Improved Simvastatin Analysis Using Agilent ZORBAX Eclipse Plus C18 5 µm, 3.5 µm and 1.8 µm Columns.

Application Note

Pharmaceutical

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Abstract

Simvastatin belongs to a class of cholesterol-lowering drugs called statins, which are among the world's most widely prescribed drugs. This application note describes adjustments that can be made to the USP method for Simvastatin tablet analysis. The USP method uses a 250-mm, 5-µm Agilent ZORBAX Eclipse Plus C18 column, and has a 10-min run time. Simple modifications to this method can make it appropriate for use with a Rapid Resolution Eclipse Plus C18 4.6 mm x 100 mm, 3.5-µm or a Rapid Resolution HT Eclipse Plus C18 4.6 mm x 50 mm, 1.8-µm column, providing a shorter analysis time with significant time and solvent savings. All columns are run at pressures under 400 bar, so that they can be run on almost any instrument. Robustness, linearity and column longevity are described within this note.

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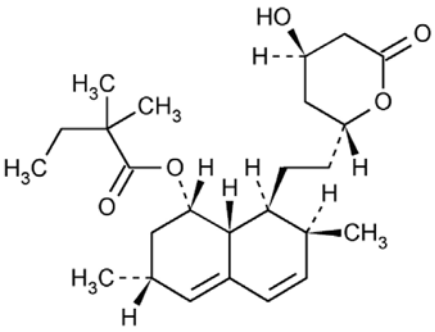
Introduction

Simvastatin belongs to a class of cholesterol-lowering drugs called statins, which are among the world's most widely prescribed drugs. The structure of Simvastatin is shown in Figure 1. After the drug is ingested it is hydrolyzed to a material that interferes with the production of cholesterol in the liver. Simvastatin lowers overall cholesterol levels as well as LDL cholesterol. Medical studies have linked high LDL cholesterol to coronary artery disease. Other major statin products available in the United States include: Atorvastatin, Fluvastatin, Lovastatin, Pravastatin and Rosuvastatin [1]. Many of these are also found in combination medications [1]. Simvastatin is typically administered as a tablet in 5 to 80 mg dosages together with inactive excipients such as cellulose, lactose and starch. It is presently produced around the world as a generic drug.



CAS: 79902-63-9, WM = 418.57

2,2-Dimethylbutyric acid, 8-ester with (4*R*, 6*R*)-6-2-[(1*S*, 2*S*, 6*R*, 8*S*, 8α*R*)-1, 2, 6, 7, 8, 8a-hexahydro-8-hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetrahydro-4-hydroxy-2*H*-pyran-2-one

Figure 1 Structure of Simvastatin.

Reduction of cost while producing a high quality product is a goal of pharmaceutical and generic pharmaceutical manufacturers. Using the USP Simvastatin tablet method (250 mm × 4.6 mm, 5-µm column) as a basis of comparison, Eclipse Plus C18 columns are shown to offer a time savings of 60 to 80 % as well as an associated saving in solvent. Since the solvent used is acetonitrile the adjustment to smaller particle, shorter columns may be especially attractive.

Experimental

Glacial acetic acid, sodium hydroxide solution, phosphoric acid, monobasic sodium phosphate and Simvastatin were obtained from Sigma-Aldrich. USP Simvastatin was obtained from United States Pharmacopeia.

High-performance liquid chromatography (HPLC) analysis was performed with the Agilent 1200 Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL, Mobile phase Channel A: pH 4.5 phosphate buffer in water, Channel B: Acetonitrile, (35:65); Flow rate was 1.5 mL/min (conditions varied for robustness testing)
- G1376C automatic liquid sampler SL (ALS), injection volume was 10 µL for the 250 mm column, 6 µL for the 150-mm column, 4 µL for the 100 mm column, 2 µL for the 50-mm column
- G1316B Thermostated Column Compartment SL(TCC), Temperature was 45 °C
- G1316C Diode Array Detector SL (DAD), wavelength used was 238, 4 nm 360, 50 mm with a G1315-60024 micro flow cell (5-mm path, 6 µL volume)

ZORBAX Columns:

- Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm p/n 959990-902
- Rapid Resolution Eclipse Plus C18, 4.6 mm × 100 mm, 3.5 µm p/n 959961-902
- Rapid Resolution HT Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 µm p/n 959941-902

Sample and mobile phase preparation from the USP Simvastatin Tablet analysis method [2]

Sample Preparation

1. Add 3 mL of glacial acetic acid to 900 mL water in a 1-liter volumetric flask.
2. Adjust the pH to 4.0 with a 5-N solution of sodium hydroxide.
3. Combine 200 mL of this solution and 800 mL of acetonitrile to form the dilution solution.
4. Prepare a standard at 0.1 mg/mL using USP Simvastatin standard in a 100 mL volumetric flask.

Assay Preparation

1. Transfer 10 Simvastatin tablets to a 250-mL volumetric flask and add water (5 to 10 mL).
2. Swirl the tablets until dissolved.
3. Dilute the tablet solution to volume with dilution solution.
4. Centrifuge a portion of the mixture and dilute a portion of the clear supernatant to produce a solution with a final concentration of 0.1 mg/mL Simvastatin.

Mobile Phase Preparation

1. Dissolve 3.9 g of monobasic sodium phosphate in 900 mL of water.
2. Confirm the pH as 4.5 and adjust according to USP procedure with either 50 % sodium hydroxide or 85 % phosphoric acid if necessary.
3. Dilute the solution with water to 1000 mL and mix. Mobile phase consists of a 65:35 mixture of acetonitrile and buffer.

The chromatographic and performance requirements of the method are listed in the USP method. These are summarized below [2]

- 238 nm detection
- 4.6 mm × 250 mm column, L1 column (C18), 10 µL injection
- 45 °C
- 1.5 mL/min flow rate
- k' not less than 3
- N ≥ 4500 plates
- USP Tailing factor (T_f) not more than 2.0

As can be seen in Figure 2, the efficiency and other chromatographic performance requirements of the USP method are easily met. During the course of a day using the USP method as written, an analysis can be performed every 10–12 minutes. This leads to a throughput of 5 to 6 analyses per hour, or approximately 120 injections that can be made per day at 12 minutes per injection. Over the course of a week, 1000 injections can be performed using a 250 mm, 5-µm column. In many applications this throughput is sufficient. An increased throughput can be achieved by adjusting the method.

The USP updated chapter <621> presents recommendations on how much a method can be modified such that the changes are considered an adjustment.

- Column length: ± 70 %
- Column internal diameter: ± 25 %
- Column material particle size: Reduction of up to 50 %, no increase
- Flow rate: ± 50 %
- Injection volume: Changes are allowed as long as system suitability testing (SST) criteria are met
- Column temperature: ± 10 %
- pH of mobile phase: ± 0.2
- UV wavelength: no change outside manufacturer specifications
- Concentration of salts in buffer: ± 10 %

Modifications outside these ranges are considered changes and require re-validation. If the analyst chooses to use a shorter column, such as a 4.6 mm × 100 mm, 3.5-µm column as shown in Figure 3a, the same analysis could be accomplished in 40 % of the time (approximately 4 minutes per

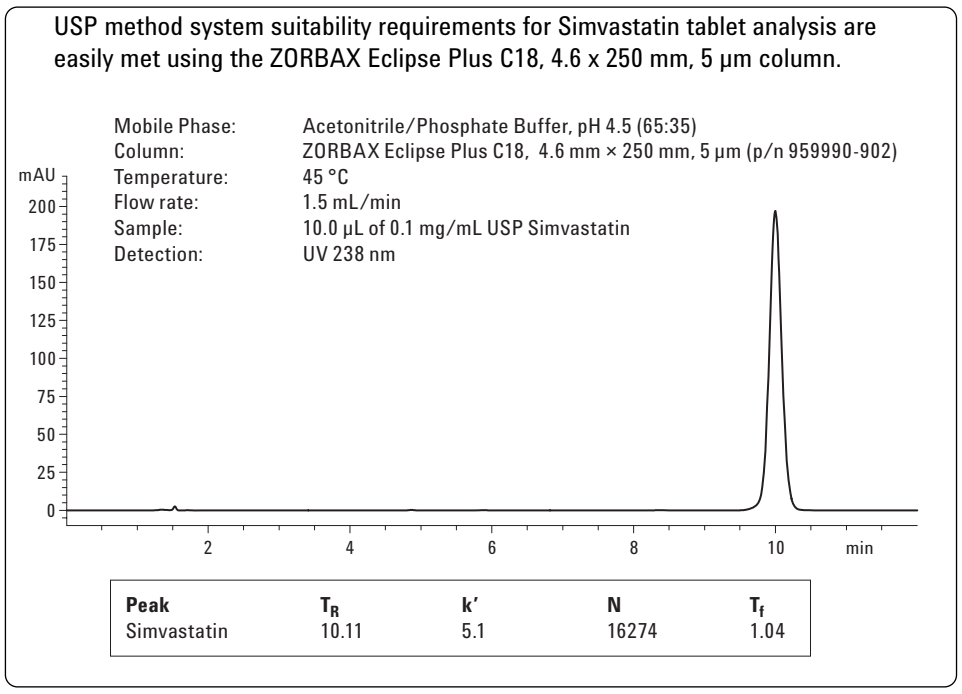


Figure 2. USP method for Simvastatin tablets using ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm 5 µm columns.

injection). Further only 40 % of the solvent would be used. USP guidelines allow certain "adjustments" without revalidation of methods. These points include length (± 70 %), diameter (± 25 %), and particle size (reduction of particle size only by 50 %, no increase allowed). As can be seen in Figure 3a, performance requirements are met within the allowable adjustments of length, diameter, and particle size. Even so, when most laboratories implement a new method some validation takes place.

The analysis could also be performed using a 4.6 mm × 50 mm, 1.8-µm Eclipse Plus C18 column as shown in Figure 3b. While the performance requirements of the USP method are easily met, it is necessary to revalidate the method as it is outside the limits of 621 method adjustment. The length has been reduced by 80 %, and the particle size has been reduced by 62 %. Validation steps that are required include demonstration of linearity and robustness testing for temperature, pH and organic content.

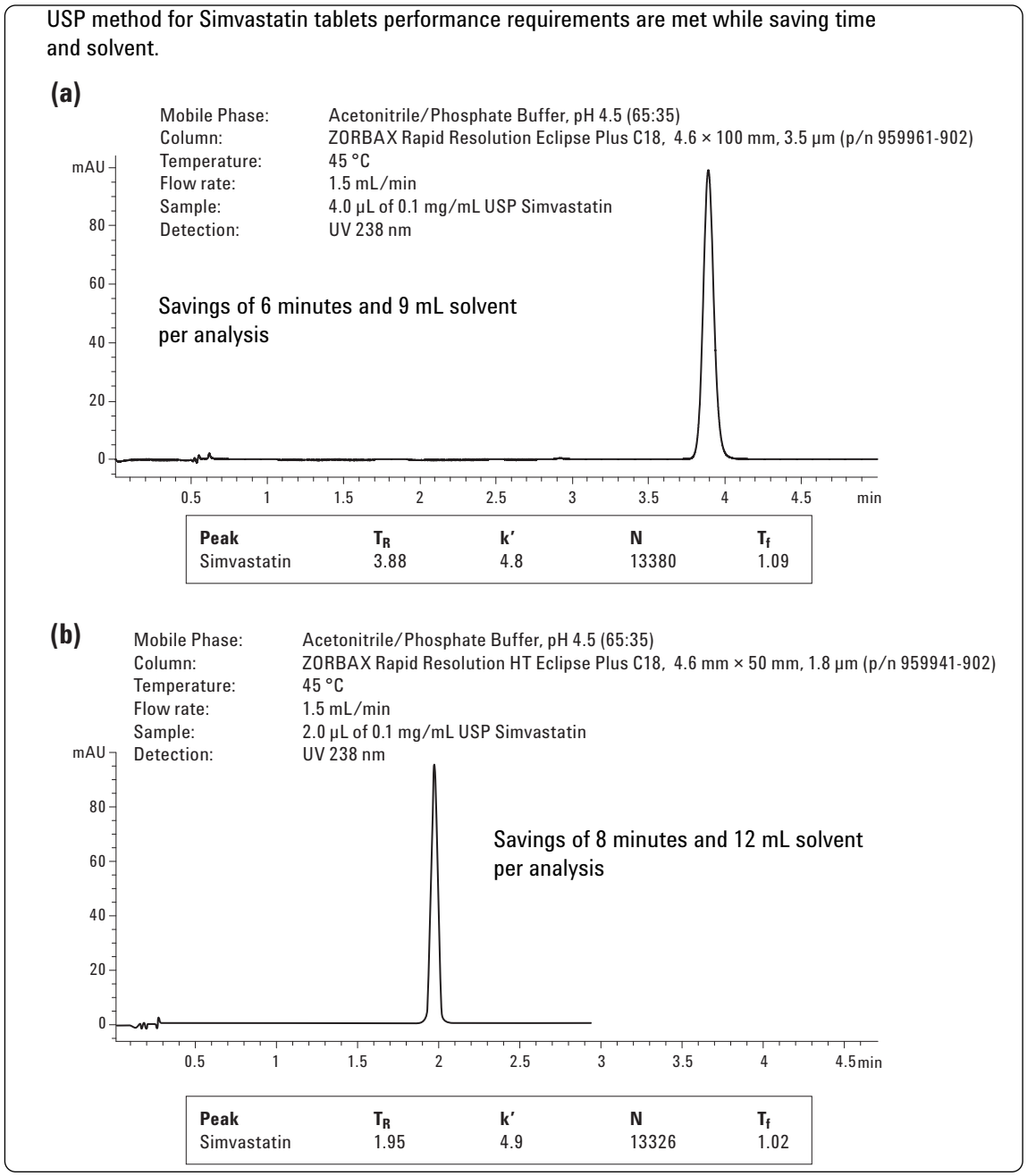
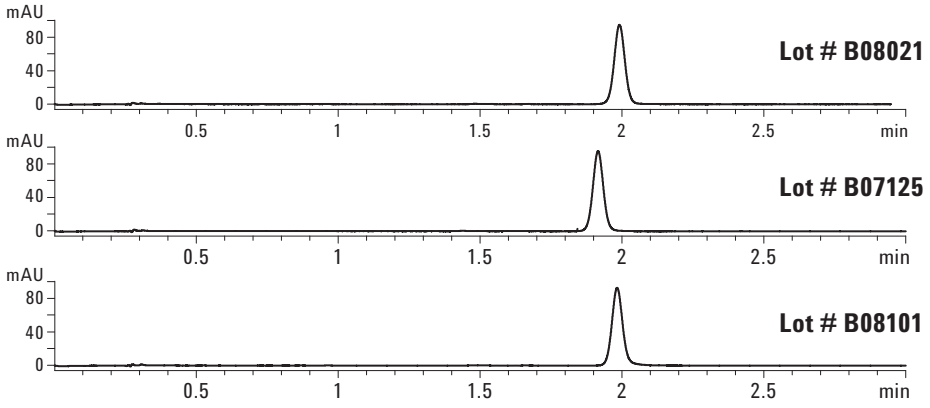


Figure 3. Overlay of chromatograms showing scaled USP Simvastatin Tablet Method on shorter columns with smaller particle size.

The demonstration of stable column batch to batch reproducibility is a critical step that is frequently misunderstood. This requirement in validation verifies that different batches of packing material yield the same separation results. The purpose of this step is to ensure that no single production of manufactured column packing has unique selectivity to make a method successful. It is a good idea to understand how much variability in separation may occur for columns that will be purchased for the life of a method. The column batch numbers of Agilent ZORBAX columns appear on the outside of the box, as well as on the data sheet inside the box, for the convenience of customers. Figure 4 shows three columns with different lot numbers. As illustrated, the consistency of the performance data achieved by the Eclipse Plus C18, 4.6 mm x 50 mm, 1.8-µm columns (approximately N = 13,000, $T_f \geq 1.0$ and $k' \geq 4.9$) is substantially better than the requirement for the analysis.

Robustness testing with 3 column lots is successful and accomplished quickly using ZORBAX RRHT Eclipse Plus C18 columns.

Mobile Phase:	Acetonitrile/Phosphate Buffer, pH 4.5 (65:35)
Column:	ZORBAX RRHT Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 µm (p/n 959941-902)
Temperature:	45 °C
Flow rate:	1.5 mL/min
Sample:	2.0 µL of 0.1 mg/mL USP Simvastatin
Detection:	UV 238 nm

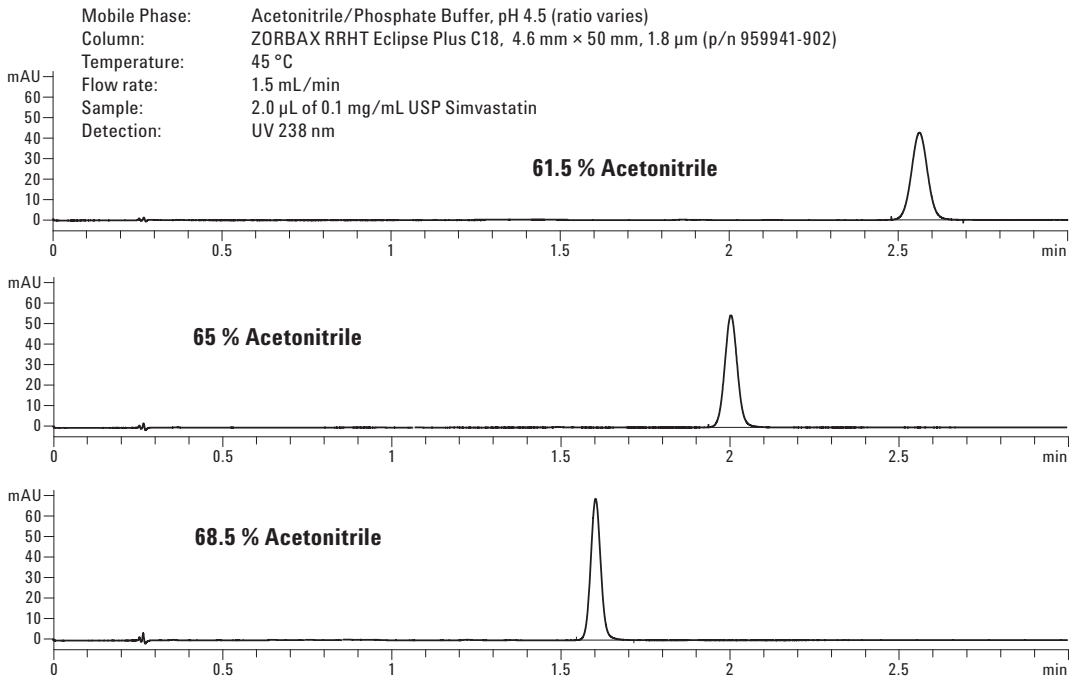


Peak	T_R	k'	N	T_f
B08021	1.99	5.0	13455	1.02
B07125	1.92	4.9	13001	1.03
B08101	1.98	5.0	13743	1.02

Figure 4. Overlay of scaled USP Simvastatin Tablet Method using columns from three production lots.

Figure 5 shows the effect of varied organic content on the analysis. The USP allows adjustment of the ratio of the solvents in the mobile phase: ± 30 % relative or ± 2 % absolute, whichever is larger, but no change can exceed 10 % (based on mobile phase component of 50 % or less) nor can the final concentration of any component be reduced to zero. Since our mobile phase is rather high in organic (65 % acetonitrile), our adjustment is based on the minor component (the buffer). 30 % of the buffer content is 10.5 %. The maximum allowed adjustment is ± 10 %. The allowed range for adjustment is between 31.5 and 38.5 % buffer or 68.5 % and 61.6 % acetonitrile. Retention decreases as expected with decreasing organic content. While the performance data for organic variance fluctuates more than the column lot data, it is still within the requirement of the method.

Adjustment of mobile phase composition across allowable range meets method requirements.



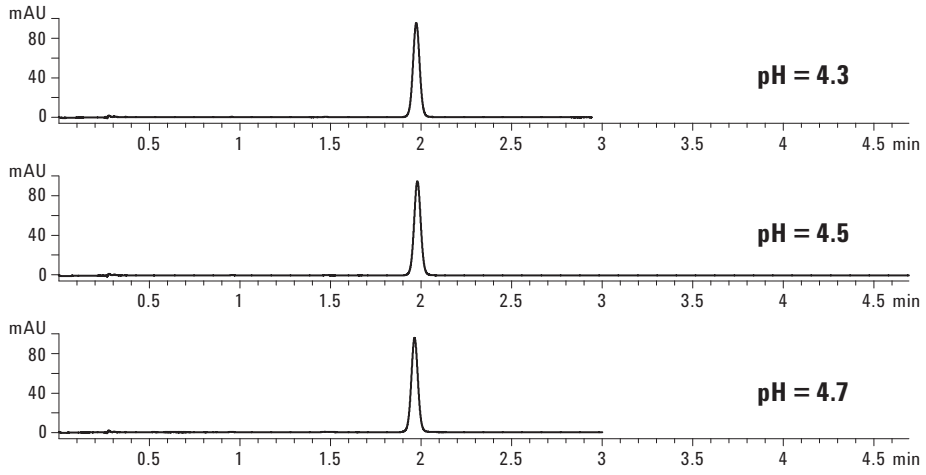
% Acetonitrile	T_R	k'	N	T_f
61.5	2.56	6.71	13852	1.03
65.0	2.00	5.03	14002	1.02
68.5	1.60	3.83	14275	1.02

Figure 5. Overlay of scaled USP Simvastatin Tablet Method showing adjustment of binary mobile phase.

Figure 6 shows the effect of pH on the Simvastatin analysis. Over the test range almost no change in pH is detected. In the case of many other polar compounds this can have a far greater effect on the analysis.

Adjustment of pH across allowable range has little effect.

Mobile Phase:	Acetonitrile/Phosphate Buffer (pH varies) (65:35)
Column:	ZORBAX RRHT Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 µm (p/n 959941-902)
Temperature:	45 °C
Flow rate:	1.5 mL/min
Sample:	2.0 µL of 0.1 mg/mL USP Simvastatin
Detection:	UV 238 nm



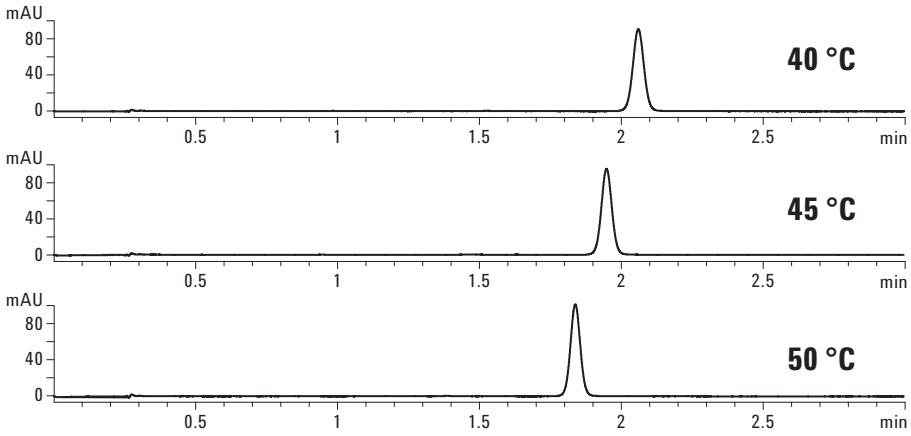
Phosphate buffer	T_R	k′	N	T_f
pH = 4.3	1.97	4.9	13168	1.02
pH = 4.5	1.98	4.9	13363	1.02
pH = 4.7	1.96	4.9	13350	1.02

Figure 6. Overlay of scaled USP Simvastatin Tablet Method showing adjustment of pH.

Finally, the effect of temperature variance on the method is shown in Figure 7. A ± 10 % change in temperature is investigated. Over a 10 °C range, performance requirements are still met.

Temperature adjustment meets method requirements across range.

Mobile Phase:	Acetonitrile/Phosphate Buffer, pH 4.5 (65:35)
Column:	ZORBAX RRHT Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 µm (p/n 959941-902)
Temperature:	Varies
Flow rate:	1.5 mL/min
Sample:	2.0 µL of 0.1 mg/mL USP Simvastatin
Detection:	UV 238 nm



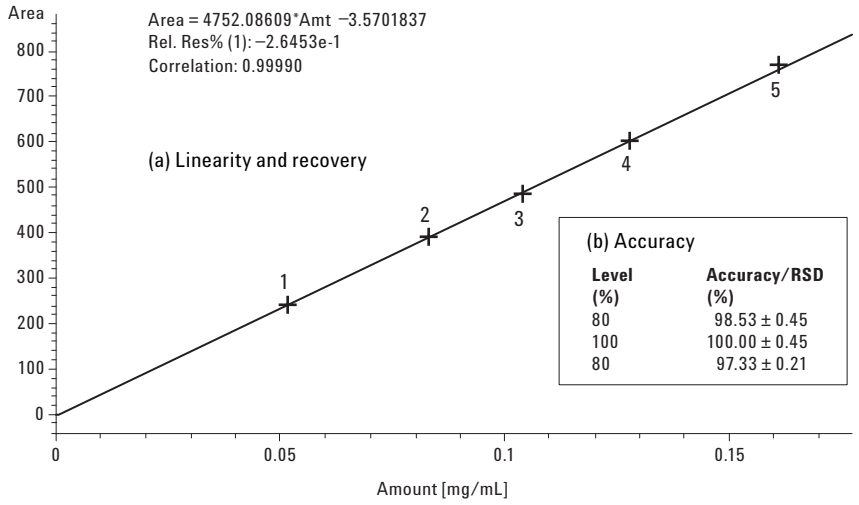
Peak	T_R	k′	N	T_f
40 °C	2.06	5.2	13176	1.02
45 °C	1.92	4.9	13333	1.02
50 °C	1.85	4.6	13289	1.03

Figure 7. Overlay of scaled USP Simvastatin Tablet Method showing adjustment of temperature.

Figure 8a shows the linearity and recovery data for the analysis. The requirements of 80 to 120 % of the expected range of the prepared sample (0.1 mg/mL) are exceeded using the 50 to 150 % calibration. Accuracy is determined by calculating the % recovery of known amounts added as samples. This is performed above, below, and within the expected range and compared. Three tests were performed, one at each level, per the International Conference on Harmonization (ICH) recommendation, since the USP does not specify a number of samples. The results are shown in Figure 8b and are close to 100 % accuracy, indicating that the method is exact enough for its expected use. Finally, ruggedness measurements were made by three chemists using separate instruments, and are shown in Figure 8c.

The testing required to do this revalidation is minimal. Essentially if approximately 1000 samples were to be analyzed using this faster method, all validation costs would be covered.

Simvastatin testing using RRHT Eclipse Plus C18 columns show excellent linearity, robustness and reproducibility.



(b) Accuracy

Level (%)	Accuracy/RSD (%)
80	98.53 ± 0.45
100	100.00 ± 0.45
80	97.33 ± 0.21

(c) Ruggedness/Reproducibility

Level (%)	Chemist 1 Accuracy/RSD (%)	Chemist 2 Accuracy/RSD (%)	Chemist 3 Accuracy/RSD (%)
120	98.53 ± 0.45	99.57 ± 0.24	100.21 ± 0.22
100	100.00 ± 0.45	99.08 ± 1.44	100.03 ± 0.35
80	97.33 ± 0.21	99.86 ± 0.42	100.22 ± 0.22

Figure 8. Linearity and recovery for scaled USP Simvastatin Tablet Method a) linearity, b) accuracy, and c) recovery.

A concern of most laboratory managers is the lifetime of a column when running real samples. In order to verify the effect and the stability of this new method, 1100 injections of a real sample are injected onto a 4.6 mm × 50 mm Eclipse Plus C18 column. Ten 40 mg Simvastatin tablets (Teva Pharmaceuticals) are disintegrated in water and dissolved using the dilution solution, as outlined in reference 1. Several aliquots of the solution are then centrifuged in two centrifuge tubes per the USP procedure. A clear aliquot of each solution is diluted to 0.1 mg/mL Simvastatin and transferred to autosampler vials. 4 liters of phosphate buffer and 4 liters of acetonitrile were used to fill the solvent reservoirs of the Agilent 1200 Series SL System. As can be seen in Figure 9, the method is run for over 1100 injections without loss of efficiency or increase in pressure. The pressure generated by this method is under 400 bar making this method accessible to all modern HPLC systems. The number of injections made is limited to the 4-liter quantity of acetonitrile. If this analysis had been performed using a 250-mm, 5-µm column, 20 liters of acetonitrile would have been required for this analysis. In addition, the analysis of the 1000 injections took approximately 33 hours. If the analysis had been carried out on a 250 mm column as specified by the USP method, the tests would have taken over 165 hours or 6.85 days.

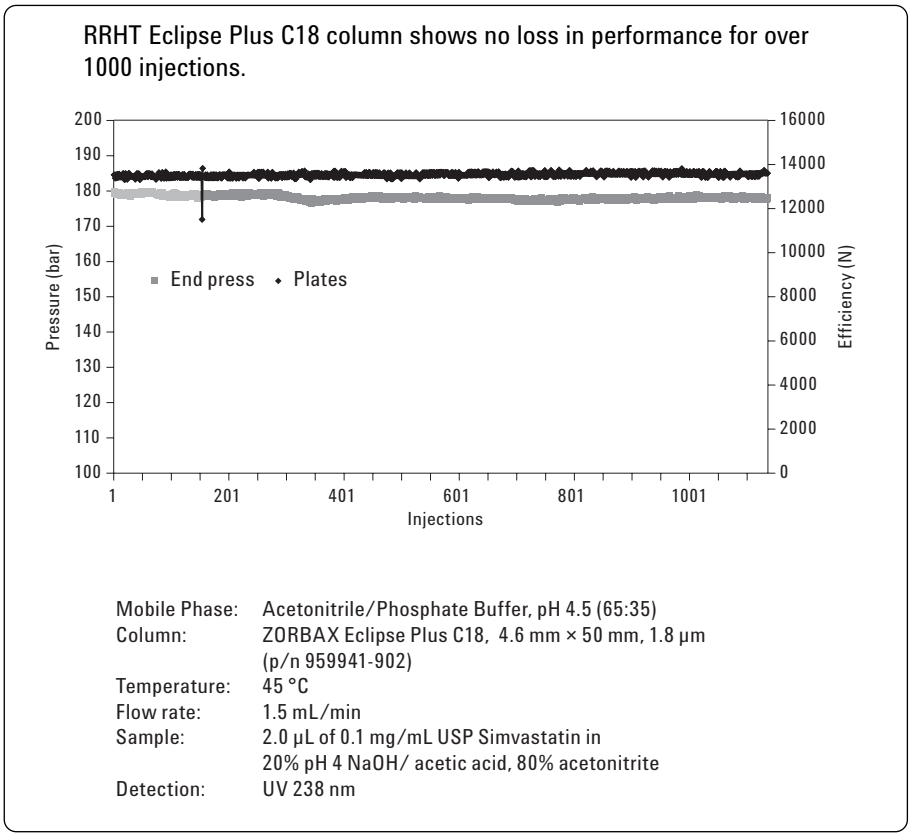


Figure 9. Lifetime study of scaled USP Simvastatin Method showing 1100 injections on a column with no efficiency loss or pressure gain.

Conclusion

While the current FDA and USP recommendations allow for a method adjustment of ±25 percent change in column diameter, a proposed USP revision that will allow adjusting the column internal diameter (ID) as much as required, provided linear velocity is constant [2]. This proposal is described in the USP 30 second supplement revisions, PF34 number 5, and is expected to be final near the end of 2009. The new guidelines will apply to methods that are final after that time [4].

In addition a change in allowable particle size adjustments is also found in the above proposal. Currently in a method adjustment, one can only reduce particle size by as much as 50 percent, which means a switch from 5 µm to 3.5 µm particles, or from 3.5 to 1.8 µm particles. A change from 5 µm to 1.8 µm particles is too large and would require method revalidation. In future methods finalized after December 2009, methods developed on columns with 3.5 µm particles can be adjusted to increase or decrease particle size (while maintaining performance metrics) and stay within the recommended guidelines [4].

The Agilent 1200 SL system as described in this application easily transitioned from the USP tablet method for Simvastatin to adjusted methods using a 4.6 mm × 100 mm, 3.5-µm column, or a 4.6 mm × 50 mm, 1.8-µm column. In all cases, the efficiency requirements were exceeded by over 100 %. Injection volumes were scaled according to the volume of the columns. An 80 % decrease in time and solvent usage was attainable, with system pressures under 400 bar, when using the Eclipse Plus C18 RRHT column. Finally, this column was able to analyze over 1000 injections with no loss of efficiency or increase in pressure proving that the 4.6 mm × 50 mm, 1.8-µm Eclipse Plus C18 column is an excellent choice for the analysis of Simvastatin.

References

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3. USP Method Validation Guidance. "United States Pharmacopeia XXX Supplement 2: System Suitability Testing, Rockville, MD. 2007, Chapter <621>
4. USP 30 Second Supplement Revisions, PF34 (5), in process and expected to be final Dec 2009.

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