Adapting Pharmaceutical Monographs for Capillary Scale HPLC Using USP 621 Guidelines

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Abstract

Compact capillary scale liquid chromatography (LC) instrumentation offers a platform for high performance separations with considerable reduction in solvent consumption, operating cost, and benchtop footprint when compared to analytical scale instrumentation. To enter more regulated environments, such as those often found in the pharmaceutical industry, systems must demonstrate their ability to operate under the conditions of existing pre-validated workflows. The United States Pharmacopeia (USP) provides monographs for the HPLC analysis of pharmaceutical compounds, as well as guidelines for adapting these monographs to analysis under modified HPLC conditions. Using USP 621 guidelines, a monograph for the analysis of hydrochlorothiazide was adapted for capillary scale analysis, passing system suitability while reducing solvent consumption by over 99%. For comparison, the analysis was also performed at analytical scale, with chromatographic figures of merit for both systems passing system suitability criteria. This work is an example that illustrates the ability of the Axcend Focus LC to satisfy USP monograph methods comparable to analytical scale chromatographic performance.

Introduction

The United States Pharmacopeia (USP)–National Formulary (USP-NF) offers monographs for thousands of pharmaceutical compounds. These monographs are used around the world for sample validation. The conditions reported in these monographs (in this case, HPLC) can be modified for different column geometries, flow rates, and elution gradients if the results obtained are within validation specifications. To ensure the validity of a monograph post modification, USP has released guidelines in USP Chapter 621[1], outlining acceptable ranges for the parameters of the chromatographic process to be changed. The Axcend Focus LC is a compact capillary scale HPLC system that has been adopted for use in pharmaceutical laboratories. To demonstrate the validity of using this instrument for pharmaceutical analysis, a USP monograph for HPLC of hydrochlorothiazide [2] was adapted to capillary LC using USP 621 guidelines. Comparisons of system performance between analytical and capillary scales are given.

Experimental

Method Adaptation

Column Dimensions

The particle size and or length of the column may be modified provided that the ratio of the column length (*L*) and the particle size (*dp*) remains within a range of -25% to +50% of the *L/dp* ratio given in the monograph. The resulting column adaptation parameters are listed below:

Monograph column:	4.6 x 50 mm column with 3.5 µm L1 particles	L/dp Ratio: 14.29
Capillary column:	0.3 x 50 mm column with 3.0 µm L1 particles	L/dp Ratio: 16.66 (+16.6%)
Analytical column:	2.1 x 50 mm column with 2.6 µm L1 particles	L/dp Ratio: 19.23 (+34.6%)

Flow Rate

When particle size and/or column diameter is changed, the flow rate must also be adjusted according to the equation:

$$F_2 = F_1 \frac{dc_2^2 * dp_1}{dc_1^2 * dp_2}$$

Where F_1 represents the flow rate listed in the monograph (mL/min), dc_1 represents the internal diameter of the column listed in the monograph (mm), dp_1 represents the particle size listed in the monograph (µm), F_2 represents the adjusted flow rate (mL/min), dc_2 represents the internal diameter of the column used (mm), and dp_2 represents the particle size of the column used (µm).

Monograph flow rate:	1.0 mL/min
Capillary flow rate:	0.0050 mL/min or 5.0 µL/min
Analytical flow rate:	0.28 mL/min

Gradient Adjustments

Changes in column dimensions also change column volume, which impacts gradient performance. To accomplish this, the total gradient time in the monograph (t_{G1}) is scaled to a new gradient time (t_{G2}) with each gradient step being adjusted proportionally using the following equation:

$$t_{G2} = t_{G1} * \frac{F_1}{F_2} * \frac{L_2 * dc_2^2}{L_1 * dc_1^2}$$

USP Monograph		Capillary Scale		Analytical Scale	
Time (min)	*Solution A (%)*	*Time (min)*	*Solution A (%)*	*Time (min)*	*Solution A (%)*
0	3	0	3	0	3
5	3	4.29	3	3.72	3
14	36	12.00	36	10.42	36
18	3	15.44	3	13.39	3
20	3	17.15	3	14.88	3

Injection Volume

Injection volume must also be scaled to account for differences in column volume. The injection volume given in the monograph (V_{inj1}) is adjusted to the modified condition (V_{inj2}) using the following equation:

$$V_{inj2} = V_{inj1} * \frac{L_2 * dc_2^2}{L_1 * dc_1^2}$$

Unlike previous conditions, injection volume can be further modified beyond this equation provided system suitability criteria are still met. When injection volume is decreased further, the limit of detection and peak response repeatability must be considered. When injection volume is increased, the linearity and peak resolution must remain consistent. The injection volumes are listed below:

Monograph injection volume[2]	10 µL
Capillary injection volume	42 nL
Analytical injection volume	2 µL

It is important to note that even if a modification falls within these ranges, the instrument must still meet the system suitability criteria described in each monograph to be considered acceptable.

Operating Conditions

Sample Preparation

A diluent solution of 70:30 water to acetonitrile with 16 mM sodium phosphate (pH 2.7) was prepared as directed in the USP monograph.[2] The system suitability solution consisted of a mixture of 0.32 mg/mL hydrochlorothiazide and 0.0032 mg/mL of chlorothiazide, and benzothiadiazine-related compound A was dissolved in the dilution solution. The quantitation limit solution consisted of 0.16 µg/mL hydrochlorothiazide. As the goal of this study was the determination of system suitability when adapting monographs across scales, standard and assay samples were not prepared or analyzed.

Chromatographic Conditions

	Monograph	Capillary Scale	Analytical Scale
Column	4.6 x 50 mm packed with 3.5 µm L1 material	0.3 x 50 mm packed with 3.0 µm L1 material	2.1 x 50 mm packed with 2.6 µm L1 material
Mobile phase	Solution A: 75:25 ACN/MeOH Solution B: Water with 0.5% Formic Acid	Solution A: 75:25 ACN/MeOH Solution B: Water with 0.5% Formic Acid	Solution A: 75:25 ACN/MeOH Solution B: Water with 0.5% Formic Acid
Flow rate	1.0 mL/min	4.96 µL/min	0.28 mL/min
Injection volume	10 µL	42.5 nL	2.08 µL
Temperature	35 ˚C	35 ˚C	35 ˚C
Detection wavelength	275 nm	275 nm	275 nm
Gradient	See above	See above	See above

Results and Discussion

Capillary Scale Monograph Performance

A system suitability test for hydrochlorothiazide was performed using the Axcend Focus LC as well as using an analytical scale LC across 6 injections. The resulting chromatograms are shown in Figure 1 and Figure 2, respectively. To meet system suitability criteria, the test solution must meet specific performance metrics for resolution, tailing, and repeatability, as well as verification of quantitation limit solution run in triplicate to be considered an acceptable adaptation of the monograph. A table of these limits, and the performance of each system are given in Table 1. Although there were differences in performance between the analytical and capillary scale systems, both met system suitability criteria, and are therefore considered valid platforms for complying with the USP monograph. Due to the reduced solvent consumption of capillary scale LC, the monograph separation generates only 85 µL of solvent waste. This represents a 98% reduction in solvent consumption from the translated analytical scale method and a 99.6% reduction compared to the original monograph conditions. There are differences in detector sensitivity between the two scales as seen in the impurities (benzothiadiazine-related compound A and chlorothiazide); however, these differences still fall within acceptable USP monograph guidelines, making both systems still suitable for performing these assays.

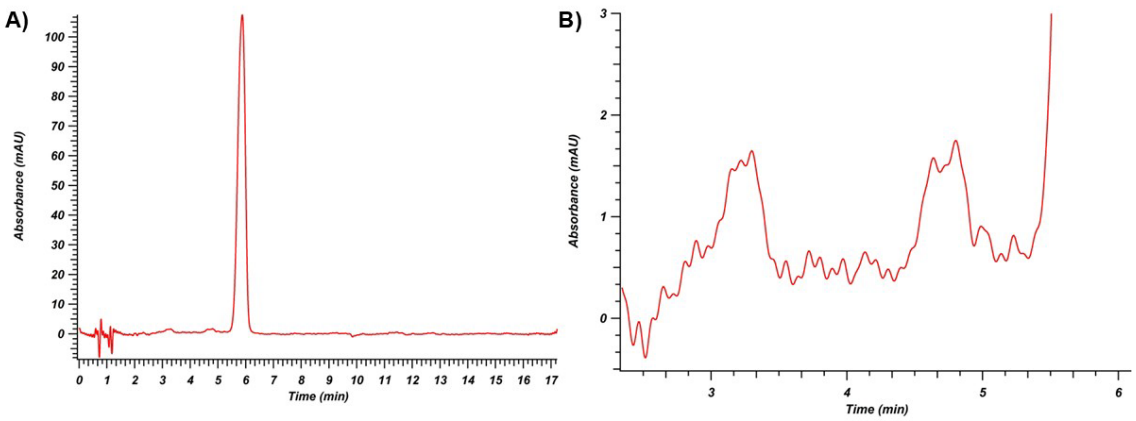

Figure 1. Capillary scale chromatograms obtained following the USP monograph: (A) full test mixture separation and (B) zoomed-in time window of the separation focusing on benzothiadiazine-related compound A, and chlorothiazide.

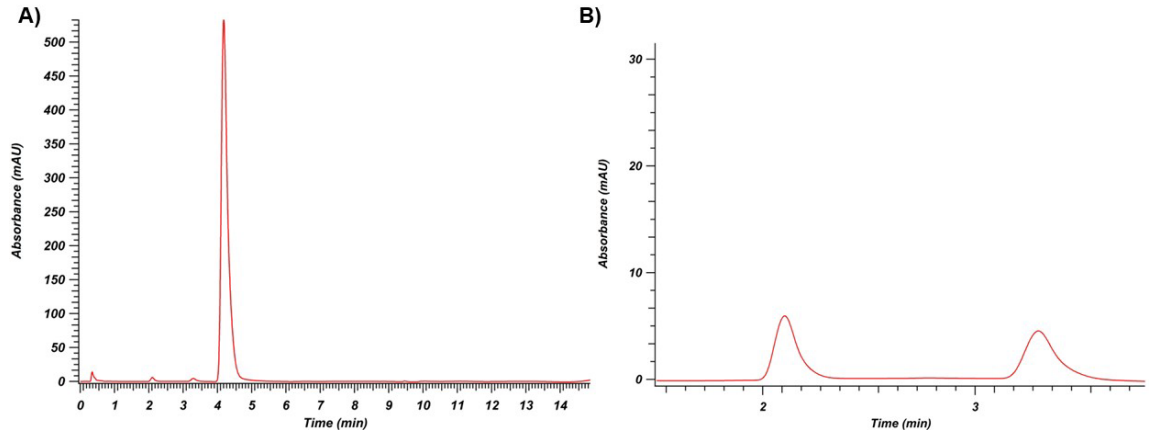

Figure 2. Analytical scale chromatograms obtained following the USP monograph: (A) full test mixture separation, and (B) zoomed in time window of the separation focusing on benzothiadiazine-related compound A and chlorothiazide.

Criteria	USP Monograph Specification	Axcend Focus LC Performance	Analytical Scale Performance
Resolution between benzothiadiazide-related compound A and chlorothiazide	>2.0	4.40	5.29
Resolution between chlorothiazide and hydrochlorothiazide	>1.5	2.60	3.40
Tailing of all peaks	<1.5	Meets suitability	Meets suitability
Relative standard deviation of peak areas for benzothiadiazine-related compound A and chlorothiazide	<5%	2.78%	1.40%
Relative standard deviation of quantitation limit solution	< 25%	0.44%	21.11%

Conclusions

A USP monograph for hydrochlorothiazide was adapted to capillary and analytical scales using USP 621 guidelines. The Axcend Focus LC demonstrated performance within the acceptable ranges provided by the monograph as well as its ability to generate comparable data to existing analytical scale instrumentation. Although there were differences in performance across the two scales, both systems were able to meet system suitability criteria, making them valid platforms for the monograph. During a single run, the Axcend Focus LC generates 99.6% less waste when compared to the original monograph conditions. This demonstrates the viability of using the Axcend Focus LC as a greener, cheaper, and more compact platform that meets the monograph specifications.

References

[1] USP General Chapter <621> Chromatography. United States Pharmacopoeia Convention Inc., Rockville, MD.

[2] United States Pharmacopeial Convention. Hydrochlorothiazide. United States Pharmacopoeia National Formula (USP 40-NF 35) 2017, 3421–3422.