Running fast LC within USP limits

Case study with USP pravastatin sodium chromatographic purity method using the Agilent 1200 Series Rapid Resolution LC system

Application Note

Manufacturing QA/QC

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Abstract

Recent revisions in United States Pharmacopeia (USP) general chapter <621> allow for adjustments to be made in monographs to enhance the quality of the chromatogram in meeting system suitability requirements. These adjustments can be made use of to produce a fast method utilizing the Agilent 1200 Series Rapid Resolution LC (RRLC) system and Agilent Method Translator software. There are various ways to produce a fast method and one of them is to start with an established method such as a USP method.

This Application Note shows how to start from a USP method and incorporate the allowed adjustments to produce a fast method that meets system suitability requirements. The chromatographic purity test for pravastatin as per the USP method suggests a 30-minute gradient with a 3.5 µm particle size column and 1 mL/min flow rate. These chromatographic conditions can be adjusted to use a 1.8 µm particle and 1.5 mL/min flow rate, which will provide faster run times. Besides particle size and flow rate, column dimensions and column temperature can also be adjusted to produce a faster method. The transition to the fast method was quickly and effectively achieved by the use of Agilent Method Translator. Such fast methods derived from the USP can be used in high-throughout environments as the new method is closest to the validated monograph method.

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Introduction

Pravastatin sodium helps to reduce cholesterol biosynthesis, thereby preventing cardiovascular disease. The USP chromatographic purity test for pravastatin recommends using a L1 column with dimensions of 4.6 mm × 75 mm, 3.5 µm particle size and 1 mL/min flow rate. However, under the revised general chapter <621>, the USP allows adjustments in the chromatographic parameters to be made but only when, "adjustments have improved the quality of the chromatogram in meeting system suitability requirements."[1] In this Application Note, we have taken advantage of the permittable adjustments in the USP to produce a faster method and test system suitability parameters. Faster runs are possible by starting with any HPLC method and determining what parameters are required if choosing smaller id and smaller particle column. Further increase in speed is possible by increasing the temperature and flow rate while decreasing the gradient time.[2] The faster LC run has the advantage of saving money in terms of solvent consumption as well as higher productivity in analyzing a larger number of samples.

Table 1 shows the permittable adjustments in the chromatographic parameters and its application in the pravastatin method.

As per the revised general chapter <621>, the pH of the mobile phase, the concentration of the salts in the buffer, the ratio of the components in the mobile phase, and the wavelength can also be adjusted but were not done so in this experiment.

Pravastatin sodium has six impurities that are reported by the USP:

1. 3″-Hydroxypravastatin
2. 6′-Epipravastatin
3. 3α-Hydroxyisocompactin (also called Related Compound A or RCA)
4. Pentanoyl impurity
5. Pravastatin lactone
6. Compactin

Only impurities 2, 3, 5, and 6 were used to demonstrate the fast impurity method on the Agilent RRLC system.

Permitted adjustments	Original pravastatin chromatographic method	Permitted limits	Fast gradient conditions
Column length (± 70%)	75 mm	22.5–127.5 mm	30 mm
Column id (± 25%)	4.6 mm	3.45–5.75 mm	4.6 mm
Particle size (–50%)	3.5 µm	1.75 µm	1.8 µm
Flow rate (± 50%)	1.0 mL/min	0.5–1.5 mL/min	1.5 mL/min
Injection volume (reduced until it is consistent)	10 µL	Variable	4.0 µL*
Column temperature (±10 °C)	Not specified	Variable	35 °C

*Value obtained from Agilent Method Translator

Table 1
The permittable adjustments in the USP pravastatin chromatographic purity method.

Experimental

Sample preparation

- **Diluent:** A 1:1 mixture of methanol and water was prepared. The methanol was HPLC grade (J.T. Baker). Milli-Q water (Millipore) was used for the experiment.
- **Relative retention time (RRT) test sample:** Pravastatin sodium and four of its impurities (6′-epipravastatin, RCA, pravastatin lactone, and compactin) were dissolved in diluent to a concentration of 65 µg/mL for pravastatin and 7.5 µg/mL for the impurities. The RRT test sample was prepared to determine the RRT of each impurity, for comparison with values reported in the USP. Pravastatin lactone and compactin were obtained from Varda Biotech (Mumbai, India). RCA was obtained from the USP and 6′-epipravastatin was obtained from the European Pharmacopoeia (EP).
- **System suitability sample:** As described in the USP, pravastatin 1,1,3,3-tetramethylbutylamine (pravastatin-TMBA) and RCA were dissolved in diluent to obtain a concentration of 0.6 mg of pravastatin-TMBA and 0.001 mg of RCA per milliliter. The system suitability sample was used to determine the value of the RRT of RCA versus pravastatin and the resolution between them. Both pravastatin-TMBA and RCA were obtained from the USP.
- **Standard sample:** Pravastatin-TMBA was dissolved in diluent to a concentration of 1.25 µg/mL. The standard sample was used to determine the relative standard deviation (RSD) of the peak areas.

- **Linearity test sample:** Six concentration levels of RCA from 0.25 to 1.10 µg/mL were prepared as spiked amounts in a 0.5 mg/mL sample of pravastatin sodium.

Equipment

The Agilent 1200 Series Rapid Resolution system included:

- Agilent 1200 Series binary pump with degasser
- Agilent 1200 Series autosampler SL and Agilent 1200 Series autosampler SL Plus with thermostat. Both modules were used interchangeably in the same LC system
- Agilent 1200 Series diode-array detector
- Agilent 1200 Series thermostatted column compartment SL
- Agilent 1200 Series RRLC mode: Binary pump in low delay volume configuration (without the mixer and damper in the flow path), and low delay volume capillaries (0.12 mm id) beyond the injection valve were used. Selecting a low delay volume reduction in the autosampler did not make any major difference in elution of the peaks. Modules were stacked in two parts such that the pump and degasser were separate from the rest of the modules to minimize delay volume as described earlier[3].
- HPLC mode: No delay volume configuration was made in the binary pump and standard green tubing (0.17 mm) was used at all points. Module stacking was similar to the RRLC mode.
- Agilent Method Translator, version 2.0

Parameters		Detail	
Wavelength for DAD		238 nm BW 4, Ref 300, 20	
Column		USP method: Agilent ZORBAX SB-C18, 4.6 mm × 75 mm, 3.5 µm Fast method: Agilent ZORBAX SB-C18, 4.6 mm × 30 mm, 1.8 µm	
Diluent		50:50 methanol:water	
Needle wash		Flush port, 3 sec using diluents	
Sample temperature		15 °C	
Mobile phase		Buffer pH 7.0: 0.08 M phosphoric acid solution adjusted with triethylamine to pH 7.0 Buffer A: Water:buffer pH 7.0:acetonitrile (52:30:18) Buffer B: Water:buffer pH 7.0:acetonitrile (10:30:60)	
USP method		Time	% Buffer B
Flow rate	1.0 mL/min	0	0
Post time	3.0 min	3.0	0
Column temperature	25 °C	26.5	100
Injection volume	10 µL	26.6	0
Response time	2 sec	30	0
Fast method		Time	% Buffer B
Flow rate	1.5 mL/min	0	0
Post time	0.8 min	0.8	0
Column temperature	35 °C	7.07	100
Injection volume	4.0	7.09	0
Response time	1 sec	8.0	0

Results and discussion

Retention time of impurity relative to the pravastatin retention time in fast run

Agilent Method Translator was used to convert the USP pravastatin chromatographic method into a fast method. The output method from Agilent Method Translator was used without any modifications. The desired flow rate can be input into the translator, which calculates the respective gradient condition. The advantage in using the Agilent 1200 Series RRLC system is that it is interconvertible between HPLC mode and RRLC mode. In this Application Note, HPLC mode was used to run the conventional USP method and RRLC mode was used in the fast method.

An RRT test sample containing a mixture of four impurities along with pravastatin was tested in both HPLC and RRLC modes. Figure 1 shows the chromatographs of the RRT test sample tested in both modes. A fast chromatograph show adequate separation of all the four impurities in a manner similar to the conventional method.

The RRT test sample was used to calculate RRTs and for comparison with the RRT provided in the USP. Table 2 shows the comparison and excellent match between the RRTs obtained experimentally and those reported in the USP method. The results show that the RRT values as reported in the USP are about the same as those obtained by the fast method.

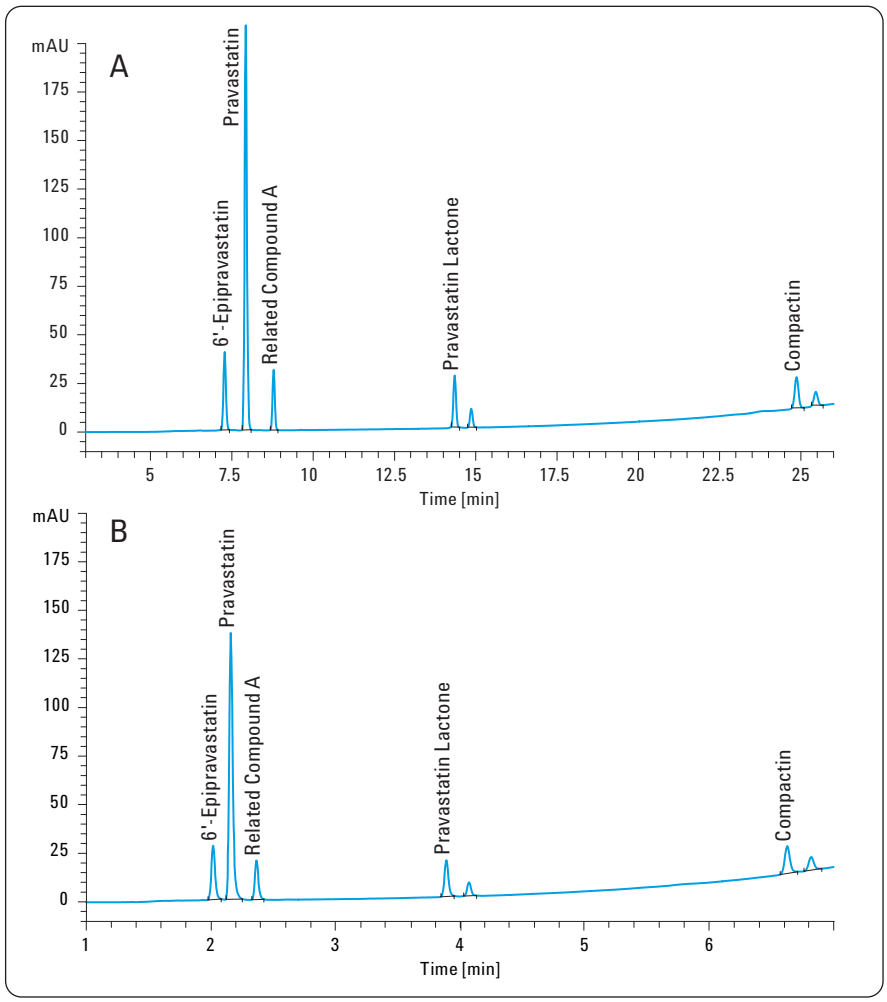

Figure 1
A) Pravastatin chromatogram performed as per the USP method. B) Pravastatin fast method based on Agilent Method Translator.

Name	USP-reported RRT	Experimentally obtained USP method (4.6 mm × 75 mm 3.5 µm column)	Experimentally obtained fast method (4.6 mm × 30 mm, 1.8 µm column)
3"-Hydroxypravastatin	0.33	_*	_*
6'-Epipravastatin	0.92	0.92	0.94
3α-Hydroxyisocompactin	1.1	1.1	1.1
Pentanoyl impurity	1.2	_*	_*
Pravastatin lactone	1.8	1.8	1.8
Compactin	3.1	3.1	3.2

* Impurity not included in the experiment

Table 2
RRTs of the four out of six impurities reported in the USP method along with the results obtained experimentally as performed by the USP method and the fast method.

System suitability of fast run using autosampler SL plus

According to the USP, a system suitability mixture containing pravastatin-TMBA and RCA spiked at the limit concentration of 1 µg/mL should show a resolution of not less than (NLT) 2.0 and RCA should have an RRT of about 1.1. Figure 2 shows the overlay of three replicate injections of the system-suitable sample performed by the fast method. The results show that RCA has an RRT of about 1.1 and a resolution of 3.8, which meets the system suitability requirements.

Standard deviation of pravastatin peak area at low concentration

The standard sample containing 1.25 µg of pravastatin-TMBA was injected six times to test the RSD of the peak area of pravastatin. According to the USP method, six replicate injections of the standard sample should have an RSD for the peak area of not more than (NMT) 10.0%. A value of 1.0% was obtained, which meets the precision requirement.

Linearity of spiked impurity in fast run

The USP method specifies an upper limit concentration of 0.2% for RCA, which is 1 µg/mL, assuming pravastatin has a concentration of 0.5 mg/mL. To test the detector response and determine the injector's precision, RCA was prepared at a concentration ranging from 0.25 to 1.1 µg/mL and spiked into 0.5 mg/mL pravastatin sodium solution. Table 3 shows the signal-to-noise (S/N) ratios obtained at each of the spiked levels.

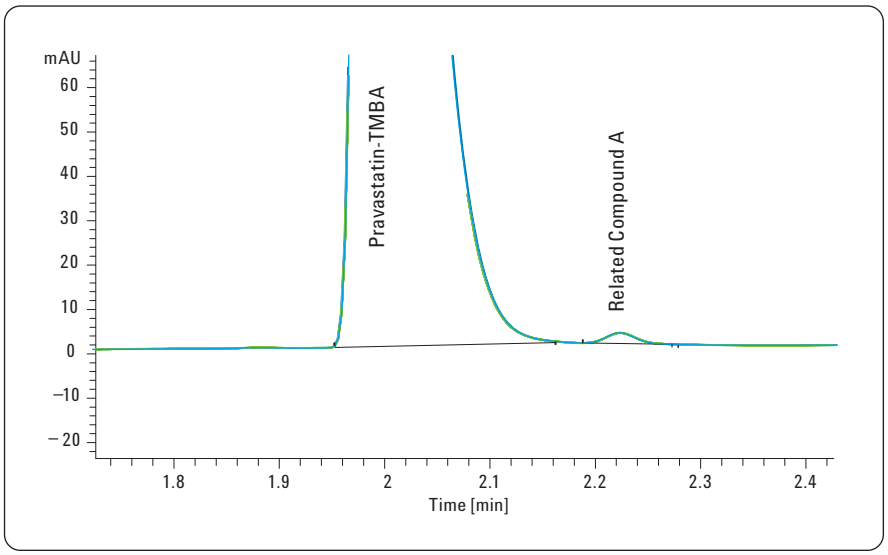

Figure 2
Overlay of three replicate injections of the system suitability sample.

Level	RCA in µg/mL (% impurity)	S/N ratio
1	0.25 (0.05)	11.8
2	0.5 (0.10)	25.0
3	0.7 (0.14)	32.3
4	0.9 (0.18)	34.9
5	1.0 (0.20)	38.6
6	1.1 (0.22)	43.3

Table 3
RCA spiked at levels below and above the limit concentration of 1.0 µg/mL.

The spiked level with a concentration of 0.25 µg/mL that corresponds to 0.05% is the limit of quantification (LOQ) for the fast LC experiment. Figure 3 shows the overlaid chromatogram at each of the injection levels. A uniform rise in the injection amount is seen with the increase in the level of spiked sample. The linearity plot of the injection level is shown in Figure 4, where the correlation coefficient (R^2) for the linearity is 0.998.

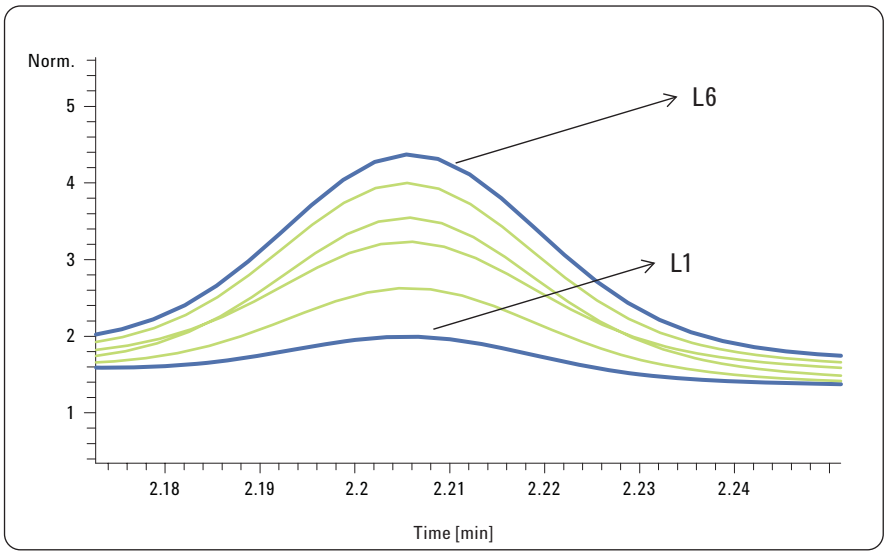

Figure 3
The linearity of spiked sample of RCA at spike levels of 0.25 µg/mL (L1) to 1.1 µg/mL (L6).

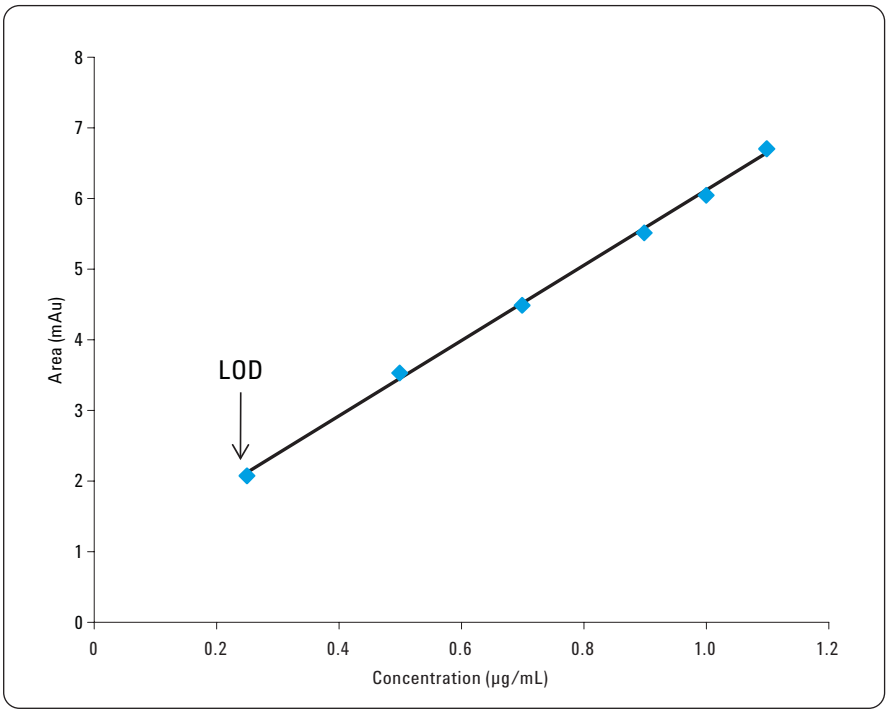

Figure 4
The graph of linearity solution for RCA shows that the concentration of 0.25 µg/mL is the concentration closest to the limit of detection (LOD).

Conclusion

Validating analytical methods can be less time consuming if run time is reduced without compromising chromatographic properties. Fast runs contribute to cost savings in routine quality assurance/quality control. A faster 8-minute pravastatin chromatographic method was determined from the original 30-minute USP pravastatin chromatographic purity method. This faster method was initiated by modifying only those parameters that were permitted in recent USP adjustments for chromatographic parameters. The new gradient conditions were obtained from Agilent Method Translator and were used without any modifications. This new fast method meets all the system suitability requirements. The resolution between RCA and pravastatin was 3.8, which is well above the NLT 2.0 limit. Precision test with standard solution obtained an RSD of 1.0% which is less than the 10% acceptable limit. A detector linearity test for the fast method obtained an R^2 value of 0.998. The fast method obtained by modifying only within the USP limits shows that the cost per sample is reduced by more than three times.

References

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3. M. Woodman, "Improving the Effectiveness of Method Translation for Fast and High Resolution Separations," Agilent Application Note, Publication number 5989-5177EN, **2006**

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