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Note d'application

Improved Injection Precision of USP Methods with an Arc HPLC System

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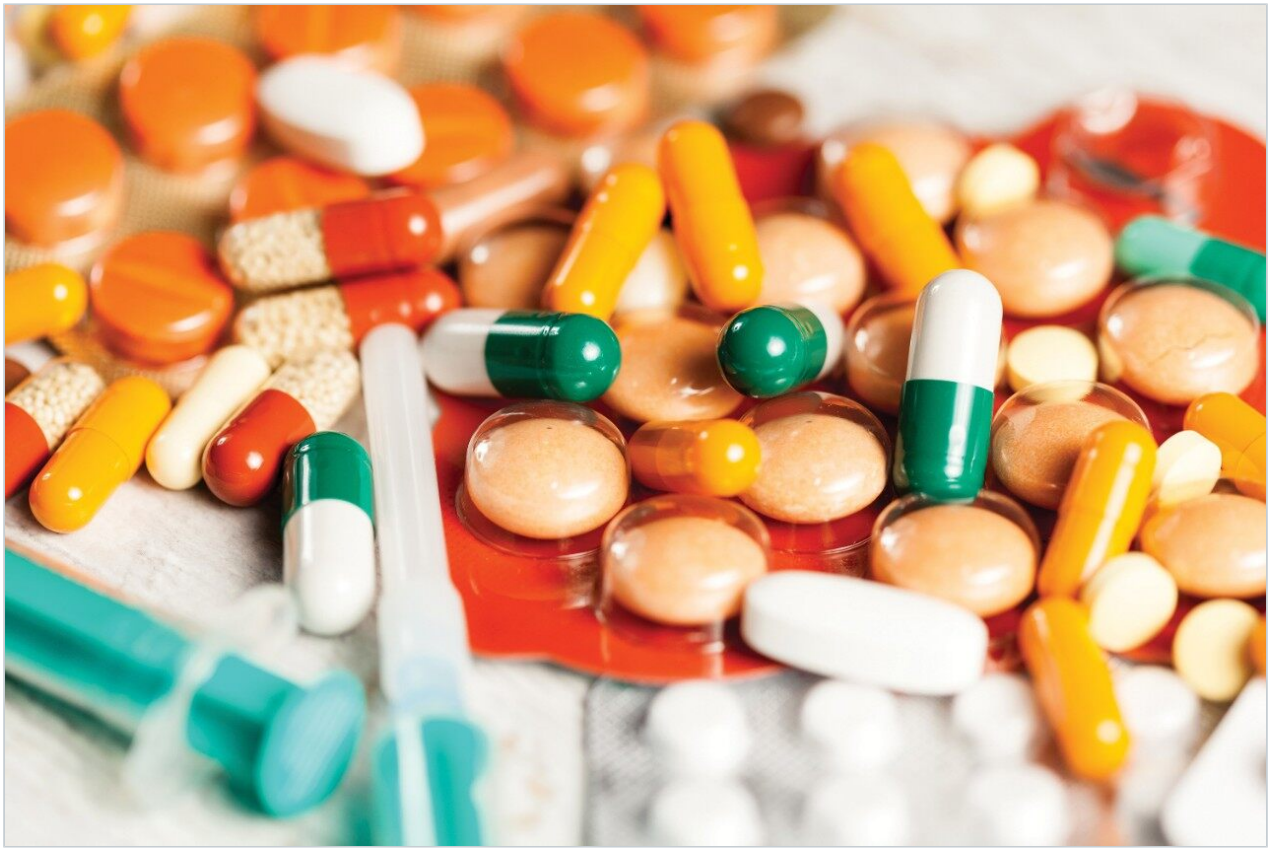

Abstract

Injection precision is a critical method attribute and, as such, is typically part of system suitability for regulated methods. While modern injectors are designed to meet injection precision requirements for most assays, many regulated methods have strict requirements employing conditions that differ from those described in vendor's methods used to determine specifications. These factors can lead to systems not meeting system suitability routinely. In this application note, the injection precision of the Arc HPLC System will be demonstrated, specifically with a focus on the system suitability requirements of United States Pharmacopeia (USP) monographs. The selected monographs were chosen for one of several criteria: 100% organic diluents, strict precision requirements (e.g., RSD ≤0.5%), and high injection volumes coupled with low sample concentrations. Any one of these variables, or a combination, can result in unacceptable injection variability. Additional studies will compare the performance of the Arc HPLC System and comparable HPLC systems, demonstrating the high injection precision of the Arc HPLC System.

Benefits

- Arc HPLC System meets strict injection precision requirements for USP monographs
- High precision for 100% organic diluent injections on Arc HPLC System

Introduction

Injection precision is critical for any quantitative HPLC method. As such, for many regulated methods there are strict requirements regarding the injection precision, to ensure that the values reported are within the expected measurement uncertainty and thus accurate. However, several instrument and method characteristics can impact the injection precision on any LC system. These can include sample diluent, aspiration rate, accuracy, and range of aspiration mechanics, etc.

One common characteristic of regulated methods is the need for highly organic diluents due to the solubility of the formulation and/or active pharmaceutical ingredient. These volatile diluents can impact the repeatability or injection precision of the method, due to potential evaporation causing changes in sample concentration over time. In addition, the combination of organic diluent with large injection volumes, often used for low concentration samples, can cause additional variability to injection precision, resulting in failure to meet system suitability criteria.

In this work, the injection precision for several regulated methods will be assessed using multiple HPLC systems. The various methods cover previously described attributes that make meeting system suitability on

a routine basis very challenging. For these examples, we will demonstrate the superior performance of the Arc HPLC System autosampler and its design in ensuring low variability from the instrument mechanics.

Experimental

Generic LC Conditions

LC system:	Arc HPLC with 30 CHC
Detection:	2998 (PDA) or 2489 (TUV)
Purge solvent:	90:10 Water/acetonitrile
Seal wash:	90:10 Water/acetonitrile
Needle wash:	50:50 Water/acetonitrile
Seal wash time:	0.5 min
Sample temp.:	10 °C
Flow cell:	Analytical (10 mm)

Data Management

Chromatography software:	Empower 3 FR3 Hotfix 1

Note: The Arc HPLC System autosampler uses a syringe and an independent (degassed) purge solvent to aspirate the sample. The syringe volume in combination with an extension loop dictates the maximum volume the autosampler can inject. The standard Arc HPLC System utilizes a 50-µL extension loop and a 30-µL needle, allowing for high precision of volumes up to 60 µL with a 100-µL syringe (75% of needle and loop volume).

USP Monograph for Azithromycin, Organic Impurities

Sample Description

As described in the USP monograph,[1] the stock solution of USP Azithromycin RS was prepared at 1 mg/mL in 7:6:7 methanol, acetonitrile, and 1.73 mg/mL anhydrous dibasic sodium phosphate in water, adjusted with ammonia to pH 10. Stock was further diluted to 86 µg/mL with same solvent.

Method Conditions

LC system:	Arc HPLC with 30 CHC
Detection:	2998 (PDA)
Wavelength:	210 nm
Column(s):	XBridge, 5 µm, 4.6 x 250 mm (p/n: 186003117)
Column temp.:	60 °C
Sample temp.:	15 °C
Injection volume:	50 µL
Flow rate:	1.0 mL/min
Mobile phase A:	1.8 mg/mL Dibasic sodium phosphate in water, adjusted with 10% phosphoric acid to pH 8.9
Mobile phase B:	3:1 Acetonitrile/methanol

Gradient

Time (min)	%A	%B
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

USP Monograph for Quetiapine

Sample Description

As described in the USP monograph,[2] the standard stock solution of USP Quetiapine Fumarate RS was prepared at 0.16 mg/mL in mobile phase. Standard solution was subsequently prepared from stock at 0.08 mg/mL solution in mobile phase.

Method Conditions

LC system:	Arc HPLC with 30 CHC
Detection:	2998 (PDA)
Wavelength:	230 nm
Column(s):	XBridge BEH C_8, 5 µm, 4.6 x 250 mm (p/n: 186003018)
Column temp.:	25 °C
Sample temp.:	15 °C
Injection volume:	50 µL
Flow rate:	1.3 mL/min

Mobile phase:	54:7:39 Methanol/acetonitrile/2.6 g/L dibasic ammonium phosphate, pH 6.5 with phosphoric acid
Run time:	15 min

USP Monograph for Losartan Potassium

Sample Description

As described in the USP monograph,[3] Losartan Potassium RS was prepared at 0.25 mg/mL in methanol.

Method Conditions*

LC system:	Arc HPLC with 30 CHC
Detection:	2489 (TUV)
Wavelength:	254 nm
Column(s):	XSelect HSS T3, 5 µm, 4.6 x 250 mm (p/n: 186004793)
Column temp.:	35 °C
Sample temp.:	15 °C
Injection volume:	13.2 µL
Flow rate:	1.32 mL/min
Mobile phase:	2:3 acetonitrile:0.1% phosphoric acid in water
Run time:	20 min

*Original monograph specified 4.0 x 250 mm column, method was scaled to 4.6 x 250 mm, with scaling of

injection volume and flow rate.

Results and Discussion

Injection Precision for USP Monographs

For this study, USP monographs were performed as described by <621>,[4] and the individual chapter to assess the performance of the Arc HPLC System. Azithromycin and quetiapine analyses were performed using the conditions and sample prep as described by the individual USP monographs. The method for losartan potassium required adjustments. Specifically, this USP assay specifies a 4.0 x 250 mm column, which was not available in a modern, robust packing material. For this method, the column was scaled to a 4.6 x 250 mm HSS T3 Column and corresponding adjustments were made for flow rate and injection volume.

The results on the Arc HPLC System demonstrated high precision for all monographs tested and were well within system suitability criteria (if applicable). Azithromycin, organic impurities, had no USP requirements for injection precision, however, this method produced precision with a value of 0.6% relative standard deviation (RSD) on the Arc HPLC System with a run time of 93 minutes.

Monograph	Diluent	Injection volume	Injection precision system suitability (RSD)	Results (RSD)
Azithromycin, organic impurities	7:6:7 methanol/acetonitrile/buffer	50 µL	N/A	0.6%
Losartan potassium, assay	Methanol	13.2 µL*	0.5%	0.08%
Quetiapine, assay	54:7:39 methanol/acetonitrile/buffer	50 µL	2.0%	0.11%

Table 1. Injection precision criteria and results (n=5) on Arc HPLC for USP monographs.

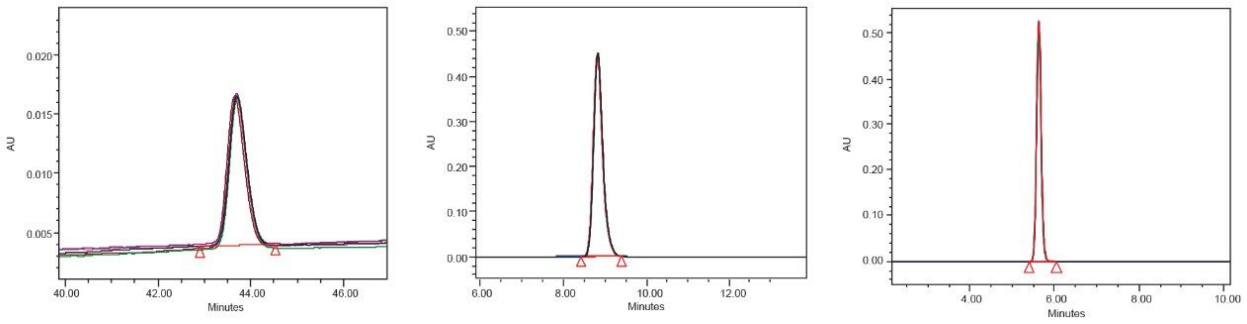

Figure 1. Injection overlay of azithromycin, losartan, and quetiapine standards (n=5).

Inter-assay Performance of Arc HPLC System

To assess the inter-assay injection precision of the Arc HPLC System, the USP monograph for losartan potassium, with strict injection precision requirements, was analyzed over a series of three days. System suitability criteria for the monograph included retention time, and area RSD, and USP tailing, and efficiency for five replicate injections. As shown in Table 2, all values were within the specifications on each of the three days. Of particular note is the area %RSD for this assay, given the strict requirement: Not More Than (NMT) 0.5%. These results demonstrated the highly reproducible injection precision of the autosampler on the Arc HPLC System.

System suitability	Criteria	Day 1	Day 2	Day 3	Mean
Retention time %RSD	NMT 0.5%	0.115	0.134	0.143	0.131
Area %RSD	**NMT 0.5%**	**0.082**	**0.105**	**0.058**	**0.082**
USP tailing	NMT 1.4	1.1	1.1	1.1	1.1
Efficiency	NLT 5600	12404	11460	11416	11760

Table 2. System suitability results for analysis of losartan potassium (n=5) over three days.

Inter-Assay Injection Precision of Arc HPLC System and Comparable HPLC Systems

For any HPLC system, inter-assay results are critical to ensure a method will meet the system suitability criteria on a routine basis. To determine the comparative performance of other HPLC systems and the Arc HPLC System, inter-assay injection precision was evaluated using the losartan potassium monograph. Each

sample set contained five replicate injections, as described in the USP monograph. All tests were performed in triplicate on each system, on separate days. The RSD values of each day were calculated.

In this study, each autosampler used a different design for sample aspiration. For the Arc HPLC System, the autosampler uses a syringe and an independent purge solvent to aspirate the sample. Other autosamplers employed metering devices, syringes or pump heads to aspirate the samples, with or without separate purge solvents. Regardless of the manner, to ensure optimal performance, each autosampler was purged or primed with recommended settings. These steps were performed to reduce failure due to improper preparation of the purge line or syringe, from air bubbles or non-degassed solvents.

The results for multiple HPLC systems are shown in Figure 2. The injection precision on the Arc HPLC System was superior to that observed on comparable systems (W–Z), with values well below the specification. For the three sets of data on the Arc HPLC System, all system suitability requirements were met. Of the other systems tested, only System W met the system suitability criteria for all three sets of injections, while Systems X, Y, and Z did not. In addition, the Arc HPLC System had the lowest %RSD values. These results demonstrate the ability of the Arc HPLC System to routinely meet these narrow precision requirements as compared to other HPLC systems.

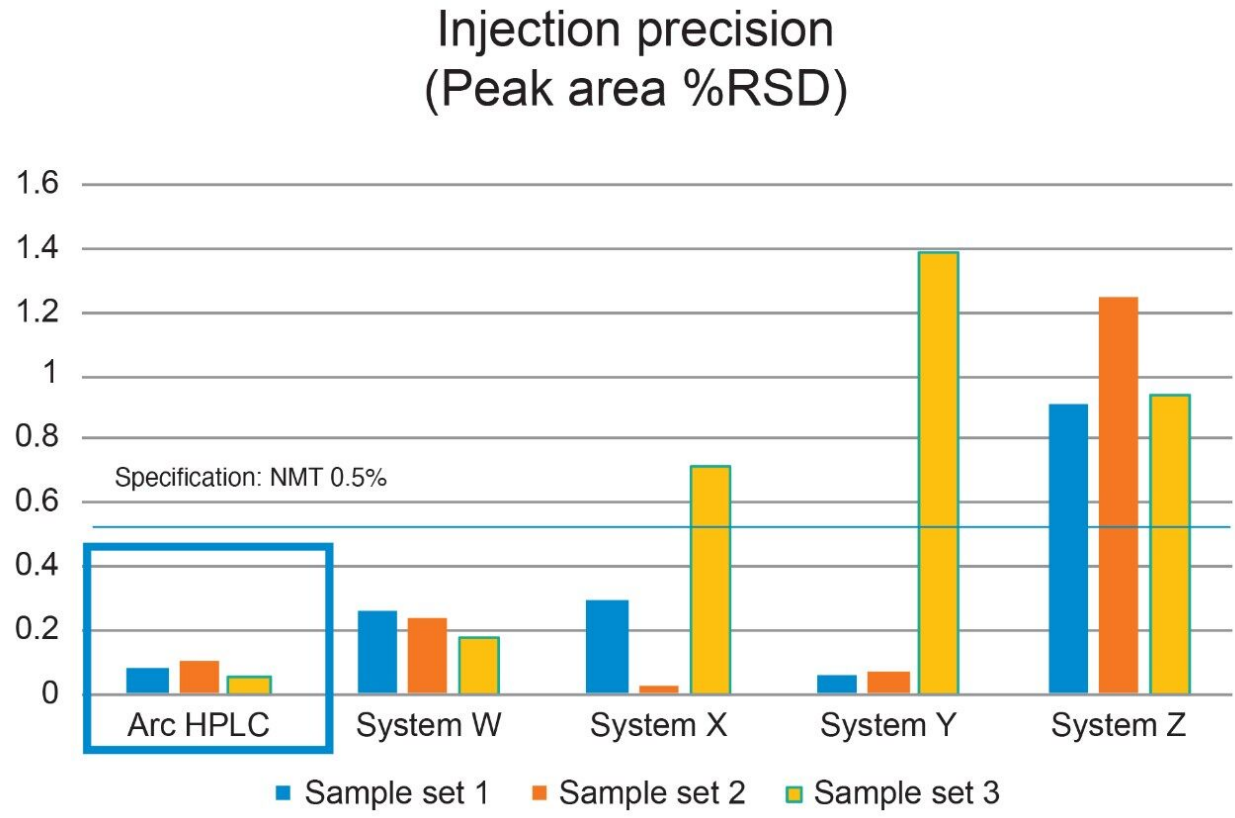

Figure 2. Injection precision of Arc HPLC System and comparable HPLC systems for analysis of losartan potassium.

Conclusion

The Arc HPLC System is designed to ensure highly reproducible injection volumes, meeting strict system suitability criteria. The autosampler takes advantage of a separate degassed purge line to ensure reproducible peak areas. This ensures a high level of reproducibility even for HPLC methods with highly organic diluents and larger injection volumes – characteristics that can impact injection precision.

References

1. USP, Azithromycin, Organic Impurities. *United States Pharmacopeia and National Formulary* (USP 43-NF38) 2020, (GUID-34C99575-55AF-407A-8C43-79D17394E453_5_en-US), 446.
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4. <621> CHROMATOGRAPHY. In *United States Pharmacopeia and National Formulary* (USP 43 NF38), United Book Press, Inc.: Baltimore, MD, 2020; 6853.

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