an example.

Simvastatin belongs to the group of cholesterol-lowering lactones known as statins, which in 2007 have been among the most widely prescribed drugs in the world. Statins lower cholesterol by inhibiting the synthesis of mevalonic acid, which is a key precursor in cholesterol synthesis. Dropping mevalonic acid levels triggers the expression of more low-density lipoprotein (LDL) receptors in the liver, which then removes LDL from the bloodstream.[1] Originally isolated from molds such as Aspergillus, several newer statins are synthetically produced, including fluvastatin, atorvastatin and pravastatin. Useful physiochemical properties of several statins are presented in Table 1, and their structures are shown in Figure 3.

Statin	Mevastatin	Lovastatin	Simvastatin
CAS	73573-88-3	75330-75-5	79902-63-9
Formula (lactone)	$C_{23}H_{34}O_5$	$C_{24}H_{36}O_5$	$C_{25}H_{38}O_5$
MW (g/mol)	390.52	404.55	418.57
LogP	3.98	4.26	4.68
Formula (acid)	$C_{23}H_{36}O_6$	$C_{24}H_{38}O_6$	$C_{25}H_{40}O_6$
MW (acid)	408.53	422.56	436.58
Water solubility	4.8 mg/L	0.4 mg/L	0.03 mg/L
Ethanol solubility	20 mg/mL	10 mg/mL	10 mg/mL

Table 1: Useful properties of the statins

The USP method for Simvastatin Tablets (30-NF25) employs HPLC with UV detection at 238 nm.[2] Simvastatin elutes at about 10 min on a 250 mm × 4.6 mm L1 column with an isocratic mobile phase containing 35:65 (v/v) 38 mM phosphate buffer (pH 4.5): acetonitrile flowing at 1.5 mL/min. The USP method requires the chromatographic performance to meet the following criteria: capacity factor (k′) >3; Efficiency (N, no. plates) > 4500; Asymmetry < 2.0; Precision (Peak area %RSD, n = 3), 2.0%. For a definition of these parameters, see Reference 2.

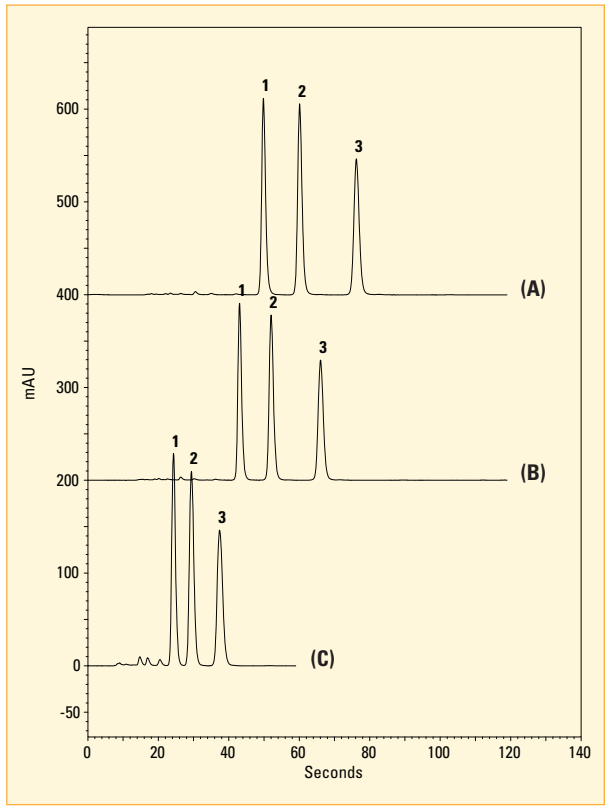

Figure 1. Separation of three statins on the Accela High Speed LC by reversed phase chromatography with UV absorbance detection at 232 nm. Peak 1, mevastatin; peak 2, lovastatin, peak 3, simvastatin, 100 mg/L each. Conditions: top trace (A) High Speed LC Method 1; middle trace (B), High Speed LC Method 2; bottom trace, High Speed LC Method 3; see text for details.

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Experimental

Instrumentation

Accela™ High-Speed Liquid Chromatography system with PDA Detector, ChromQuest™ 4.2 Chromatography Data System (CDS).

Chromatographic conditions

Columns: Hypersil GOLD, 1.9 µm, 100 × 2.1 mm
(P/N 25002-102130)
Hypersil GOLD, 1.9 µm, 50 × 2.1 mm
(P/N 25002-052130)
Mobile phase: A: 38 mM phosphate buffer, pH 4.5
B: acetonitrile
Isocratic: 35:65
Flow rate: 823 or 1000 µL/min
Detector: PDA, D_2 lamp, 238 nm, 10-mm flow cell, 11 nm bw, 20 Hz, 0s rise time.
Column temp.: 45 °C
Injection: 5 µL sample loop, 1 µL partial loop injection

Chemicals

Water, HPLC-grade	Thermo Fisher Scientific W5
Acetonitrile, HPLC-grade	Thermo Fisher Scientific A998-1
Sodium phosphate monobasic monohydrate	JT Baker 4011-01
Lovastatin, 25 mg	Calbiochem 438185, La Jolla, CA
Mevastatin, 50 mg	Calbiochem 474700
Simvastatin, 50 mg	Calbiochem 567020
Simvastatin Tablet	Teva Pharmaceutical Industries Ltd., Petach Tikva Israel

Consumables

Nalgene Filter Unit, 0.2 µm Nylon	Thermo Fisher Scientific 09-740-26A
Syringe filters, 0.45 µm Nylon	Thermo Fisher Scientific A5307-010
Autosampler vials, 1.8 mL glass	Thermo Fisher Scientific A4954-010
50 µL in-line static mixer	Thermo Fisher Scientific 109-99-00032

Mobile Phase

Phosphate buffer (38 mM, pH 4.5): Dissolve 3.9 g of sodium phosphate monobasic monohydrate in 800 mL of HPLC-grade water. Measure the pH and adjust to 4.5 ± 0.2. Bring to volume with HPLC-grade water in a 1-L volumetric flask and thoroughly mix.

Premixed mobile phase: Combine 350 mL of phosphate buffer with 650 mL of HPLC grade acetonitrile. Mix, filter through a 0.45 µm Nylon filter unit and degas by sonicating under vacuum for 5 min. Transfer to Solvent Reservoir Bottle A of the Accela pump and purge the solvent line with at least 30 mL of fresh mobile phase.

Proportioned mobile phase: If desired, proportion the phosphate buffer and acetonitrile 35:65 to produce the mobile phase. First, filter the phosphate buffer through a 0.45 µm Nylon filter unit and degas by sonicating under vacuum for 5 min. Transfer to Solvent Reservoir Bottle A of the Accela pump and purge the solvent line with at least 30 mL of fresh buffer. Connect a fresh bottle of HPLC-grade acetonitrile to Reservoir B and purge as above.

Dilution solution: Add 3.0 mL of glacial acetic acid to 900 mL of HPLC-grade water. Measure pH and adjust to 4.0 with 5N sodium hydroxide. Bring to volume with HPLC-grade water in a 1-L volumetric flask. To 200 mL of this solution, add 800 mL of HPLC-grade acetonitrile, and mix.

Calibration Standards

Simvastatin, 200 mg/L: Accurately weigh 10 mg of Simvastatin into a 50-mL volumetric flask, dissolve in a small quantity of Dilution solution, and bring to volume with Dilution solution.

Mixed statins, 200 mg/L: Accurately weigh 10 mg each of Simvastatin, Mevastatin and Lovastatin into a 50-mL volumetric flask, dissolve in a small quantity of Dilution solution, and bring to volume with Dilution solution.

Calibration standards: Use a calibrated pipette to dilute the 200 mg/L standards with mobile phase in volumetric glassware to 100, 50, 20, 5, 1, 0.2, and 0.05 mg/L.

Samples

Dissolve a Simvastatin tablet (40 mg) by following the USP procedure to yield a sample solution nominally containing 200 mg/L Simvastatin. Filter the sample through a 0.45 µm nylon syringe filter into a glass autosampler vial and inject into the Accela system.

HPLC Method Transfer Calculator

The HPLC method transfer calculator is available at the following site: *http://www.unige.ch/sciences/pharm/fanal/lcap/divers/telechargements.php*

This tool is free to use and distribute in accordance with the guidelines provided at the site. For this application note, we used the isocratic method transfer calculator.

To transfer the USP Method for Simvastatin Tablets to High Speed LC, enter the following parameters into the Method Transfer Calculator:

Original Column Length, Original Column Diameter, Original Column, Particle size, Original Column Flow rate, Original Injection Volume, Transferred Column Length, Transferred Column Diameter, Transferred Column Particle Size (the calculator refers to the new column as "Transferred column").

The Method Transfer Calculator ouputs the Transferred Column Flow Rate and Transferred Column Injection Volume; it also calculates the expected improvements in separation efficiency and analysis time. Figure 2 displays the inputs and outputs for the USP method, with the outputs backshaded in light blue.

If you do not have access to the online method transfer calculator, you can perform two simple calculations to determine the flow rate and injection volume required for the High Speed LC methods.

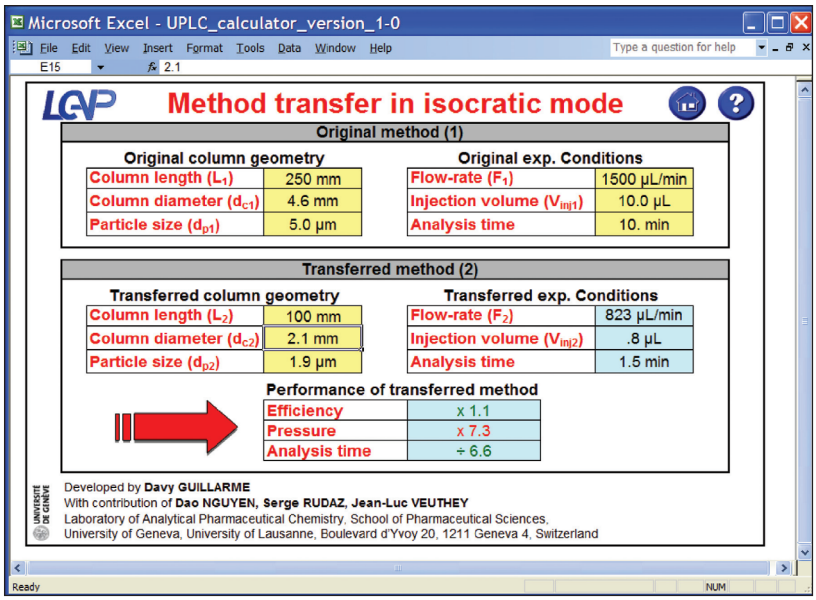

Figure 2: Online Transfer calculator

For the flow rate, the goal is to maintain the "reduced flow rate" constant between the conventional method and high speed method. Reduced flow rate is the linear flow rate divided by the particle size. Use the following equation:

Transferred flow rate = (original flow rate) x (transferred column diameter/original column diameter)^2 x (original column particle size/ transferred column particle size)

For High Speed Method #1,

Transferred flow rate = (1000 µL/min) x (2.1 mm/4.6 mm)^2 x (5 µm/1.9 µm) = 823 µL/min

For the injection volume, the goal is to maintain the ratio of injection volume to column volume constant between the conventional method and high speed method. Use the following equation:

Transferred injection volume = (original injection volume) x (transferred column diameter/original column diameter)^2 x (transferred column length/ original column length)

For High Speed Method #1,

Transferred injection volume = (10 µL) x (2.1 mm/4.6 mm)^2 x (250 mm/100 mm) = 0.8 µL

System Preparation

To ensure good performance of this application, prepare the system as directed in Appendix A.

Results and Discussion

The first step when transferring a conventional HPLC method to High Speed is to start with the conditions calculated by the method transfer calculator. A chromatogram obtained under the recalculated USP conditions is shown in Figure 1a. The performance obtained with this method (High Speed LC method #1) easily exceeds the USP requirements, as summarized in the third column of Table 2, and the analysis time is reduced 6-fold. High Speed Method #1 was also used to assay a drug tablet for simvastatin. As seen in Figure 4, Simvastatin is well resolved from impurities and degradation products that elute earlier in the chromatogram.

In step two of the method transfer, increase the flow rate to 1000 µL/min in order to complete the separation in 1 minute. A chromatogram obtained under these conditions is shown in Figure 1b; the performance obtained by High Speed method #2 still exceeds the USP requirements, as summarized in the fourth column of Table 2, and the analysis time is reduced 10-fold.

Table 3 highlights three important features of the Hypersil GOLD 1.9 µm stationary phase. First, Hypersil GOLD 1.9 µm columns operate at a higher optimal flow rate (362 µL/min) than competitive columns. Second, Hypersil GOLD 1.9 µm columns maintain high efficiency better than competitive columns as flow rate is increased. At 45 °C, an increase in flow rate from the optimum flow rate to 1000 µL/min is expected to cause a reduction in plate number on the Hypersil GOLD column of 14%, while the same change in flow rate reduces the plate number on the Competitor A column by 26%. Third, the Hypersil GOLD column's highly uniform packing profile minimizes backpressure; at 1000 µL/min, the pressure drop is only 753 bar, compared to 1030 bar for the Competitor A column and 1209 bar for the Competitor B column. Note that the Competitor E columns, limited to 600 bar, cannot be used at 1000 µL/min under the conditions of the USP Method for Simvastatin.

Simvastatin	USP 30-NF 25[2]	High-Speed LC #1 2.1 x 100 mm 45 °C 823 µL/min	High-Speed LC #2 2.1 x 100 mm 45 °C 1000 µL/min	High-Speed LC #3 2.1 x 50 mm 45 °C 823 µL/min
Retention time	9 min	81.6 s	63.8 s	37.8 s
k'	> 3	4.1	3.97	3.8
N, no. plates	> 4500	11463	11124	5793
Asymmetry	< 2.0	1.2	1.2	1.2
Precision, n = 3	< 2.0%	0.63%	0.24%	0.17%

Table 2: Performance criteria of USP Method for Simvastatin Tablets and measured performance of High Speed LC methods #1-3 performed on Hypersil GOLD 1.9 µm columns. USP criteria from Reference 2.

	Optimal flow rate (µL/min)	Working efficiency at 1000 µL/min (% of optimal N)	Plate loss at 1000 µL/min (% of optimal N)	ΔP at 1000 µL/min (bar)
Hypersil GOLD	362	86	–14	753
Competitor A	304	74	–26	1030
Competitor B	251	51	–49	1209
Competitor C	280	~~73~~	~~–27~~	~~959~~
Competitor D	338	~~88~~	~~–12~~	~~1105~~
Competitor E	228	~~49~~	~~–51~~	~~852~~

Table 3: Performance of sub-2 µm 2.1×100 mm Ultra High Pressure HPLC Columns, from Reference 3.

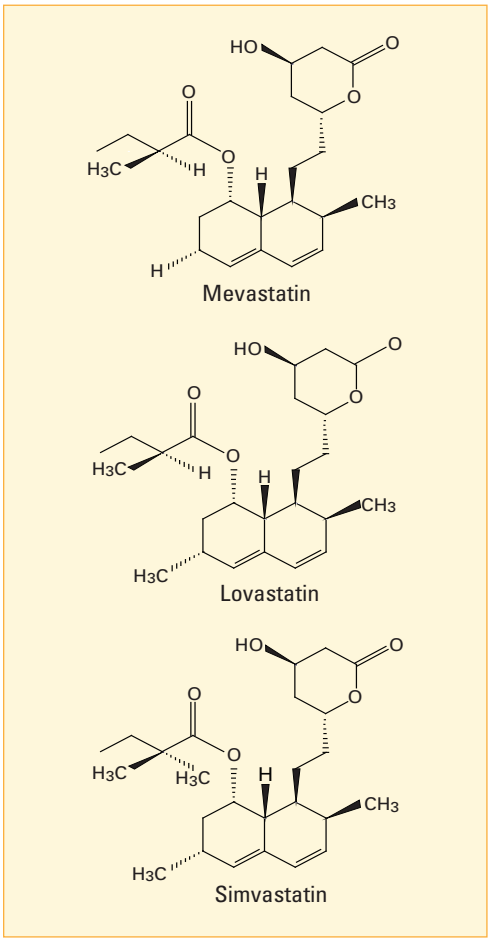

Figure 3: Chemical structures of Mevastatin, Lovastatin, and Simvastatin [redrawn after Ref. 1.]

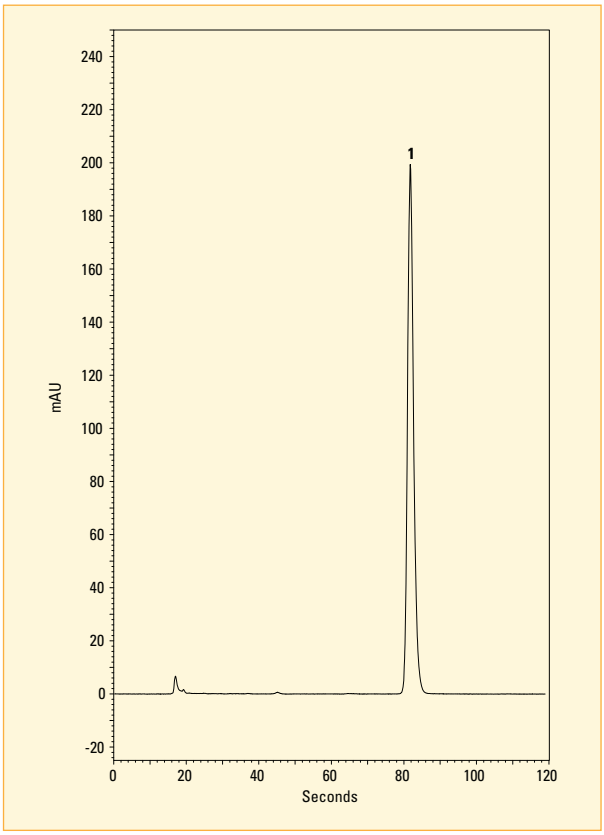

Figure 4: Determination of Simvastatin in Tablets on the Accela High Speed LC by reversed phase chromatography with UV absorbance detection at 232 nm. Peak 1, simvastatin, 100 mg/L. Conditions: High Speed LC Method 1 using Hypersil GOLD 1.9 µm, 2.1×100 mm column at 45 °C and 823 µL/min; see text for details.

For a routine analysis on well characterized samples, such as Simvastatin tablets, many chromatographers will want to push the limits of speed and throughput with a shorter column. For step three of the method transfer, perform this separation on a Hypersil GOLD 1.9 µm, 2.1 × 50 mm column to reduce the analysis time to 41 seconds. Although the 50 mm column develops only half the efficiency of the 100 mm column, the performance summarized in columns of Table 2 still exceeds the USP requirements. This column could be used to perform the USP method for Simvastatin Tablets 20X faster than the original method.

Although the USP method does not set requirements for resolution, linear calibration range and limits of detection, these performance parameters were measured for the high speed methods by including two other statins, Mevastatin and Lovastatin, in a mixture at the same concentration as Simvastatin. Table 4 summarizes the performance of the 100 mm column at 823 µL/min, Table 5 summarizes the performance of the 100 mm column at 1000 µL/min, and Table 6 summarizes the performance of the 50 mm column at 823 µL/min.

Conclusion

The Accela High Speed Chromatography system equipped with a Hypersil GOLD 1.9 µm column increases the throughput of a Simvastatin Tablet assay 10-fold with performance that exceeds the USP requirements. The conditions required for the high-speed method are conveniently calculated by using a web-based Method Transfer calculator or simple formulas for flow rate and injection volume.

References

[1] Endo, A., The discovery and development of HMG-CoA reductase inhibitors. *Journal of Lipid Research*, 33, 1992, 1569-1582.

[2] United States Pharmacopeia 30-National Formulary 25, United States Pharmacopeia, Rockville, Maryland 20852-1790, USA.

[3] Dao T.-T. Nguyen, Davy Guillarme, Serge Rudaz and Jean-Luc Veuthey, *J. Chromatogr. A*, 1128, 2006, 105-113

[4] From the "Chromatographic Performance" section found at http://www.unige.ch/sciences/pharm/fanal/lcap/divers/telechargements.php.

Method 1 N = 30	K′	R[a]	N # plates	Linear range, mg/L	r^2	MDL[b]	Precision, retention time % RSD	Precision, peak area % RSD
Mevastatin	3.2		8910	0.05–200	0.99987	n.d.	0.89	0.44
Lovastatin	4.1	4.8	10734	0.05–200	0.99992	n.d.	1.05	0.40
Simvastatin	5.5	6.4	11861	0.05–200	0.99989	n.d.	1.22	0.42

Table 4: Performance of High Speed Method #1 using Hypersil GOLD 1.9 µm, 2.1 × 100 mm column at 45 °C and 823 µL/min.
[a] Resolution (R) calculated according to Reference 2.
[b] n.d. indicates value not determined for this method.

Method 2 N = 30	K′	R[a]	N # plates	Linear range, mg/L	r^2	MDL mg/L	Precision, retention time % RSD	Precision, peak area % RSD
Mevastatin	2.6		7573	0.05–200	0.99963	n.d.[b]	0.88	0.81
Lovastatin	3.4	4.8	8615	0.05–200	0.99964	n.d.	0.94	0.78
Simvastatin	4.5	5.6	9293	0.05–200	0.99951	n.d.	0.94	0.82

Table 5: Performance of High Speed Method #2 using Hypersil GOLD 1.9 µm, 2.1 × 100 mm column at 45°C and 1000 µL/min.
[a] Resolution (R) calculated according to Reference 2.
[b] n.d. indicates value not determined for this method.

Method 3 N = 30	K′	R[a]	N # plates	Linear range, mg/L	r^2	MDL[b]	Precision, retention time % RSD	Precision, peak area % RSD
Mevastatin	3.2		1843	0.05–200	0.99972	0.032	1.03	0.15
Lovastatin	2.6	2.0	1996	0.05–200	0.99985	0.019	0.96	0.19
Simvastatin	3.4	2.6	4854	0.05–200	0.99996	0.037	0.29	0.67

Table 6: Performance of High Speed Method #3 using Hypersil GOLD 1.9 µm, 2.1 × 50 mm column at 45 °C and 823 µL/min.
[a] Resolution (R) calculated according to Reference 2.
[b] Minimum Detection Limit (MDL) calculated as the standard deviation times the students' t value for n = 7 replicates of a low level standard.

Appendix A.

System Preparation

Pump: Always plumb the Accela system with precut and polished 0.005″ i.d. high-pressure tubing and high pressure fittings as shown in Figure 15 of the Accela Pump Hardware Manual (Document 60157-97000 Revision B). For all tubing connections that you make, ensure that the tubing end is square-cut and burr-free. Firmly push the tubing into the injection valve port as you tighten the high-pressure fitting, in order to maximize peak efficiency. Prime the pulse dampener and purge the solvent lines as instructed in Chapter 4 of the Accela Pump manual. Verify that the pump is performing well by monitoring the pressure pulsation and by testing the pump proportioning accuracy as described in Chapter 5 of the pump manual.

Autosampler: Open the Instrument Configuration and verify that the Accela AS Configuration entry for "Dead volume" is correct (the calibrated volume in µL written on the transfer between the injection port and injection valve). Verify that the entry for "Loop size" is correct for the currently installed sample loop. Fill the Flush reservoir with 90:10 (v/v) methanol:water and flush the syringe with solvent to purge any air bubbles from the syringe and tubing.

Install the Hypersil GOLD, 1.9 µm 2.1×100 mm column, using a 10-cm length of precut and polished 0.005" i.d. high-pressure tubing and the high pressure fitting consisting of a nut, back ferrule and front ferrule. Ensure that the tubing is fully pushed into the column inlet when you tighten the high-pressure fitting. Consult the Accela Getting Connected manual (Document 60057-97001 Revision A) for details.

Detector: Use a 10 mm LightPipe™ flow cell. Add a short section of 0.005″ PEEK backpressure tubing to the flow cell outlet to suppress bubble formation in the flow cell. Verify that the deuterium lamp has been used for less than 2000 hours.

Use Direct Control or a downloaded method to equilibrate the Accela system under the conditions shown in Table 3: 45°C, 823 µL/min, and 0.8 µL injection. Create a method based on these operating conditions and then create a Sequence to make several injections of HPLC grade water. The system is ready to run standards and samples when the peak-to-peak baseline oscillation is between 50–200 µAU/min (average of ten 1-min segments) and no significant peaks elute in the retention time window of the analytes.

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