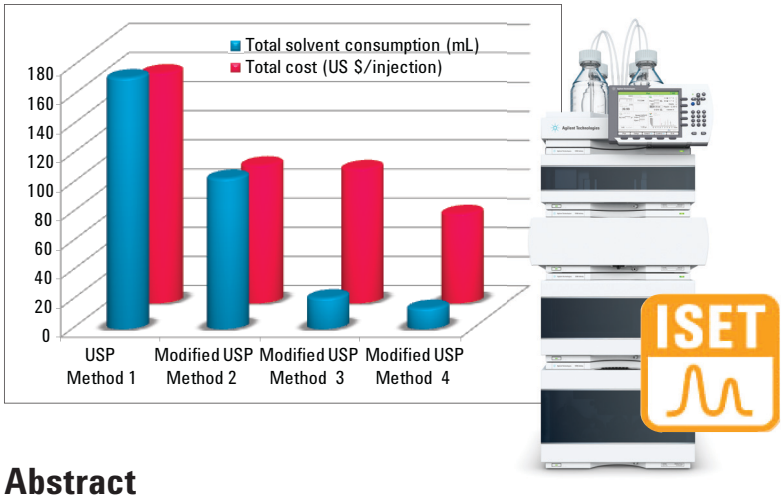
Effective use of pharmacopeia guidelines to reduce cost of chromatographic analysis

Optimized, cost-effective HPLC analysis of atorvastatin by varying column dimensions within the USP <621> allowed limts

Application Note

Pharmaceutical QA/QC



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Abstract

This Application Note describes an efficient way to significantly reduce the cost of analysis of liquid chromatography (LC) under United States Pharmacopeia (USP) guidelines using the atorvastatin calcium assay method. Time and solvent savings were achieved by varying column dimensions used for chromatographic separation, resulting in reduction of total cost of analysis per injection. The column dimensions such as length, diameter, and particle size were modified within the allowed deviations defined by the USP guidelines, thereby eliminating the need for method revalidation. The Agilent Method Translator and Cost Savings Calculator tool was used to derive new gradient parameters. The Agilent 1290 Infinity LC System with Intelligent System Emulation Technology (ISET) was the instrumentation used. A 61% reduction in cost of analysis per injection with a reduction of 91.7% in solvent consumption was achieved only by varying column dimensions within USP guidelines.

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Introduction

Atorvastatin is one of the top three blockbuster drugs used for lowering blood cholesterol. The USP method for organic impurities of atorvastatin takes approximately 115 minutes and uses a 4.6 × 250-mm column with a 5-µm L7 packing[1]. Considering a cost of US $ 60/L for organic solvents like acetonitrile and tetrahydrofuran, and time/labor costs of US $ 80/hour for running an instrument, the total cost of analysis for atorvastatin amounts to US $ 158.2 per injection. This includes an expense for solvent waste disposal of US $ 1.5/L.

Table 1[2] shows USP <621> guidelines on permitted column dimension deviations for LC methods. A significant amount of solvent and analysis time can be saved by reducing column length, diameter, and particle size within this deviation limit.

Column parameter	USP limit for deviation
Length	± 70%
Internal diameter	No limit, but keep constant linear velocity
Particle size	- 50%

Table 1
Allowed column deviations according to the USP <621> recommendation.

An Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5-µm column was used in this Application Note to carry out the standard USP analysis (Experiment 1). To demonstrate reduction of analysis cost, three additional methods (Experiments 2, 3, and 4) were carried out with modified column dimensions within the allowed deviation limit. However, for the adoption of any column dimension modification, a system suitability test as per USP should be performed. To meet this USP requirement, system suitability testing was performed using all the column dimensions. A 1290 Infinity LC System with ISET was used to emulate different instrument modes according to the column dimensions. The ISET algorithm delivered identical gradient mixing conditions upon selecting other instruments, and eliminated the variation due to differences in delay volumes and mixing behavior[3].

Experimental

Instruments

The Agilent 1290 Infinity LC System used for the experiment included the following modules:

- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (G4220A) and 35-µL Jet Weaver mixer.
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light flow cell (1.0 µL dispersion volume, 10 mm path length) (G4212-60008)

Software

- The system was controlled using the Agilent ChemStation OpenLAB CDS ChemStation Edition C.01.03

Columns

Table 2 shows the column details for the columns used in the four experiments.

	Experiment 1 (Original USP method)		Experiment 2		Experiment 3		Experiment 4	
Column parameter	Actual	% Deviation	Actual	% Deviation	Actual	% Deviation	Actual	% Deviation
Length	250 mm	0	150 mm	- 40	150 mm	- 40	100 mm	- 60
Diameter	4.6 mm	0	4.6 mm	0	2.1 mm	- 54	2.1 mm	- 54
Particle size	5 µm	0	2.7 µm	- 46	2.7 µm	- 46	2.7 µm	- 46

Table 2
Column dimensions used for various experimental conditions and their deviations from the original USP method.

Reagents and materials

The USP reference standards for atorvastatin and corresponding impurities were purchased from USP-India Private Ltd, Hyderabad, India. Acetonitrile was of super gradient grade and was purchased from Lab-Scan (Bangkok, Thailand). Highly purified water from a Milli Q water purification system (Millipore Elix 10 model, USA) was used for the experiment. Other chemicals such as tetrahydrofuran, ammonium acetate, and glacial acetic acid were purchased from Aldrich (India).

Chromatographic parameters

The buffers and mobile phases were prepared according to the USP method. The buffer solution was prepared by dissolving 3.9 g of ammonium acetate in 1 L of water and adjusting the pH to 5.0 ± 0.1 using glacial acetic acid. Mobile phase A was prepared by mixing acetonitrile, tetrahydrofuran, and buffer in the ratio 21:12:67. For mobile phase B, these solvents were mixed in a ratio 61:12:27 respectively. The column temperature was maintained at 35 °C and the detection was set at 244 nm. The standard USP analysis with the 4.6-mm id column was performed by emulating a conventional Agilent 1100 Series LC System with a 400-bar pressure limit. This is done by selecting the corresponding modules from a drop-down list with a few mouse clicks. For Experiment 2, a smaller particle size column was used and a 1260 Infinity LC System with a 600-bar pressure limit was emulated. For Experiments 3 and 4, the 1290 Infinity LC System was the best choice and no emulation was used since the analysis was using a narrow bore column with smaller particle size. Table 3 shows the detailed chromatographic method parameters for each experiment.

	Agilent 1290 Infinity Binary LC with ISET			
Parameter	**Experiment 1 Emulated as Agilent 1100 Series LC**	**Experiment 2 Emulated as Agilent 1260 Infinity LC**	**Experiment 3 Without ISET**	**Experiment 4 Without ISET**
Injection volume	20 µL	12 µL	2.7 µL	1.7 µL
Column	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm (p/n 959990-906)	Agilent Poroshell SB-C8, 4.6 × 150 mm, 2.7 µm (p/n 683975-906)	Agilent Poroshell SB-C8, 2.1 × 150 mm, 2.7 µm (p/n 683775-906)	Agilent Poroshell SB-C8, 2.1 × 100 mm, 2.7 µm (p/n 685775-906)
Flow rate	1.5 mL/min	1.5 mL/min	0.31 mL/min	0.31 mL/min
Gradient	At 0 min : 0% B At 40 min : 0% B At 70 min : 80% B At 85 min : 100% B At 100 min : 100% B At 105 min : 0% B At 115 min : 0% B	At 0 min : 0% B At 24 min : 0% B At 42 min : 80% B At 51 min : 100% B At 60 min : 100% B At 63 min : 0% B At 69 min : 0% B	At 0 min : 0% B At 24 min : 0% B At 42 min : 80% B At 51 min : 100% B At 60 min : 100% B At 63 min : 0% B At 69 min : 0% B	At 0 min : 0% B At 16 min : 0% B At 28 min : 80% B At 34 min : 100% B At 40 min : 100% B At 42 min : 0% B At 46 min : 0% B
Acquisition rate	5 Hz	5 Hz	10 Hz	10 Hz

Table 3
Chromatographic parameters for all four experiments.

Procedure

The system suitability mix and standard solution of atorvastatin are prepared as per USP assay method described in USP 34–NF 29[1]. The atorvastatin calcium USP system suitability mixture contains 0.05 mg/mL of active pharmaceutical ingredient (API) and approximately 0.06 mg/mL of atorvastatin calcium related compound B while standard solution contains only 0.4 mg/mL of atorvastatin calcium.

The system suitability test for the atorvastatin USP assay method includes:

a) Measurement of the resolution between atorvastatin calcium related compound B and atorvastatin calcium using system suitability mix (limit: not less than 1.5)

b) Calculation of the tailing factor of atorvastatin peak using standard solution (limit: not more than 1.5)

c) Calculation of the relative standard deviation (RSD) of atorvastatin peak retention time using standard solution (limit: not more than 0.6%)

System suitability testing for assay method was performed under all four experimental conditions. Emulation of the 1290 Infinity LC System to other suitable instrument models appropriate for each selected column dimension was done using ISET. Shorter gradient time parameters were calculated using the Agilent Method Translator in simple conversion mode. Savings in total time and solvent were calculated for each experiment. The chromatographic parameters for atorvastatin USP organic impurities are the same as the assay method.

To verify the effectiveness of the newly developed methods for organic impurity analysis, a spiked mix of atorvastatin and related impurities (A, B, C, D, H, I) was prepared and injected in all experimental conditions. The impurity and API concentrations used to prepare this spike mix were similar to the assay method system suitability mix (0.05 mg/mL of API and 0.06 mg/mL of each impurity).

Results and discussion

Separation and detection

Figure 1 shows the separation of atorvastatin and impurities using a standard USP method. All peaks are well separated and the observed resolution between related compound B and atorvastatin was approximately 1.5. The assay method system suitability test results were within the acceptance criteria using the new experimental conditions. The chromatograms of the system suitability test that resulted using the new cost effective experimental conditions are shown in Figure 2, and results are tabulated in Table 4.

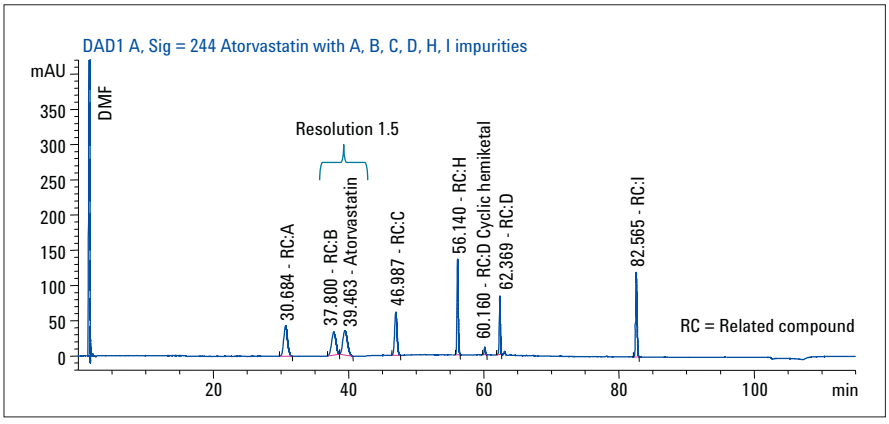

Figure 1
Separation of atorvastatin and all impurities per standard USP method.

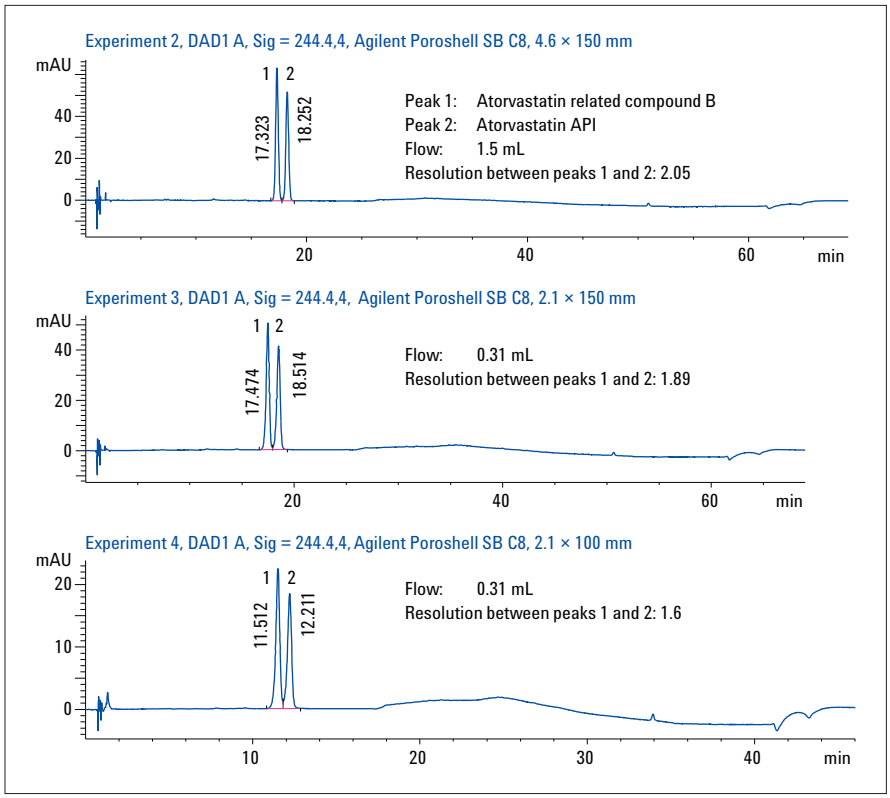

Figure 2
Separation of the atorvastatin system suitability mix using newly developed cost effective experimental conditions.

			Result			
Sl no.	**USP system suitability tst**	**Limit**	**Experiment 1 (USP)**	**Experiment 2**	**Experiment 3**	**Experiment 4**
1	Resolution between atorvastatin and RCB	NLT* 1.5	1.5	2.05	1.88	1.6
2	USP tailing factor for atorva peak (0.4 mg/mL)	NMT** 1.6	1.3	1.5	1.2	1.1
3	RSD retention time, atorvastatin peak (%)	NMT** 0.6	0.24	0.05	0.15	0.18
4	RSD area, atorvastatin peak (%)	NMT** 0.6	0.14	0.23	0.34	0.49

*NLT: not less than
**NMT: not more than

Table 4
System suitability results for all four experiments.

Cost of analysis

The cost of analysis was calculated for all four experimental conditions. The approximate time required to complete one injection for the atorvastatin USP assay method is approximately 115 minutes and approximately US $ 158.2/injection was calculated as the total analysis cost. However, by adopting Experiment 2 conditions, the total cost of analysis could be reduced to US $ 94.9 per injection with a time saving of 40%. In Experiment 3, the column id was reduced to 2.1 mm thereby achieving a total solvent saving of 87.6%. In Experiment 4, further cost reduction was achieved by reducing the gradient time with a shorter 100-mm column while maintaining a 2.1-mm id. The total analysis expense using Experiment 3 and Experiment 4 conditions was calculated as US $ 92.4/injection and US $ 61.6/injection respectively.

Figure 3 shows the graphical representation of total solvent consumption, analysis time, and total cost of analysis for all four experimental conditions. Figure 4 shows the excellent separation of atorvastatin and related impurities using Experiment 4 conditions with a solvent saving of 91.7% and a time saving of 60%. The results described demonstrate that the chromatographers have a wide choice of options to reduce the cost of analysis significantly by reducing column dimensions within the pharmacopeia deviation limits.

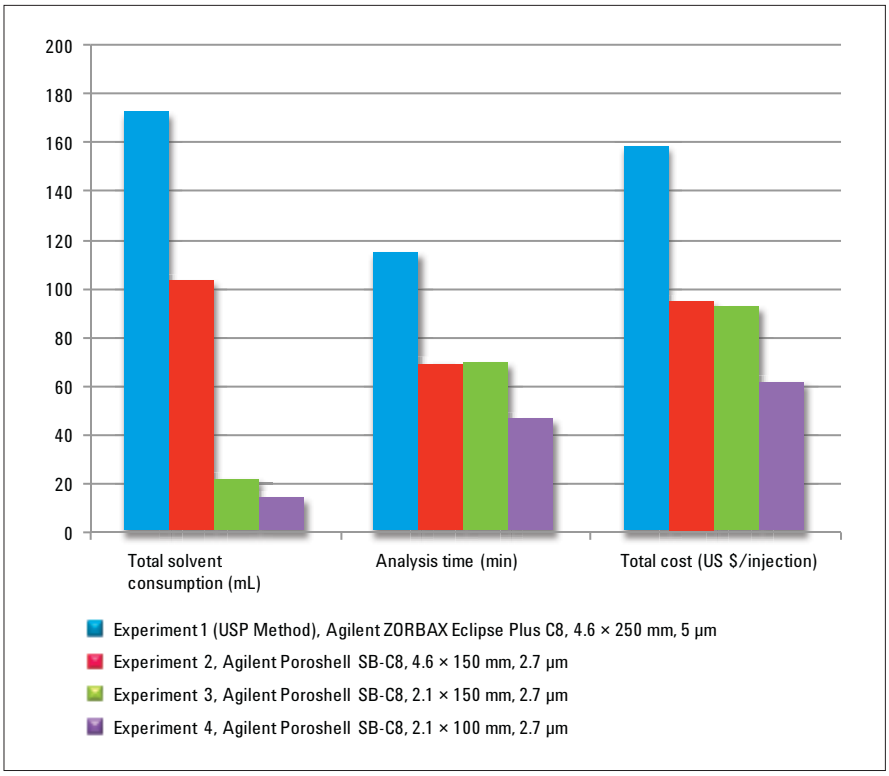

Figure 3
Solvent, time, and total cost calculations for USP and newly developed cost effective experimental conditions.

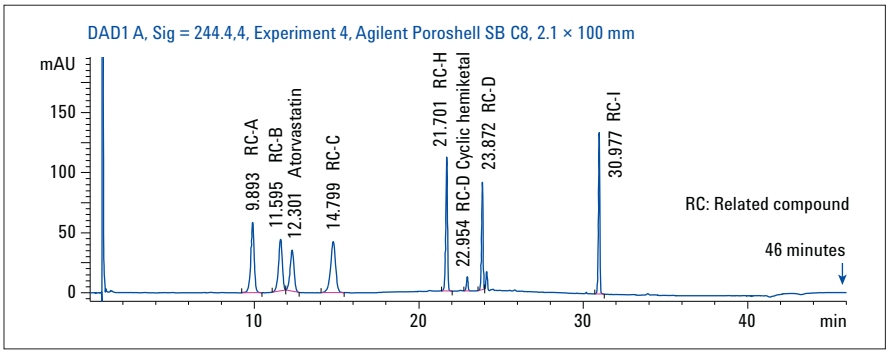

Figure 4
Chromatographic elution profile of atorvastatin and related impurities using Experiment 4 conditions.

Conclusion

A simple and acceptable approach to reduce the total cost of analysis for a generic drug like atorvastatin using the Agilent 1290 Infinity LC System with ISET is demonstrated. The cost reduction was achieved by varying USP required column dimensions within the allowed pharmacopeia deviation limits, thereby avoiding the need for method revalidation. Compared to the original USP method, 91.7% of solvent and 60% of analysis time could be saved with the newly developed methods, translating to a 61% reduction in analysis cost. The effectiveness of the new methods for routine use was confirmed by performing system suitability analysis. Using ISET, the Agilent 1290 Infinity LC System was easily emulated from one instrument model to another with respect to the column dimension used for the analysis.

References

1.
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2.
"Validation of analytical methods", Agilent Publication Number 5990-5140EN, **2011**.

3.
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www.agilent.com/chem/1290

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