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APPLICATION NOTE 72716

Method transfer of a USP-derived acetaminophen assay from an UltiMate 3000 SD system to a Vanquish Flex UHPLC system

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Keywords
HPLC method transfer, Vanquish Flex quaternary system, UltiMate 3000 Standard quaternary system, gradient delay volume, acetaminophen

Application benefits

- Flexible system volume adjustment in Thermo Scientific™ Vanquish™ UHPLC systems facilitates straightforward transfer of analytical HPLC methods from Thermo Scientific™ UltiMate™ 3000 HPLC systems
- Fine tuning of retention times can be achieved by adjustment of the idle volume of the autosampler metering device

Goal

To demonstrate the straightforward transfer of analytical HPLC methods between the UltiMate 3000 platform and the Vanquish platform.

Introduction

The transfer of analytical liquid chromatographic (LC) methods from one instrument to another is a frequent but challenging task in most industries and is of particular importance in regulated environments.[1,2] Transfers are performed between identical instruments as well as instruments of different configurations, vendors, or generations. The true complexity thus is highly dependent on the robustness of the method that needs to be transferred as well as on instrumentational differences of the systems in concern.[1,2] To obtain equivalent results with the sending and receiving systems, specific technical characteristics like gradient delay volume (GDV), hydrodynamic

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behavior, or thermostatting mode need to be accounted for, preferably without modification of method parameters to avoid elaborate revalidation.[3]

According to this, the current application note demonstrates the straightforward transfer of a USP-derived assay[4] of the active pharmaceutical ingredient (API) acetaminophen, a common pain killer, and its impurities from an UltiMate 3000 SD system to a Thermo Scientific™ Vanquish™ Flex UHPLC system by means of unique features of that platform.

Experimental

Reagents and materials

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Optima™ Methanol, LC/MS grade (P/N 10767665)
- Fisher Scientific™ Sodium phosphate dibasic anhydrous (P/N 10182863)
- Fisher Scientific™ Potassium dihydrogen orthophosphate (P/N 10429570)
- Acetaminophen, 4-aminophenol, N-(4-hydroxyphenyl) propanamide (impurity B), 2-acetamidophenol (impurity C), acetanilide (impurity D), and 4'-chloracetanilide (impurity J) were purchased from reputable vendors.

Sample preparation

Stock solutions of acetaminophen (20 mg/mL), 4-aminophenol, and the impurities B, C, D, and J (1 mg/mL each) were prepared in methanol. By dilution with methanol and mixing of stock solutions, a sample was prepared that contained 1 mg/mL acetaminophen and 10 µg/mL of each of the other compounds (corresponding to 1% of the API).

Instrumentation

See Table 1 for the instruments used in this study.

LC conditions

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 KH_2PO_4 and 1.8 g/L of Na_2HPO_4 in water B: Methanol
Flow rate:	1 mL/min
Gradient:	0 min 1% B 3 min 1% B 7 min 81% B 7.1 min 1% B 12 min 1% B
Column temp.:	35 °C (with or without eluent pre-heating)
Autosampler temp.:	8 °C
Detection:	230 nm, 10 Hz data collection rate, 0.5 s response time
Inj. volume :	1 µL
Needle wash:	Off

Table 1. Instruments used in this study

Standard configurations		
	UltiMate 3000 SD Quaternary	**Vanquish Flex Quaternary**
		System Base (P/N VH-S01-A-02)
Pump	Standard Quaternary Pump LPG-3400SD (P/N 5040.0031)	Quaternary Pump F (P/N VF-P20-A)
Sampler	Well Plate Autosampler WPS-3000TSL (P/N 5822.0020)	Split Sampler FT (P/N VF-A10-A)
Column Compartment	TCC-3000SD (P/N 5730.0010) with or without 7 µL eluent pre-heater (P/N 6722.0540)	Column Comp. H (P/N VH-C10-A)
Detector	Diode Array Detector DAD-3000 (P/N 5082.0010)	Diode Array Detector FG (P/N VF-D11-A)
Flow Cell	Analytical (10 mm, 13 µL, P/N 6082.0100)	Standard Bio (10 mm, 13 µL, P/N 6083.0540)
Modifications applied for method transfer		
		Modify idle volume from default 25 µL

Data processing and software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2.8 was used for data acquisition and analysis.

Results and discussion

All method transfer experiments were conducted with the same column and sample, with consistent method parameters and seven repeated injections. The chromatograms in Figure 1 display the starting situation for the transfer from the UltiMate 3000 SD system to the Vanquish Flex system (both quaternary). The corresponding retention times are summarized in Table 2. As was shown in a recent Thermo Scientific Application Note,[5] the effect of eluent pre-heating on retention times is not negligible, even not at moderate separation temperatures. Hence, a method transfer should be conducted under equivalent conditions. A method that is run on an UltiMate 3000 SD system in standard configuration (i.e. without eluent pre-heater) should be transferred to a Vanquish system with a disabled active pre-heater. If the UltiMate 3000 SD system was equipped with a 7 µL pre-heater, the active pre-heater, which is included in the Vanquish standard configuration, should be enabled for the Vanquish system. Both situations are covered in Figure 1. All gradient eluted peaks (2–6) exhibit earlier retention times with the Vanquish system. This is not surprising and can be attributed to the slightly smaller GDV of the Vanquish system. However, differences in the isocratic elution of the first peak (aminophenol) are not induced by gradient effects and might be the result of minor temperature differences or slight differences in the proportioning of the isocratic conditions with 1% of mobile phase B.

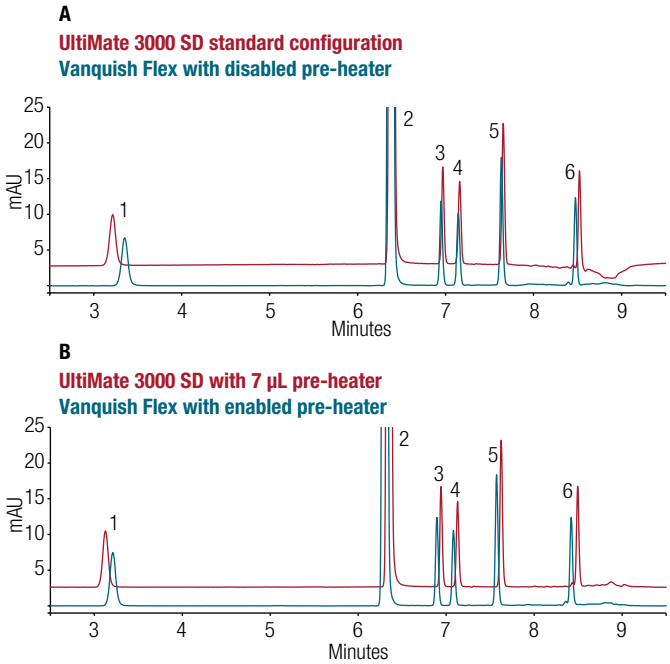

Figure 1. Starting situation of the method transfer. A) Comparison of UltiMate 3000 SD system and Vanquish Flex system without eluent pre-heating; B) Comparison of UltiMate 3000 SD system and Vanquish Flex system with enabled eluent pre-heating. For peak assignment see Table 2.

Three actions can be taken and combined to physically adapt the GDV of the Vanquish system to emulate the originating system:

1. The unique feature of adjustable idle volume of the autosampler metering device (the unit conducting sample aspiration) can be used to fine tune the GDV.
2. If the range of idle volume is not sufficient for the GDV difference compensation, the standard sample loop is replaceable by a larger volume one.
3. If GDV differences of major amount have to be emulated, a change of the static pump mixer should be considered.

Table 2. Averaged retention times in minutes over seven injections for the systems and configurations stated in Figure 1 and % deviation of Vanquish Flex target systems from originating UltiMate 3000 configurations

Peak No.	Compound	UltiMate 3000 SD w/o pre-heating	Vanquish Flex, pre-heater off	UltiMate 3000 SD w/ pre-heating	Vanquish Flex, pre-heater on
1	4-Aminophenol	3.21	3.35 (Δ -4.2%)	3.13	3.21 (Δ -2.6%)
2	Acetaminophen	6.39	6.37 (Δ 0.2%)	6.34	6.30 (Δ 0.7%)
3	Impurity B	6.97	6.95 (Δ 0.3%)	6.94	6.90 (Δ 0.7%)
4	Impurity C	7.16	7.14 (Δ 0.2%)	7.13	7.08 (Δ 0.6%)
5	Impurity D	7.66	7.63 (Δ 0.3%)	7.63	7.57 (Δ 0.7%)
6	Impurity J	8.52	8.47 (Δ 0.5%)	8.50	8.42 (Δ 0.9%)

An overview on respective volume ranges is given by Table 3. However, in the current case idle volume adaption was already sufficient. The very good retention time matches achieved are displayed in Figure 2. Without pre-heating idle volume adaption from default 25 µL to 53 µL was successful, with enabled pre-heating the idle volume was set to 79 µL. As expected, the peak that elutes under isocratic conditions was not affected by the adjustments, while gradient-eluted peaks were shifted accordingly, resulting in relative retention time differences of <0.3 % for these peaks with respect to the UltiMate 3000 SD chromatogram. The applied modulations are in full agreement with the allowed adjustments according to the USP General Chapter <621>, which states: "If adjustments are necessary, a change in […] the duration of an initial isocratic hold (when prescribed), and/or the dwell volume are allowed."[6]

All described systems (either with or without retention time adaption) easily pass the USP system suitability criteria, with a resolution of the critical peak pair of impurities B and C of 3.2 or larger, tailing factors from 0.99 to 1.1, and a relative standard deviation of peak heights of less than 0.5%. The relative areas of all impurity peaks were constant over all instruments, but signal-to-noise ratios improved during the transfer to the Vanquish Flex system (Figure 3).

Table 3. Overview of options for GDV adjustments with the Vanquish platform

1) Autosampler metering device	Adjustable 0–100 µL (default 25 µL)
2) Sample loops	10 µL (V=23 µL, P/N 6850.1915) 25 µL (default, V=50 µL, P/N 6850.1911) 100 µL (V=130 µL, P/N 6850.1913)
3) Pump mixer kits	Available with total volume of: 35 µL (P/N 6044.3870) 100 µL (P/N 6044.5100) 200 µL (P/N 6044.5110) 400 µL (P/N 6044.5310, default in quaternary pump) 800 µL (P/N 6044.5750A) 1550 µL (P/N 6044.5450A)

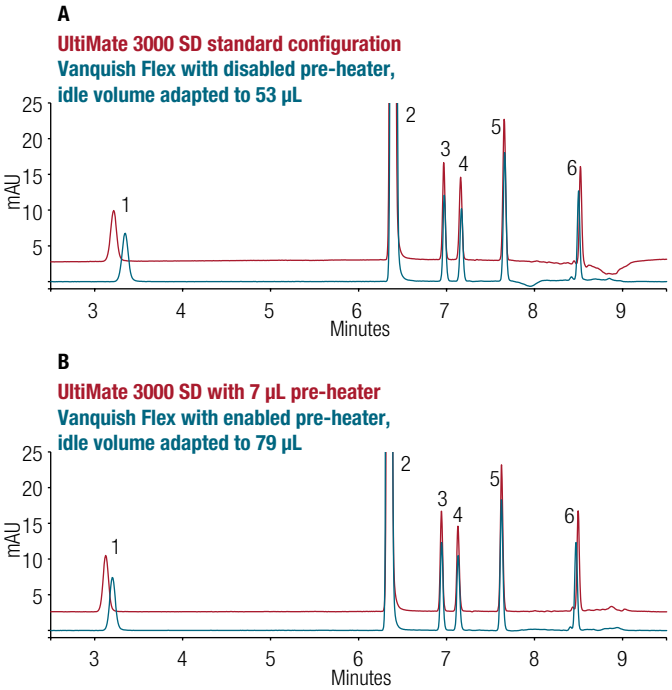

Figure 2. Method transfer from UltiMate 3000 SD system to Vanquish Flex system by idle volume adaption. A) Both systems without eluent pre-heating, idle volume adapted to 53 µL; B) both systems with enabled eluent preheating, idle volume adapted to 79 µL. For peak assignment see Table 2.

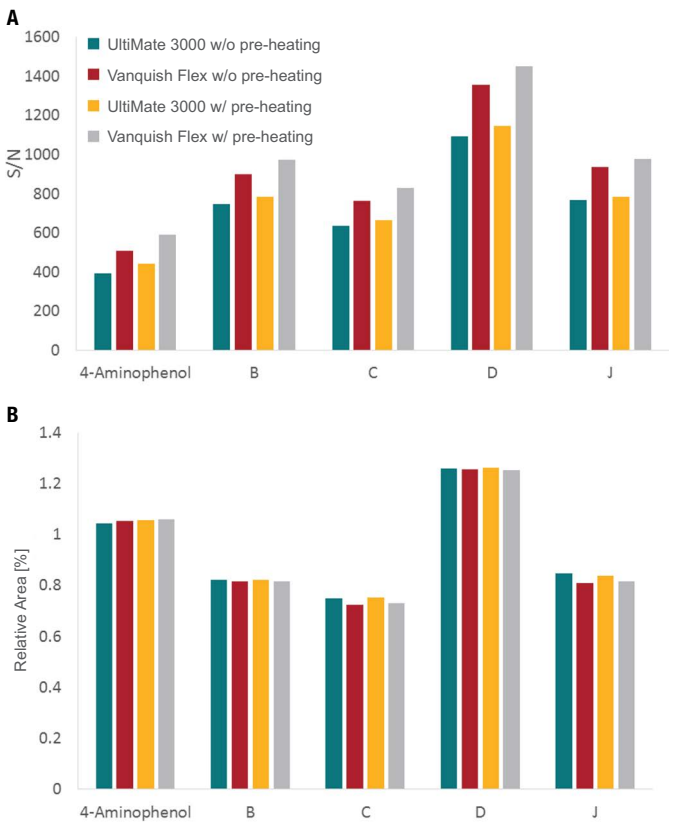

Figure 3. Averaged signal-to-noise ratios (A) and relative peak areas (B) for the originating UltiMate 3000 SD system with or without eluent pre-heater and the receiving system Vanquish Flex system with enabled or disabled eluent preheating. Noise calculated from the current chromatogram 4.1–4.6 min

In the context of method transfer, it is worthwhile to mention another unique feature of the Vanquish platform; that is the switchable thermostatting mode of the column compartment. The current experiments on the Vanquish system were conducted in forced air mode, as this reflects best the UltiMate 3000 column compartment. In a recent Application Note,[5] it was already shown that the type of thermostatting mode has a negligible effect on the acetaminophen assay. However, in applications where frictional heating of the column becomes significant due to high applied pressures, the thermostatting mode in the column compartment (still or forced air) can be important.[3]

Conclusions

- During method transfer of an acetaminophen assay from an UltiMate 3000 SD HPLC system to a Vanquish Flex UHPLC system (both quaternary), straightforward retention time matches were achieved by adjustments of the idle volume of the autosampler metering device.
- Critical chromatographic results like resolution of critical peak pair, peak asymmetries, peak height precision, and relative peak areas were easily maintained during transfer from one HPLC platform to another. Signal-to-noise ratios improved during the transfer.
- Unique features of the Vanquish platform such as the adjustable autosampler idle volume and switchable column thermostatting and eluent pre-heating modes provide helpful tools in the process of method transfer.

References

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