# STRATEGIES FOR IMPROVING INJECTION PRECISION WITH CHALLENGING USP MONOGRAPHS

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## PURPOSE

Injection precision is a common system suitability criterion for USP monographs and can be affected by method and instrument attributes on an high-performance liquid chromatography (HPLC) system. These can include sample diluent, injector draw rate, and accuracy.

Due to solubility concerns, many regulated methods require sample diluents that are highly organic. Furthermore, many methods have strict suitability criterion for injection precision. These conditions require high performing autosamplers, and optimization of the autosamplers may be required to meet the stringent system suitability criterion.

| System | Aspiration Mechanism |
|---|---|
| Alliance™ e2695 System<br>Waters™ HPLC System | Sampling Syringe |
| Alliance™ iS HPLC System<br>Comparable System X<br>Comparable System Y | Metering Device |

*Table 1: HPLC Systems and Aspiration Mechanisms.*

To examine the impact of system design on injection precision, USP monographs with challenging conditions were executed on several HPLC systems with different mechanisms for sample aspiration. USP monographs were selected based on method parameters expected to be challenging for an autosampler, such as strict precision requirements, highly organic diluents, and low sample injection volumes.

## METHODS

**Selected USP Monographs (Assay)**

| No. | Compound Name | Diluent | Area RSD Criteria (%) |
|---|---|---|---|
| 1 | **Fenofibrate** | Acetonitrile/Water pH 2.5 (70/30) | **1.0** |
| 2 | **Ketoconazole** | Methanol | **0.73** |

Standards of fenofibrate and ketoconazole and mobile phases were prepared as described in the USP monographs (assay). Sample sets consisted of six replicate injections of standards.

**Method Conditions**

| Fenofibrate | | | |
|---|---|---|---|
| **Mobile Phase** | Acetonitrile/Water pH 2.5 (70/30) | | |
| **Standard** | 1 mg/mL USP Fenofibrate RS | | |
| **Wavelength** | 286 nm, 10 Hz | | |
| **Column*** | XSelect™ CSH™ C18 4.6 × 250 mm, 5 µm | | |
| **Column Temp.** | 25°C | | |
| **Injection Vol.*** | 6.6 | | |
| **Flow Rate*** | 1.323 mL/min | | |
| **Pump Mode** | Isocratic | | |
| *Original method called for 4.0 × 250 mm column dimensions. Method was scaled using Waters Columns Calculator: flow rate adjusted from 1.0 mL/min to 1.323 mL/min and injection volume adjusted from 5 µL to 6.6 µL. | | | |

| Ketoconazole | | | |
|---|---|---|---|
| **Mobile Phase** | A: Acetonitrile/3.4 mg/mL tetrabutyl ammonium hydrogen sulfate in water (5/95)<br>B: Acetonitrile/3.4 mg/mL tetrabutyl ammonium hydrogen sulfate in water (50/50) | | |
| **Standard** | 0.1 mg/mL USP Ketoconazole RS | | |
| **Wavelength** | 225 nm, 10 Hz | | |
| **Column*** | XBridge™ Shield RP18 4.6 × 100 mm, 3.5 µm | | |
| **Column Temp.** | 25°C | | |
| **Injection Vol.** | 10.0 | | |
| **Flow Rate*** | 1.714 mL/min | | |
| **Pump Mode** | Gradient | | |
| | **Time (min)** | **A (%)** | **B (%)** |
| **Gradient Table*** | **Initial** | 100 | 0 |
| | **23.33** | 0 | 100 |
| | **29.17** | 0 | 100 |
| | **30.33** | 100 | 0 |
| | **35.00** | 100 | 0 |
| *Original method called for 4.6 × 100 mm column with 3-µm particle size. Method was scaled using Waters Columns Calculator: flow rate adjusted from 2.0 mL/min to 1.714 mL/min and adjustments to Gradient Table. | | | |

| Shared LC Parameters | |
|---|---|
| **LC System** | Flow-Through-Needle (FTN), Quaternary |
| **Sample Temp.** | 15°C |
| **CDS** | Empower™ 3 |

## RESULTS & DISCUSSION

Autosamplers in this study utilize different designs for sample aspiration, see Table 1. Alliance e2695 System and Waters HPLC System use sampling syringes. Alliance iS HPLC System and comparable systems X and Y use metering devices in-line with the flow path. Typical autosampler designs are depicted in Figure 1.

USP monographs for fenofibrate and ketoconazole were executed and peak reproducibility assessed over three days. Intra-day sample sets consisted of three sample sets of six replicate standard injections run back-to-back. Each system was primed or purged according to recommended settings.

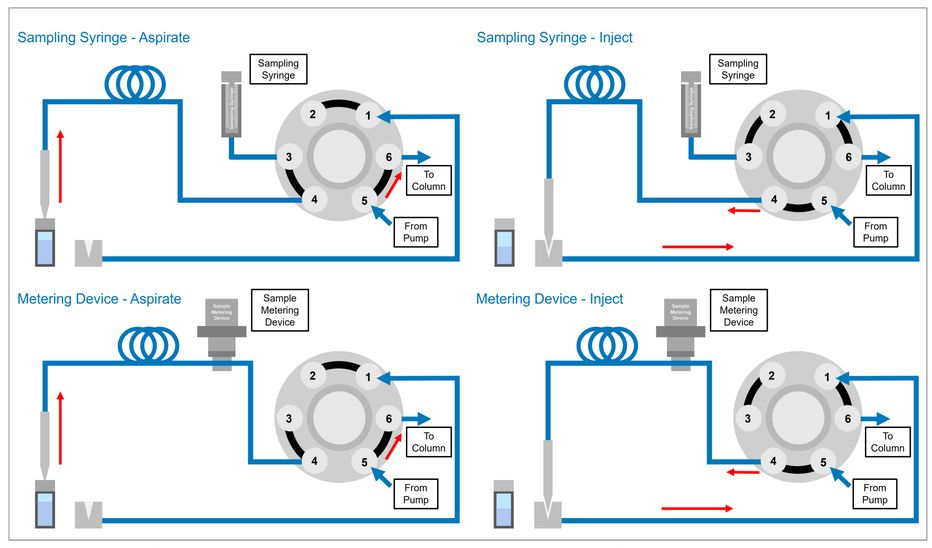


*Figure 1. Sample aspiration and injection stages for sampling syringe and metering device designs in a FTN HPLC system.*

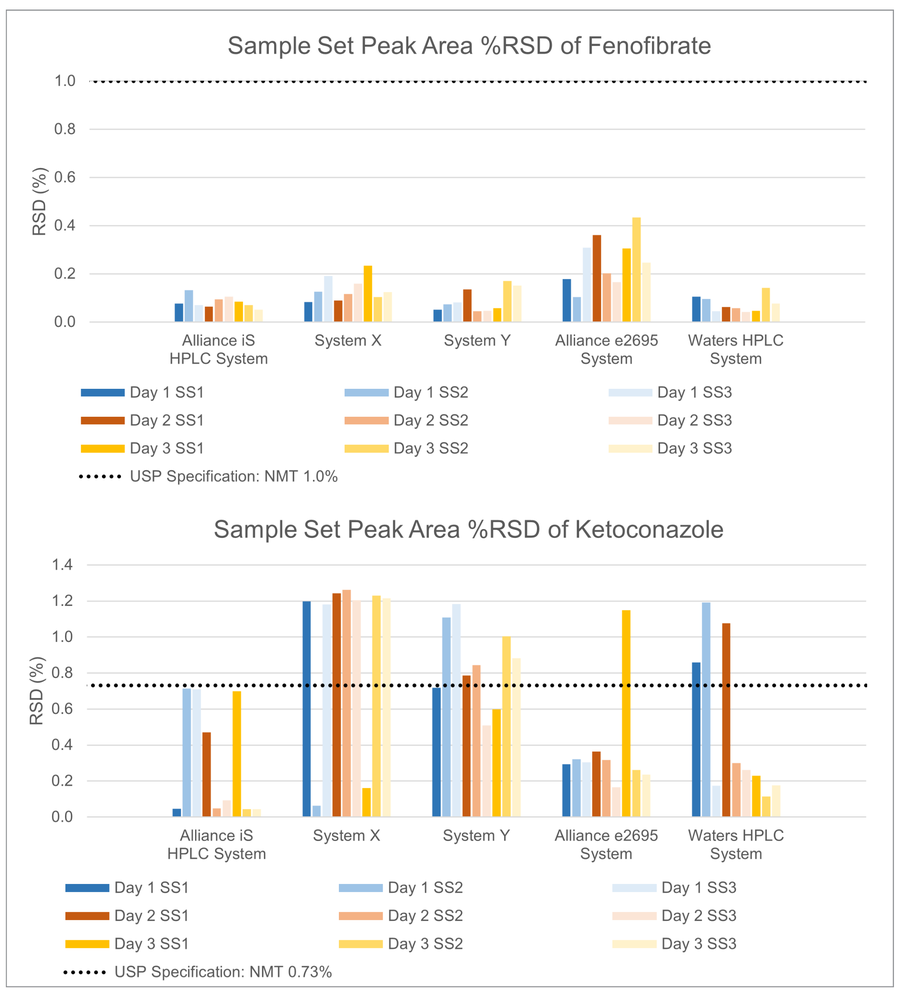


*Figure 2. Sample set peak area %RSD. Each sample set consisted of 6 replicate injections of standard. N=3 samples sets were run each day.*

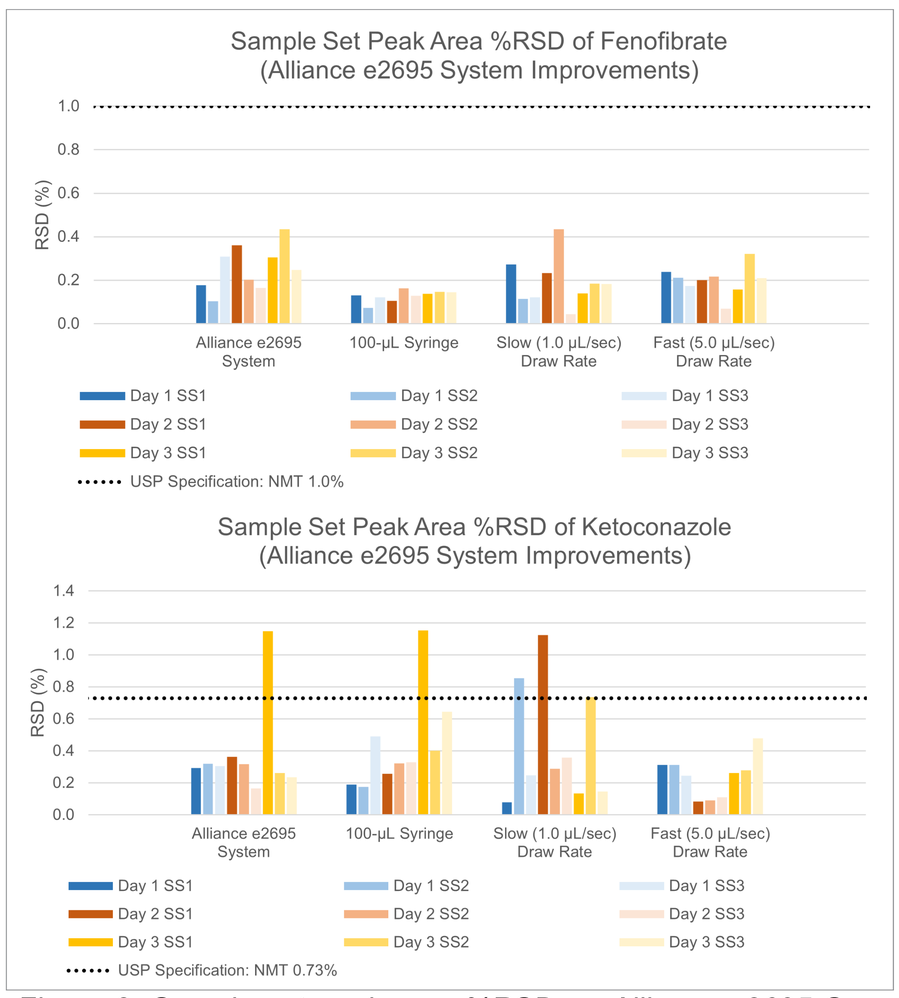


*Figure 3. Sample set peak area %RSD on Alliance e2695 System. Each sample consisted of 6 replicate injections of standard. N=3 sample sets were run each day.*

Peak area relative standard deviations (RSD) were calculated for individual sample sets in Figure 2.

Fenofibrate RSD values did not exceed 0.5% and met 1.0% RSD requirement across all systems. Alliance e2695 System, which uses a sampling syringe, had highest average RSD values overall.

Ketoconazole RSD values met the 0.73% RSD requirement only on Alliance iS HPLC System, which utilizes an in-line metering device. The ketoconazole assay proved more challenging for injector precision across the systems, with large variances between intra-day sample sets, as seen in Figure 2.

A sampling syringe is isolated from the flow path and may accumulate air bubbles over time. On the other hand, a metering device is located in-line with the flow path and is flushed with mobile phase, reducing air bubble formation during acquisition.

Some additional characteristics, such as injector draw rate and accuracy, were investigated in Figure 3, using Alliance e2695 System as a model.

In default configuration, the system uses a 250-µL syringe and a Normal (2.5 µL/sec) draw rate. For this test, different strategies were implemented, including using a 100-µL syringe and varying injector draw rates. Each change was tested independently.

Using a 100-µL syringe provided the most consistent injector performance for Fenofibrate, but not for Ketoconazole. Using a Fast Draw Rate worked best for Ketconazole, possibly due to the viscosity of the methanol diluent. The effective strategies observed differed based on assay.

## CONCLUSION

- **An in-line metering device can aid injection precision by flushing the flow path during acquisition, reducing formation of air bubbles.**
- **For instruments equipped with sampling syringes, purging the injector prior to start of sequence is critical.**
- **Additional strategies to improve performance can be implemented case-by-case. In this study, using a smaller volume sampling syringe and selecting a proper draw rate for the samples based on the viscosity of the diluent yielded positive results.**

**References**

*USP. Fenofibrate Assay. DOI: https://doi.org/10.31003/USPNF_M32710_04_01*

*USP. Ketoconazole Assay. DOI: https://doi.org/10.31003/USPNF_M43990_04_01*

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