

HPLC to UHPLC Transfer of USP Method for Amlodipine Besylate Using the Agilent 1290 Infinity II LC

Application Note

Small Molecule Pharmaceuticals

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Abstract

Over the last decades, an increasing number of generic drug formulations have been released on the pharmaceutical market. These products are generally analyzed with compendia methods. This Application Note describes the method transfer of a USP HPLC method for amlodipine besylate tablets to a state-of-the-art UHPLC method. Adjustments on column dimension and method parameters were carried out within the permitted limits in order to avoid the need for a lengthy revalidation process. The Agilent 1290 Infinity II LC facilitated smooth transfer from one method to the other. System suitability criteria were investigated and met with both HPLC and UHPLC.



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Introduction

The methods described in the various compendia such as U.S. Pharmacopeia Convention (USP), European Pharmacopoeia (Ph. Eur.), Japanese Pharmacopoeia (JP), Chinese Pharmacopoeia (ChP), and so on, are generally based on techniques and equipment that were available at the time of drug development. The availability of low dispersion UHPLC systems and high-efficiency columns packed with small fully porous particles or superficially porous particles was not taken into account at initial method development and validation. With current instrument and column technology, the chromatographic analysis of drug products and formulations can be carried out in a much more efficient and productive manner.

Amlodipine is a dihydropyridine derivative with calcium-channel blocking properties. It is used in treatment of hypertension (alone or in combination with other antihypertensive agents), and in management of Prinzmetal variant angina and chronic stable angina pectoris (alone or in combination with other antianginal agents). Amlodipine is usually administered in tablets as a salt, with an acidic counterion such as besylate or mesylate. The salt has enhanced properties in terms of stability and bioavailability. It has become available as a generic formulation with tablet dosage expressed in terms of amlodipine.

The official USP method for the active pharmaceutical ingredient (amlodipine) assay in amlodipine besylate tablets is based on an isocratic reversed-phase LC analysis with UV detection¹. In this Application Note, this method was set up on an Agilent 1290 Infinity II LC, and the assay was carried out as described in the procedure. On the same system, the method was transferred to UHPLC and the procedure was repeated. Results of both methods are compared and discussed.

Experimental

Instrumentation

An Agilent 1290 Infinity II LC system was used.

- Agilent 1290 Infinity II High-Speed Pump (G7120A) with ultralow dispersion kit (#006)
- Agilent 1290 Infinity Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) with 10-mm flow-cell

Buffer preparation

Add 7.0 mL of triethylamine into a 1,000-mL flask containing 900 mL of water. Adjust the solution with phosphoric acid to a pH of 3.0 ± 0.1 . Dilute with water to volume, and mix well.

Standard solutions

System suitability solution

0.02 mg/mL of USP Amlodipine Besylate RS and 0.002 mg/mL of USP Amlodipine Related Compound A RS, prepared in mobile phase.

Standard solution

0.02 mg/mL of Amlodipine prepared from USP Amlodipine Besylate RS, prepared in mobile phase

Sample preparation

In a volumetric flask, dissolve one tablet (5 mg amlodipine, as amlodipine besylate) in 250 mL of mobile phase. The sample solution theoretically contains 0.02 mg/mL of Amlodipine. Pass the sample through a 0.45-mm pore size syringe tip filter (regenerated cellulose).

Results and Discussion

The USP method indicates the use of a 3.9 mm column. In this Application Note, a 3 mm column was used. According to the USP², the column inner diameter can be adjusted as long as the linear velocity is kept constant. Therefore, the flow rate was reduced from 1 to 0.6 mL/min on the 3.9 and 3 mm columns, respectively. Because of the excellent sensitivity of the Agilent 1290 Infinity II DAD, a lower injection volume could be used. Injection volume can be adjusted if it is consistent with accepted precision, linearity, and detection limits². Table 1 summarizes the other method settings, and Figure 1 shows a result for the system suitability solution.

Table 1. Comparison of USP parameters and parameters used for HPLC in this Application Note.

	USP	HPLC
Column	Packing L1 3.9 × 150 mm, 5 μm	Agilent ZORBAX Eclipse Plus C18, 3 × 150 mm, 5 μm (p/n 959993-302)
Mobile phase	Methanol/acetonitrile/buffer 35/15/50, isocratic	
Flow rate	1 mL/min	0.6 mL/min
Temperature	Not indicated	30 °C
Injection	50 μL	20 μL Needle wash 3 seconds with mobile phase
Detection	UV 237 nm	DAD Signal 237/4 nm, Reference off 5 Hz
Analysis time	Approximately three times the retention of amlodipine	23 minutes

To reduce analysis time and thus increase productivity, the HPLC column was replaced with a sub-2 μm UHPLC column with the same stationary phase chemistry. Since column dimensions were significantly different, several method parameters were changed. These changes were carried out in accordance to USP guidelines to avoid the need for a complete or partial revalidation of the method². Table 2 shows the method settings. The main changes, together with comments are listed below.

The rationale of the method transfer is as follows:

Column dimensions and flow rate

The column internal diameter can be adjusted if the linear velocity is kept constant. For isocratic separations, particle size or column length may be modified provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range between -25 % to 50 %, relative to the prescribed column.

$$L/dp \text{ HPLC} = 30,000$$

$$L/dp \text{ UHPLC} = 27,778 \text{ (-7.4 \%)}$$

When a change is made from $\geq 3 \mu\text{m}$ to $< 3 \mu\text{m}$ particles in isocratic separations, an additional increase in linear velocity (flow rate) may be justified. Flow rate changes for both a change in column diameter and in particle size can be made by:

$$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$$

Where F_1 and F_2 are the flow rates for the original and modified conditions, respectively, dc_1 and dc_2 are the respective column diameters; and dp_1 and dp_2 are the particle sizes.

$$\text{Flow rate HPLC} = 0.600 \text{ mL/min}$$

$$\text{Calculated flow rate UHPLC} = 0.817 \text{ mL/min}$$

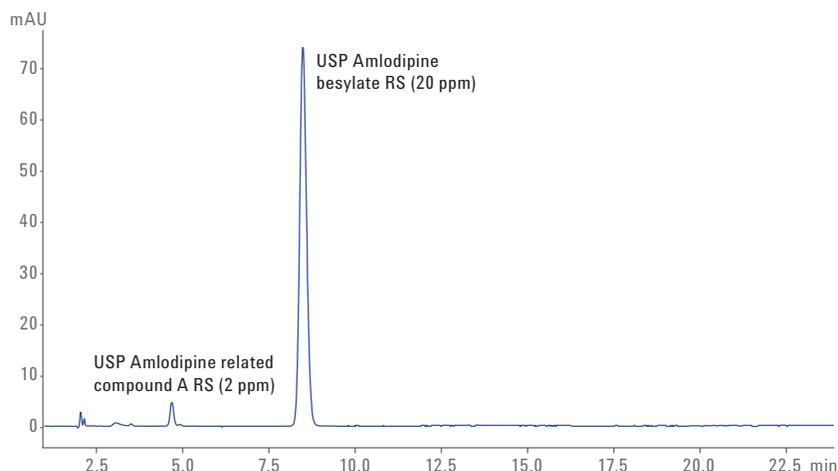


Figure 1. Analysis of the system suitability solution with HPLC.

Table 2. Comparison of HPLC and UHPLC used in this Application Note.

	HPLC	UHPLC
Column	Agilent ZORBAX Eclipse Plus C18 3 × 150 mm, 5 μm (p/n 959993-302)	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 50 mm, 1.8 μm (p/n 959757-902)
Mobile phase	Methanol/acetonitrile/buffer 35/15/50, isocratic	
Flow rate	0.6 mL/min	0.8 mL/min
Temperature	30 °C	
Injection	20 μL Needle wash 3 seconds with mobile phase	10 μL Needle wash 3 seconds with mobile phase
Detection	DAD Signal 237/4 nm, Ref off 5 Hz	DAD Signal 237/4 nm, Ref off 20 Hz
Analysis time	23 minutes	3.1 minutes

Injection volume

The injection volume can be adjusted if it is consistent with accepted precision, linearity, and detection limits.

Detector speed

The data acquisition of the detector was increased from 5 to 20 Hz for HPLC and UHPLC, respectively.

Figure 2 shows an example of the UHPLC analysis of the system suitability solution, and Figure 3 shows a comparison of an HPLC and a UHPLC result. A commercial sample (amlodipine besylate tablet, containing 5 mg amlodipine) was analyzed with each method, and the percentage of the labeled amount of amlodipine was calculated. Figure 4 shows an example of a real sample analysis. Table 3 summarizes the comparison of the outcome with different methods.

The stability of the UHPLC method was tested by a series of replicate injections. The following injection sequence was repeated 20 times, resulting in a total of 160 analyses.

- Blank solution
- System suitability solution
- Standard solution (five replicates)
- Sample solution

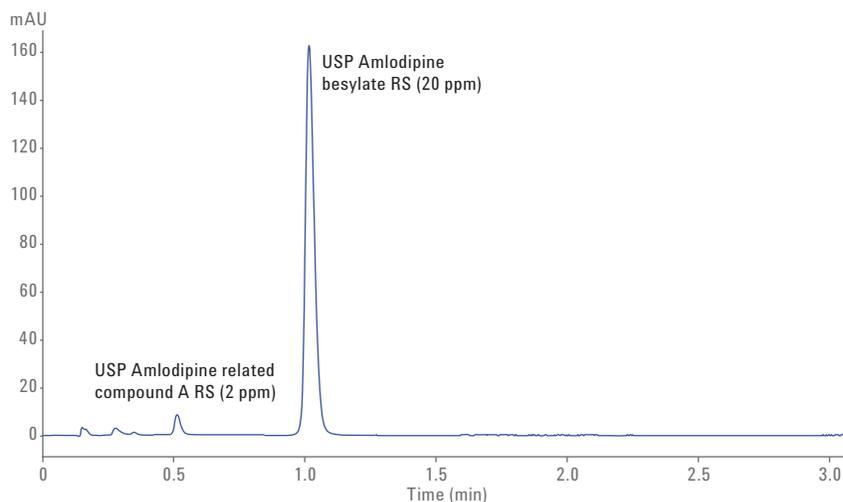


Figure 2. Analysis of the system suitability solution with UHPLC.

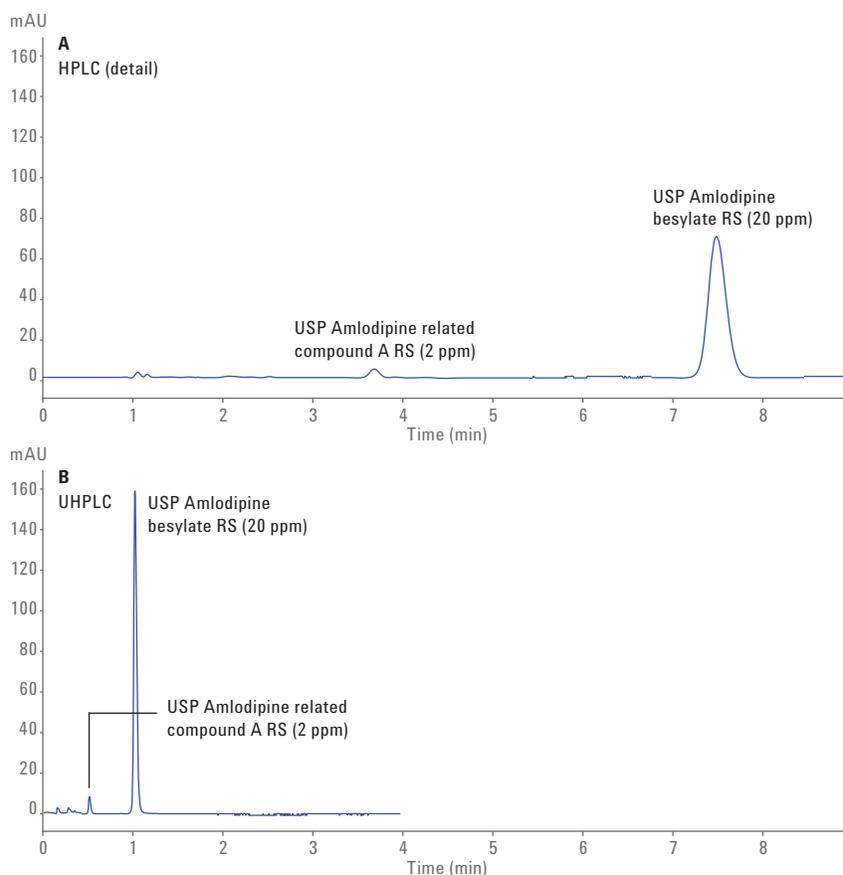


Figure 3. HPLC and UHPLC analysis of the system suitability solution.

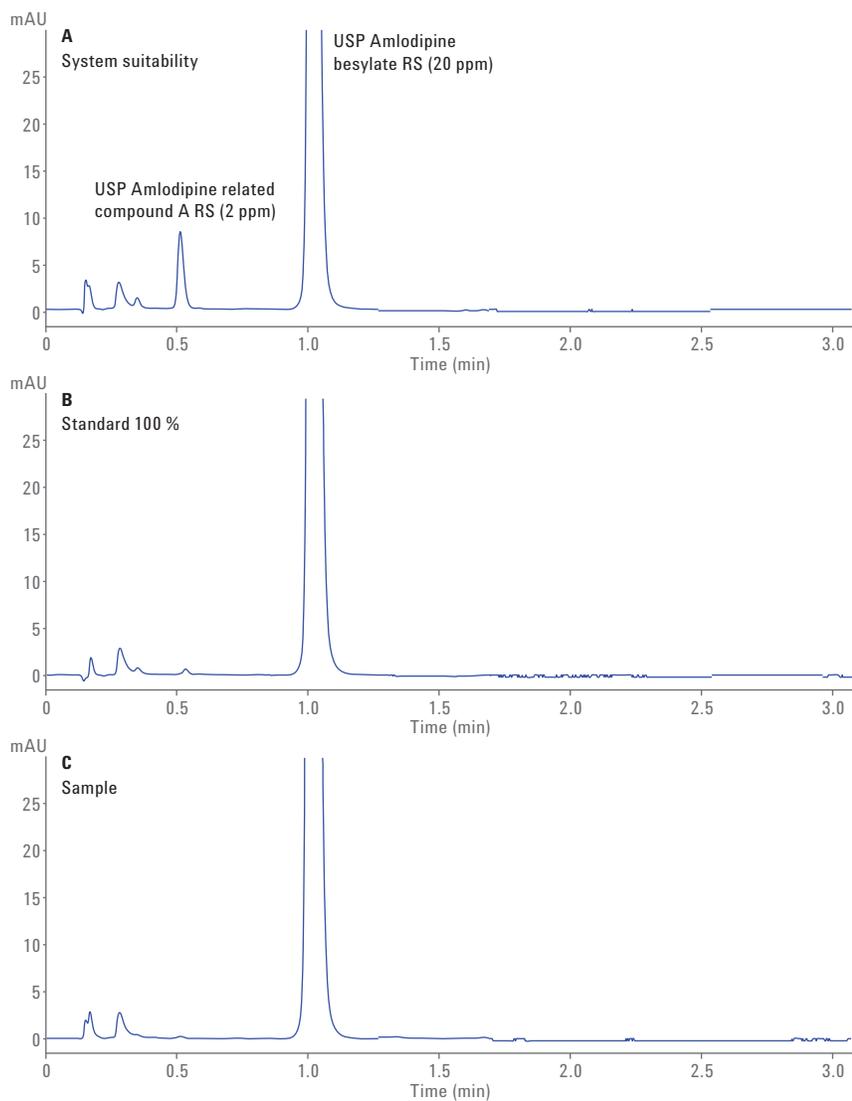


Figure 4. UHPLC analysis of the system suitability solution, standard solution, and a sample.

Table 3. Comparison of the results obtained with both methods.

	USP requirements	HPLC	UHPLC
Resolution	NLT 8.5	13.3	9.7
Tailing factor at 5 % height	NMT 2.0	1.14	1.31
Area %RSD amlodipine (n = 6)	NMT 1.0 %	0.03	0.02
Area %RSD amlodipine related compound A (n = 6)	NMT 5.0 %	0.29	0.33
Linearity (R ²)*	–	0.99999	0.99999
Amlodipine in sample	90–110 %	105.37 %	105.28 %
Retention time	–	7.52 minutes	1.02 minutes
Analysis time	–	23.00 minutes	3.10 minutes
Mobile phase consumption/analysis		13.8 mL	2.5 mL

*0.002 (10 %), 0.005 (25 %), 0.010 (50 %), 0.015 (75 %), 0.020 (100 %), 0.030 (150 %) mg/mL, one injection/concentration level

Figure 5 summarizes the results in the graph. The pressure varied between 730 and 740 bar over the entire sequence.

Conclusion

The USP method for amlodipine besylate was transferred from HPLC to UHPLC on the same Agilent 1290 Infinity II LC. The method transfer could be carried out in a short period of time taking into account the USP guidelines. System suitability criteria were evaluated and reached with both methods. Repeatability and stability were demonstrated over a set of 160 injections with the developed UHPLC method. Analysis time was reduced over 85 %, and mobile phase consumption in UHPLC was less than one fifth of the consumption in the original HPLC method. It is obvious that laboratory productivity and sample throughput can be greatly enhanced using the described approach.

References

1. USP Monograph, Amlodipine Besylate Tablets, *The United States Pharmacopeial Convention*, February 1, 2011.
2. First Supplement to USP 37–NF 32, *The United States Pharmacopeial Convention*, August 1, 2014.

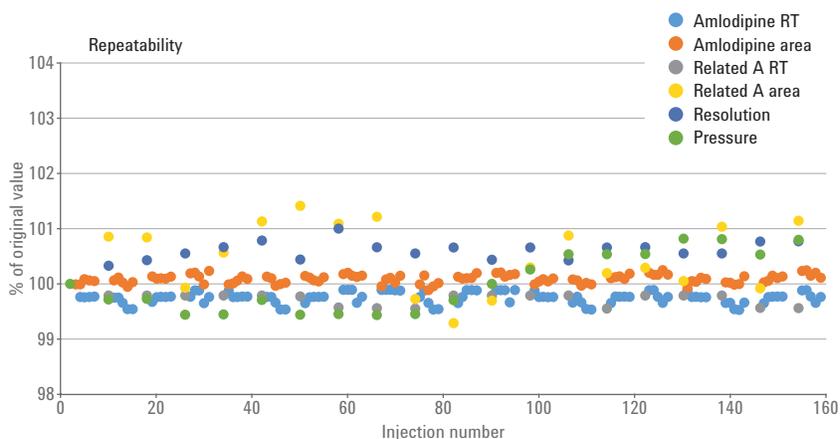


Figure 5. Method stability data for a total of 160 UHPLC analyses of the system suitability solution, standard solution, and a sample solution.

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