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Evaluation of intra- and inter-system precision for HPLC analysis of active pharmaceutical ingredients and impurities

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Goal

- Evaluate hardware-related variability of six Thermo Scientific™ Vanquish™ Core HPLC systems under highly controlled conditions
- Evaluate intra- and inter-system retention time and peak area precision for the analysis of two active pharmaceutical ingredients and their related impurities

Introduction

High performance liquid chromatography (HPLC) has been an established technique in pharmaceutical laboratories for decades. Whether in research and development or in quality control labs, HPLC users expect robust, reliable systems with equivalent performance. A high level of reproducibility between the systems greatly facilitates method transfer, a frequent task in many laboratories. In a previous work a global round-robin study of multiple Thermo Scientific™ Vanquish™ Core HPLC systems was conducted to demonstrate system-to-system variability across different operators in different locations running the same application. It was shown that retention time reproducibility is largely influenced by eluent preparation, while peak area reproducibility primarily depends on the sample preparation. However, regardless of where and by whom the HPLC system was operated, each system met the criteria set for system suitability.[1]

In this technical note, we go into more depth on the system-to-system comparability of results generated from a large number of random system samples from serial production. Multiple systems were operated in the same laboratory, under the same conditions, running the same pharmaceutical applications. This will demonstrate how similar the systems perform in intra-system and inter-system comparison. A total of six Vanquish Core systems, randomly picked from serial manufacturing, were used for this study. To minimize non-hardware related influences like mobile phase, samples, and equilibration time, these variables were strictly controlled. In addition, to reduce environmental influences such as temperature fluctuations, mobile phase, and sample aging, the experiments were carried out on two systems at a time to achieve a short overall experimental time. Two applications were run in total. The first application analyzes nevirapine with a typical gradient method and is based on a previously published technical note.[2] The second application is for the analysis of acetaminophen and related impurities. The chromatographic method is based on the United States Pharmacopeia (USP) monograph of the assay method.[3]

Experimental

Chemicals

- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (P/N A955-212)
- Fisher Scientific™ Methanol, Optima™ LC/MS grade (P/N A456-212)
- Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System, deionized water, 18.2 MΩ·cm at 25 °C (P/N 50136149)
- Fisher Scientific™ Acetic acid, Optima™ LC/MS grade (P/N A113-50)
- Fisher Scientific™ Ammonium acetate, LC/MS grade (P/N A114-50)
- Fisher Scientific™ Potassium dihydrogen orthophosphate (P/N P/4806/50)
- Fisher Scientific™ Sodium phosphate dibasic anhydrous (P/N BP332-500)

Certified standards of the following were purchased from reputable vendors:

- 2-Acetamidophenol (impurity C)
- 4-Aminophenol
- 4'-Chloroacetanilide (impurity J)
- 11-Ethyl-4-methyl-5,11-dihydro-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (impurity A)
- 4-Methyl-5,11-dihydro-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (impurity B)
- 4-Methyl-11-propyl-5,11-dihydro-6H-dipyrido[3,2-b:2′,3′-e][1,4]diazepin-6-one (impurity C)
- Acetaminophen
- Acetanilide (impurity D)
- N-(4-hydroxyphenyl) propanamide (impurity B)
- Nevirapine
- Uracil

Equipment

- Vials (amber, 2 mL), Fisher Scientific (P/N 11545884)
- Snap Cap with Septum (Silicone/PTFE), Fisher Scientific (P/N 10547445)
- Thermo Scientific™ nanoViper™ capillary, 950 mm x 50 µm (P/N 6041.5125)

Instrumentation

A Thermo Scientific™ Vanquish™ Core Quaternary HPLC system equipped with the following was used for the analysis:

- Thermo Scientific™ Vanquish™ System Base Vanquish Core (P/N VC-S01-A)
- Thermo Scientific™ Vanquish™ Quaternary Pump C (P/N VC-P20-A)
- Thermo Scientific™ Vanquish™ Sampler CT (P/N VC-A12-A)
- Thermo Scientific™ Vanquish™ Column Compartment C (P/N VC-C10-A-03)
- Thermo Scientific Vanquish™ Variable Wavelength Detector C (P/N VC-D40-A) with standard flow cell, SST, 10 mm, 11 µL (P/N 6077.0250)

Experimental setup

Six Vanquish Core HPLC systems were tested under highly controlled conditions. Two systems at a time were run in parallel. Prior to the experiments, four columns were evaluated, of which two were selected to run the parallel setup. Those columns with the most similar efficiencies were selected for the experiments. Therefore, they were tested according to the requirements of the respective certificate of analysis. The mobile phases and samples were freshly prepared by a single operator before each application and then split into two equal portions. After each sequence, the mobile phases, samples, columns, and flow cells were transferred to the next pair of systems. Ten injections of each sample were carried out per system, bracketed by two blank runs before and after the sample block.

Data processing and software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.3 was used for data acquisition and processing.

Sample preparation and chromatographic conditions for Application 1

According to the USP monograph,[4] stock solutions of nevirapine (0.24 mg/mL) and the related impurities A (0.24 mg/mL) and B (0.06 mg/mL) were each prepared. For better solubility, acetonitrile and solvent A were added in a ratio of 1:20 (nevirapine solution), 1:3 (impurity A solution), and 1:22 (impurity B solution). First, the appropriate amount of acetonitrile was added to the volumetric flask with the sample and sonicated for 10 minutes. Secondly, solvent A was added to reach ¾ of the volume of the volumetric flask and sonicated again for 10 minutes. After the solutions had cooled to room temperature, the volumetric flasks were filled to the respective volume with solvent A (Table 1).

Table 1. Chromatographic conditions for Application 1

Column	Thermo Scientific™ Acclaim™ Polar Advantage II, 150 x 3 mm, 3 µm (P/N 063705)	
Mobile phase	A: 10 mM ammonium acetate, pH 5/acetonitrile (85/15; v/v) B: Acetonitrile	
Flow rate	0.75 mL/min	
Gradient	**Time [min]**	**%B**
	0.000	16
	2.000	18
	3.533	25
	8.000	25
	8.067	16
	13.333	16
Column compartment temperature	30 °C (with passive pre-heater), forced air mode with fan speed 5	
Sample compartment temperature	10 °C	
Injection volume	5 µL	
UV detector settings	Wavelength 240 nm, data collection rate 10 Hz, response time 0.5 s	

The working solution was prepared at 0.03 mg/mL nevirapine and impurity A, and 0.015 mg/mL impurity B by diluting the individual stock solutions with the appropriate volumes of mobile phase A (equal to the system suitability sample described in the monograph).

Sample preparation and chromatographic conditions for Application 2

Individual stock solutions of the active pharmaceutical ingredient (API) acetaminophen (20 mg/mL), 4-aminophenol, and the related impurities B, C, D, and J (1 mg/mL each) were prepared in methanol. A working solution containing all analytes was prepared at a concentration of 1 mg/mL acetaminophen and 10 µg/mL of each of the other compounds (corresponding to 1% of the API) by diluting the stock solutions with methanol.

Table 2. Chromatographic conditions for Application 2

Column	Thermo Scientific™ Hypersil GOLD™ C8, 4.6 x 100 mm, 3 µm, 175 Å (P/N 25203-104630)	
Mobile phase	A: 1.7 g/L potassium dihydrogen orthophosphate in water and 1.8 g/L sodium phosphate dibasic anhydrous in water B: Methanol	
Flow rate	1 mL/min	
Gradient	Time [min]	%B
	0.000	1
	3.000	1
	7.000	81
	7.100	1
	12.000	1
Column compartment temperature	35 °C (with passive pre-heater), forced air mode with fan speed 5	
Sample compartment temperature	8 °C	
Injection volume	5 µL	
UV detector settings	Wavelength 230 nm, data collection rate 10 Hz, response time 0.5 s	

Results and discussion

Determination of system void volume equivalency

The equivalence of the systems for void time and volume should demonstrate non-application related influences on the overall study. Flow Injection Analysis (FIA) was performed to determine void time and volume of each system. Instead of a column, a nanoViper capillary was installed to generate reasonable backpressure of around 320 bar. Experiments were carried out using a mobile phase composition of water/ACN (40/60; v/v) mixed by pump at a flow rate of 0.5 mL/min. 2.5 µL of uracil standard (0.015 mg/mL) was injected in triplicate on each system.

All six systems showed similar results as can be seen in Table 3. Measured system void volumes were corrected by subtracting the nominal volume of the flow-restrictor capillary (1.9 µL).

Table 3. Overview of obtained void times and the calculated void volumes of each system. For the calculation, the mean value of the void time was considered and corrected by the volume of the nanoViper capillary (1.9 µL); FIA analysis with three consecutive injections of uracil standard.

Systems	Void time [min]	Void volume [µL]
System 1	0.088; 0.088; 0.088	42.1
System 2	0.088; 0.088; 0.088	42.1
System 3	0.090; 0.090; 0.090	43.1
System 4	0.090; 0.090; 0.090	43.1
System 5	0.089; 0.090; 0.088	42.8
System 6	0.090; 0.090; 0.090	43.1

As a result, system components including capillaries, mixers, valves, or injection parts are not expected to negatively impact system reproducibility results of the application testing further discussed in this technical note.

Intra-system performance

Results from ten consecutive injections of two pharmaceutical applications were assessed on each of the six systems. Figure 1 shows an overlay of the ten injections on system 1 for Application 1 (A) and Application 2 (B).

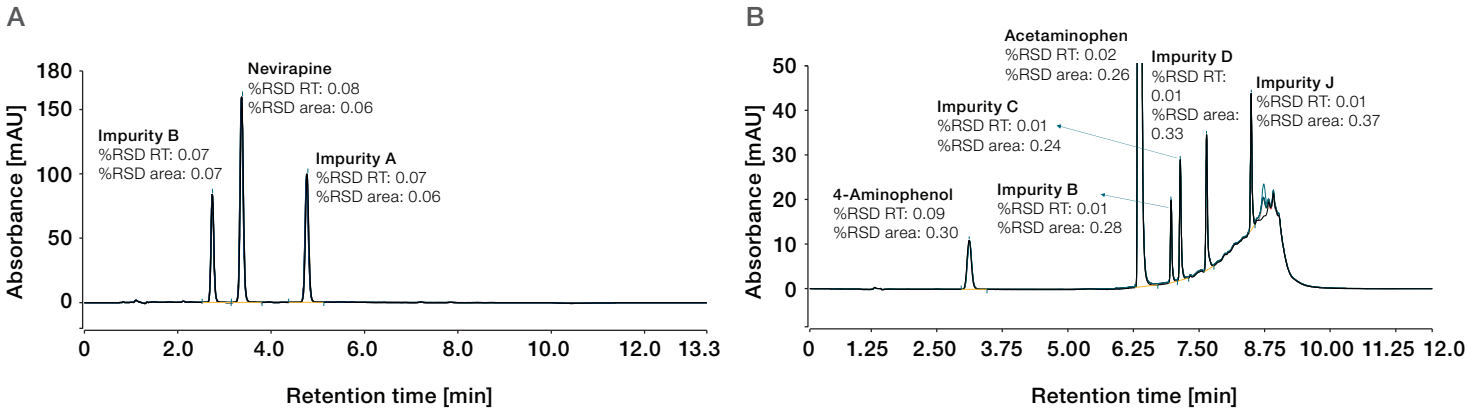

Figure 1. Overlay of ten consecutive injections (system 1). (A) Application 1 (nevirapine); (B) Application 2 (acetaminophen) with obtained relative standard deviation of retention time (%RSD RT) and peak area (%RSD area) for all compounds.

To evaluate intra-system precision, representative compounds of each application were selected. This selection was done by considering the specific character of each method. The nevirapine application (Application 1) is a typical gradient method on a standard HPLC system (Table 1). Here, one compound eluting within the gradient ramp (nevirapine related impurity B) and one compound eluting in the isocratic hold (nevirapine related impurity A) after the gradient were chosen. The acetaminophen application (Application 2) starts with an isocratic hold at 1% B, which can be challenging for a standard quaternary HPLC pump (low-pressure gradient pump (LPG)), followed by a steep gradient ramp (from 1% B to 81% B in 4 min). For this application three compounds were selected: 4-aminophenol, which eluted during the isocratic hold, acetaminophen related impurity B (the compound with the lowest signal), and acetaminophen (the compound with the highest signal). The acetaminophen peak height was close to the upper limit of the detector linearity range.

Overall, excellent intra-system precision of retention time and peak area were observed on each system, regardless of which application was applied, as shown in Table 4. Higher relative standard deviations for retention time (%RSD RT) and peak area (%RSD area) were expected for 4-aminophenol because it was dissolved in methanol and injected into 1% organic solvent, which generally leads to distortion of poorly retained peaks and renders integration more challenging. However, the results show no difference compared to the other analytes. Moreover, Application 2 shows excellent performance over a wide linear range, which enables accurate quantitation of both acetaminophen (1 mg/mL) and the related impurities (10 µg/mL; corresponds to 1% of acetaminophen).

Table 4. Relative standard deviation ranges for retention time (%RSD RT) and peak area (%RSD area) of each compound from ten consecutive injections on the six systems

Compound name	%RSD RT (min and max of six systems)	%RSD area (min and max of six systems)
Nevirapine related impurity B	0.04–0.12	0.05–0.24
Nevirapine related impurity A	0.03–0.08	0.05–0.10
4-Aminophenol	0.04–0.09	0.13–0.31
Acetaminophen	0.01–0.02	0.15–0.26
Acetaminophen related impurity B	0.01–0.01	0.13–0.28

Excellent retention time and peak area reproducibility of the Vanquish Core system has also been demonstrated in another technical note. In that previous study, a European pharmacopeial isocratic method for thiopental was run on one system in a 3.5 month long-term robustness study. The %RSD RT resulted in less than 0.04, and the %RSD area was between 0.10 and 0.54 for ten consecutive injections in each sequence. Additionally, a %RSD RT of 0.06 was demonstrated for 170 consecutive injections over three days. Consequently, the study showed that a single system delivers stable performance over a long period.[5] The results presented here further demonstrate excellent intra-system performance across multiple systems for gradient separations.

Inter-system comparison

Solvents and samples for each application were freshly prepared and divided into portions to run two systems in parallel. Mobile phases, samples, detector flow cells, and columns were then transferred to the next pair of systems (see further details in the experimental section). A measurement time of less than three days could be achieved for all six systems per application to minimize the risk of solvent or sample aging, which could have a negative impact on the inter-system comparison.

Inter-system RSD RT values were less than 1% for all compounds analyzed. This is particularly important because processing methods do not have to be adapted from one system to another when a method is transferred. Figure 2 shows the inter-system results of RT, peak area, and relative peak area for all selected compounds. For the RSD calculation, the mean values of each system were considered.

Inter-system RSD values for peak area and relative peak area were generally good at <2.5%. As expected, the intense peak of acetaminophen, close to the detector linear response limit, and the peak of acetaminophen related impurity B with the lowest relative peak area, show higher values for peak area RSD. In contrast, 4-aminophenol shows much less variation in area, with a %RSD of 0.98, and therefore leads to the higher %RSD in relative area. While the monographs of the applications used allow up to 5% peak area RSD for intra-system results, this illustrates clearly the excellent inter-system equivalency of the Vanquish Core HPLC systems.

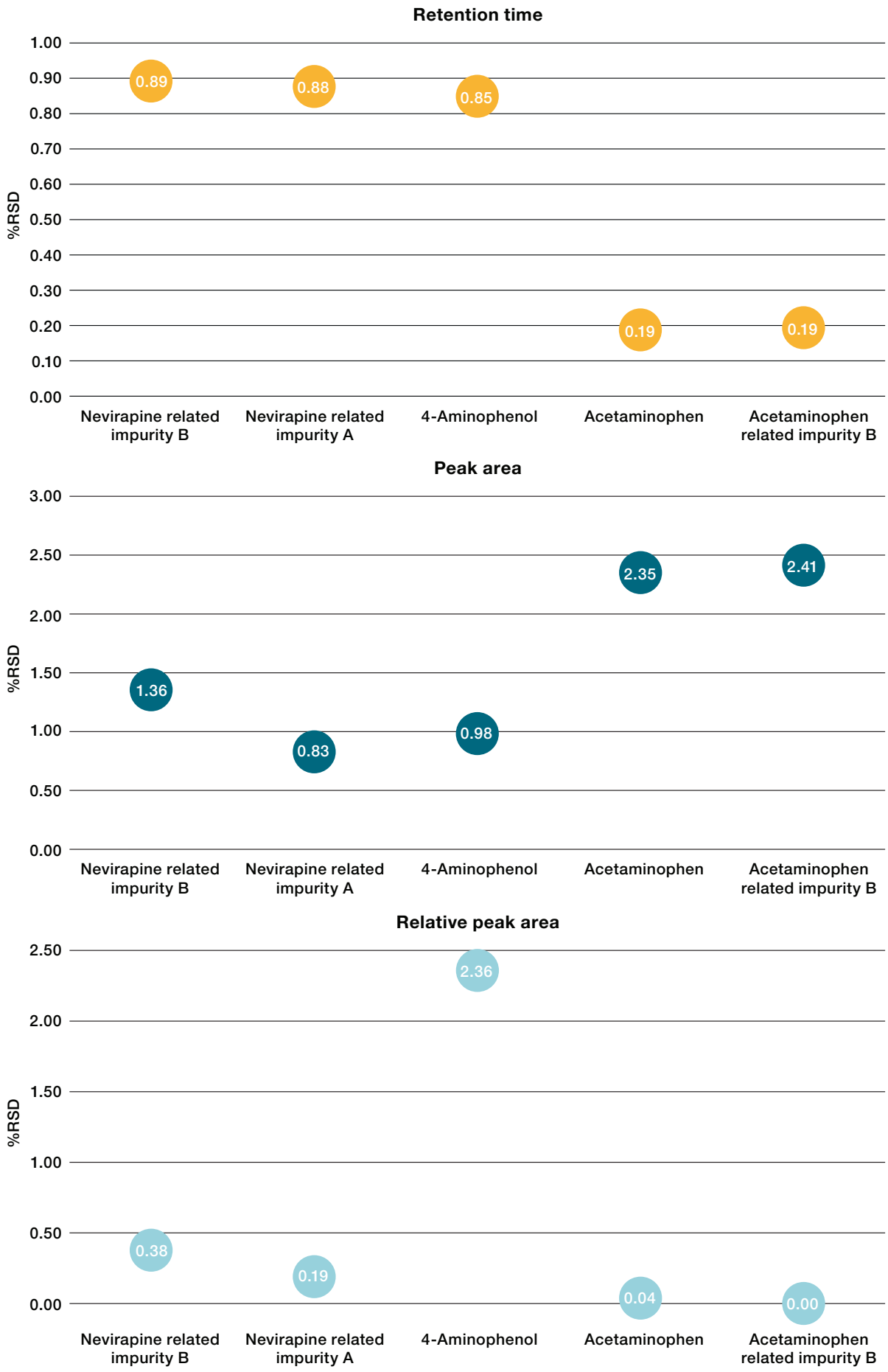

Figure 2. Inter-system comparison results; %RSD RT <1; %RSD peak area and relative peak area <2.5

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Conclusion

Two different pharmaceutical applications were carried out on six randomly selected Vanquish Core HPLC systems:

- All six HPLC systems show excellent intra-system precision with %RSD RT between 0.01 and 0.12 and %RSD peak area between 0.05 and 0.31.
- A high level of inter-system reproducibility in terms of retention time (%RSD RT ≤0.89), peak area (%RSD area ≤2.41), and relative peak area (%RSD relative area ≤2.36) allows for easy and successful method transfer.
- System components such as capillaries, mixers, and injection parts do not impact the overall inter-system performance as demonstrated by the similar system void volumes obtained.
- A laboratory standardized on Vanquish Core HPLC systems can expect highly reproducible results.

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