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APPLICATION NOTE 72717

Method transfer of a USP derived acetaminophen assay from an Agilent 1260 Infinity system to an UltiMate 3000 SD system and a Vanquish Flex UHPLC system

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Keywords
HPLC method transfer, Vanquish Flex quaternary system, UltiMate 3000 Standard quaternary system, Agilent 1260 Infinity quaternary system, gradient delay volume, acetaminophen

Application benefits

- Flexible system volume adjustment in Thermo Scientific™ UltiMate™ 3000 systems and Thermo Scientific™ Vanquish™ UHPLC systems facilitate straightforward transfer of analytical HPLC methods.
- Fine tuning of retention times can be achieved by shifting the gradient start relative to injection time.
- If detection sensitivity is a critical issue, Thermo Scientific™ LightPipe™ technology provides an excellent remedy.

Goal

To demonstrate the straightforward transfer of analytical HPLC methods from an Agilent™ 1260 Infinity system to the UltiMate 3000 platform and the Vanquish platform.

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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] Reasons for the need to transfer methods are manyfold, and procedures comprise application switching between the same or different types of instruments within the same laboratory, as well as transfers from legacy instruments to new ones due to replacement. Also, the transfer from developing laboratories to implementing laboratories of diverse location and equipment is very common. Proper transfer is only achieved if equivalent results are obtained with the sending and the receiving LC system.[1,2] The true complexity of this task highly depends on the robustness of the method to be transferred as well as on instrumentational differences of both systems.[1,2] To succeed in the challenge of maintaining retention times, resolutions, and other critical factors, specific technical characteristics of the systems like gradient delay volume (GDV), hydrodynamic behavior, or thermostatting mode must be taken into account. Additionally, as revalidation is time-consuming and expensive, modification of method parameters must be avoided as much as possible. Thus, hardware solutions become attractive features in method transfer.[3]

The current application note demonstrates the use of helpful features provided by the Thermo Scientific UltiMate 3000 and Vanquish platforms, like tunable GDVs and switchable thermostatting modes for the method transfer from another vendor's instrument (here the Agilent 1260 Infinity system). The selected application is derived from a USP assay for the analysis of the active pharmaceutical ingredient (API) acetaminophen, a common pain killer, and its impurities.[4] Analysis is performed with a Thermo Scientific™ Hypersil GOLD™ C8 stationary phase that matches the required USP level L7 and is well suited for analytes of medium hydrophobicity.

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), 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.

Table 1. Instruments used in this study

	Agilent 1260 Infinity Quaternary	UltiMate 3000 SD Quaternary	Vanquish Flex Quaternary
Standard configurations			
			System Base (P/N VH-S01-A-02)
Pump	Quaternary Pump (G1311B)	Standard Quaternary Pump LPG-3400SD (P/N 5040.0031)	Quaternary Pump F (P/N VF-P20-A)
Sampler	High Performance Autosampler (G1367E) with thermostat module (G1330B)	Well Plate Autosampler WPS-3000TSL (P/N 5822.0020)	Split Sampler FT (P/N VF-A10-A)
Column Compartment	TCC with 6 µL heat exchanger (G1316A)	TCC-3000SD (P/N 5730.0010)	Column Compartment H (P/N VH-C10-A)
Detector	Diode Array Detector DAD VL (G1315D)	Diode Array Detector DAD-3000 (P/N 5082.0010)	Diode Array Detector FG (P/N VF-D11-A)
Flow Cell	Standard: 10 mm, 13 µL (G1315-60022)	Analytical: 10 mm, 13 µL (P/N 6082.0100)	Standard bio: 10 mm, 13 µL (P/N 6083.0540)
Hardware modifications applied for method transfer			
		• Add 7 µL eluent pre-heater (P/N 6722.0540) • Replace default static mixer 350 µL (P/N 6040.5310) by static mixer 750 µL (P/N 6040.5750)	• Replace default loop 25 µL (V=50 µL, P/N 6850.1911) by loop 100 µL (V=130 µL, P/N 6850.1913) • Modify idle volume from default 25 µL
Modifications applied for additional sensitivity enhancement			
			• Replace DAD FG by DAD HL (P/N VH-D10-A) with LightPipe standard flow cell (10 mm, P/N 6083.0100B) or LightPipe high sensitivity flow cell (60 mm, P/N 6083.0200)

LC conditions

Column:	Hypersil GOLD C8, 4.6 × 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* (*when the UltiMate 3000 SD system was used with the 750 µL static mixer, equilibration was extended to 13 min)
Column Temp.:	35 °C (with 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

Data processing and software

Thermo Scientific™ Chromeleon Software 7.2.8 Chromatography Data System 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 Agilent 1260 Infinity system to the UltiMate 3000 SD system and to the Thermo Scientific™ Vanquish™ Flex system (all quaternary). The corresponding retention times are summarized in Table 2. In Figure 1a, Agilent 1260 Infinity system data are compared to data from the UltiMate 3000 Standard configuration system without an eluent pre-heater, and to data from the UltiMate 3000 system equipped with an optional 7 µL pre-heater. The distinct differences of both

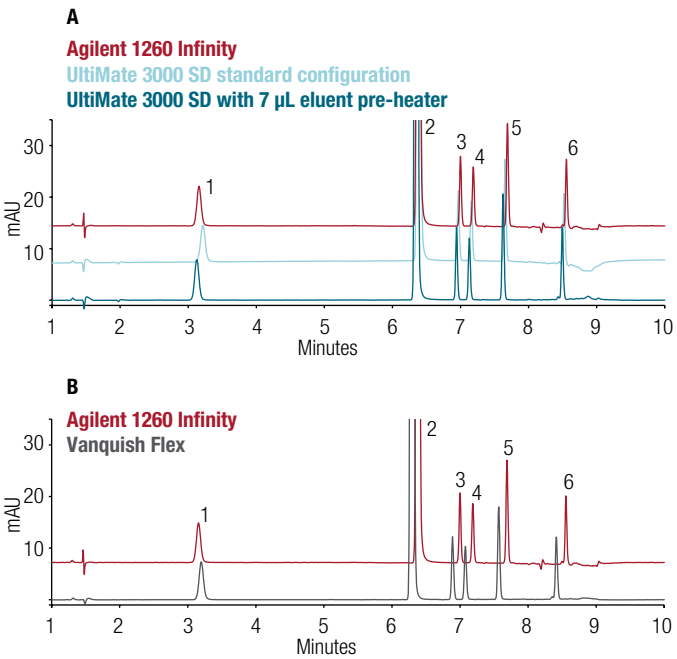

Figure 1. Starting situation of the method transfer. A) Chromatogram of Agilent 1260 Infinity system compared to UltiMate 3000 SD system in standard configuration and with optional eluent pre-heater; B) Chromatogram of Agilent 1260 Infinity system compared to Vanquish Flex system. For peak assignment see Table 2.

Table 2. Averaged retention times in minutes over seven injections for the systems and configurations stated in Figure 1 and % deviation for both pre-heated target systems from originating system

Peak No.	Compound	Agilent 1260 Infinity (originating system)	UltiMate 3000 SD system w/o pre-heating	UltiMate 3000 SD w/ pre-heating (target system)	Vanquish Flex (target system)
1	4-Aminophenol	3.16	3.21	3.13 (Δ 0.9%)	3.20 (Δ - 1.3%)
2	Acetaminophen (API)	6.38	6.39	6.34 (Δ 0.5%)	6.29 (Δ 1.3%)
3	Impurity B	7.00	6.97	6.94 (Δ 0.8%)	6.89 (Δ 1.6%)
4	Impurity C	7.19	7.16	7.13 (Δ 0.8%)	7.08 (Δ 1.5%)
5	Impurity D	7.69	7.66	7.63 (Δ 0.8%)	7.57 (Δ 1.6%)
6	Impurity J	8.56	8.52	8.50 (Δ 0.7%)	8.41 (Δ 1.7%)

UltiMate 3000 system chromatograms clearly illustrate the noticeable impact of eluent thermostatting even at moderate separation temperatures. Thus, a successful method transfer should be conducted with adjusted thermostatting conditions and an installed pre-heater on the UltiMate 3000 system. This is especially emphasized by the behavior of the first peak (4-aminophenol), which elutes under isocratic conditions and is not affected by gradient effects. Without eluent pre-heating, it elutes later than on the Agilent 1260 Infinity system and approximates when pre-heating is applied. For the Vanquish Flex system an active pre-heater is included in the standard configuration and was activated for this method transfer, yielding similar retention of aminophenol (Figure 1b). In contrast, all peaks that elute during the gradient elute earlier on both Thermo Scientific instruments than on the Agilent 1260 Infinity system with enabled eluent pre-heating for the three systems. This is mainly due to a larger (and furthermore back-pressure dependent) GDV of the Agilent 1260 instrument. For that reason, a physical GDV adjustment by several features provided by the UltiMate 3000 and Vanquish portfolio is a promising way to minimize system differences for a successful method transfer.

Tables 3 and 4 give an overview of UltiMate 3000 SD and Vanquish system accessories available to stepwise modify system GDVs. For the transfer of the acetaminophen assay from the Agilent 1260 Infinity system to the UltiMate 3000 SD system, the default static mixer (350 µL) was replaced by the larger 750 µL mixer. As this volume difference overcompensated the GDV difference between

Table 3. Available UltiMate 3000 SD system consumables that can be used to modify the system GDV

Description	P/N
Mixer kit for pump 35 µL (25 µL capillary mixer +10 µL inline filter)	6040.5000
Mixer kit for pump 100 µL (25 µL capillary mixer + 75 µL static mixer)	6040.5100
Mixer kit for pump 200 µL (50 µL capillary mixer + 150 µL static mixer)	6040.5110
Mixer kit for pump 400 µL **(default configuration quaternary pump)** (50 µL capillary mixer + 350 µL static mixer)	6040.5310
Mixer kit for pump 800 µL (50 µL capillary mixer + 750 µL static mixer)	6040.5750
Mixer kit for pump 1550 µL (50 µL capillary mixer + 1500 µL static mixer)	6040.5450
Sample loop 25 µL (V=40 µL)	6820.2452
Sample loop 100 µL (V=130 µL) **(default configuration)**	6820.2451
Sample loop 250 µL (V=344 µL)	6820.2453
Sample loop 500 µL (V=667 µL)	6820.2454

Table 4. Available Vanquish system consumables that can be used to modify the system GDV

Description	P/N
Mixing system for pump 35 µL (25 µL capillary mixer + 10 µL inline filter)	6044.3870
Mixing system for pump 100 µL (25 µL capillary mixer + 75 µL static mixer)	6044.5100
Mixing system for pump 200 µL (50 µL capillary mixer + 150 µL static mixer)	6044.5110
Mixing system for pump 400 µL **(default configuration quaternary pump)** (50 µL capillary mixer + 350 µL static mixer)	6044.5310
Mixing system for pump 800 µL (50 µL capillary mixer + 750 µL static mixer)	6044.5750A
Mixing system for pump 1550 µL (50 µL capillary mixer + 1500 µL static mixer)	6044.5450A
Sample loop 10 µL (V=23 µL)	6850.1915
Sample loop 25 µL (V=50 µL) **(default configuration)**	6850.1911
Sample loop 100 µL (V=130 µL)	6850.1913

sending and receiving instrument (see Figure 2a), a prestart of the gradient was then applied to match the retention times. With the prestart technique a smaller GDV can be emulated by shifting the point of injection relative to the method start. As the injection by definition is executed at 0.0 min, the method start is set to a negative time and all remaining steps of the method are shifted by the same value. Thus, no segment of the method is modified and the gradient table in total is not changed. For the current transfer, the extent of the time offset was -0.27 min and was derived from the average retention time difference of gradient-eluted peaks of the UltiMate 3000 system with the 750 µL static mixer and the Agilent 1260 Infinity system. Figure 2b illustrates the very good retention time match that was obtained by this technique, giving relative retention time deviations of <1% for aminophenol and <0.2% for peaks that elute in the gradient with respect to the originating method. While gradient-eluted peaks are shifted according to true or emulated GDV adjustments, peaks eluted under isocratic conditions are not affected. The minor difference in aminophenol retention thus might be the result of slightly different temperature conditions or proportioning of the isocratic conditions with 1 % of mobile phase B.

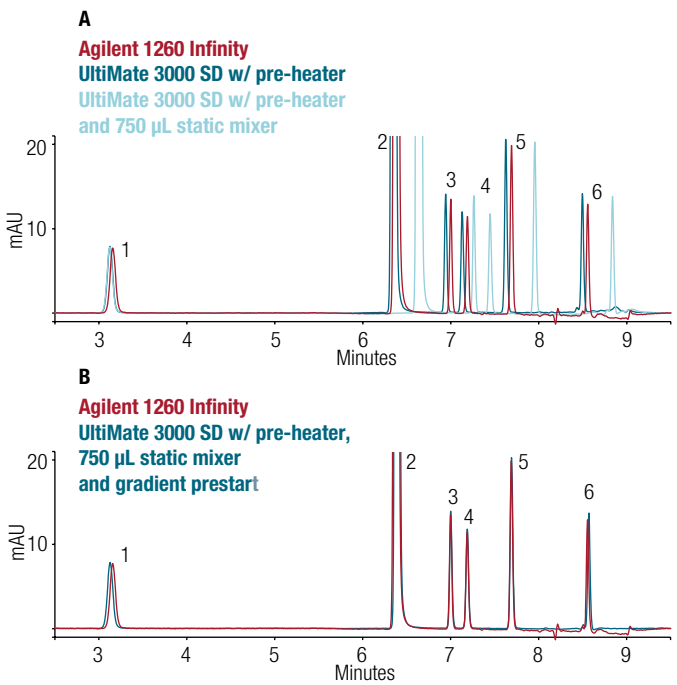

Figure 2. Method transfer from the Agilent 1260 Infinity system to the UltiMate 3000 SD system. A) Comparison of Agilent 1260 Infinity system and UltiMate 3000 SD system with eluent pre-heater and standard or larger static mixer; B) final transfer: comparison of Agilent 1260 Infinity system and UltiMate 3000 SD system with eluent pre-heater, larger static mixer, and gradient prestart. For peak assignment see Table 2.

In contrast, pre-starting the gradient was not necessary with the Vanquish Flex system to attain retention time congruence due to more flexible capabilities in GDV adjustment. At first the GDV difference of the Agilent 1260 Infinity and Vanquish Flex standard configured systems observed in Figure 1b was partially compensated by replacing the Vanquish standard sample loop by the 100 µL sample loop (actual GDV contribution 130 µL). The resulting retention times were closer to the originating instrument (see Figure 3a), and the remaining differences were in a range that could be offset by adjusting the idle volume of the autosampler metering device, the conducting unit of sample aspiration. This feature is unique to the Vanquish platform and can help in fine-tuning of the GDV as it is part of the sample loop flow path. The default idle volume setting of 25 µL was increased by 43 µL to a total of 68 µL, yielding the good alignment of retention times seen in Figure 3b with relative retention time deviations of 1.2 % for aminophenol and <0.4 % for peaks in the gradient.

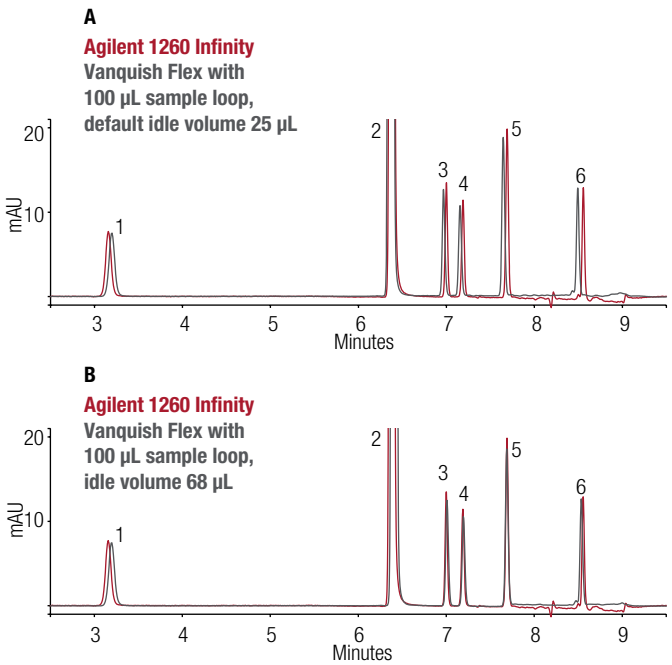

Figure 3. Method transfer from the Agilent 1260 Infinity system to the Vanquish Flex system. A) Comparison of Agilent 1260 Infinity system and Vanquish Flex system with 100 µL sample loop; B) final transfer: comparison of Agilent 1260 Infinity system and Vanquish Flex system with 100 µL sample loop and adapted idle volume to 68 µL. For peak assignment see Table 2.

Another unique feature of Vanquish instruments is the switchable thermostatting mode of the column compartment, giving the choice of still or forced air column heating. The previous chromatograms were recorded in still air mode, as this reflects the thermostatting mode of the Agilent column compartment best. Figure 4 shows that for the current application the thermostatting mode has minor influence on retention times and is negligible here. However, in applications of higher pressure ranges (ultra-high-performance LC, UHPLC, > 600 bar) where frictional heating of the column becomes relevant, the column compartment mode is of certain importance.[3]

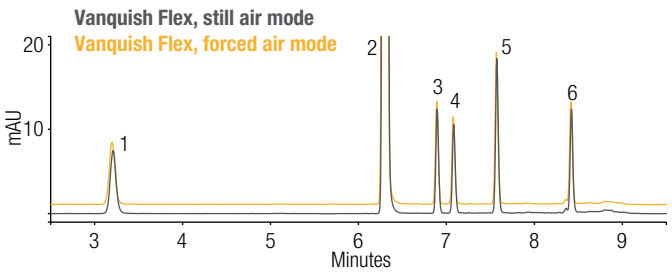

Figure 4. Negligible effect of column thermostatting mode for Vanquish Flex system in still and forced air mode. For peak assignment see Table 2.

In conclusion, retention times were successfully transferred from an Agilent 1260 Infinity instrument to an UltiMate 3000 SD instrument and a Vanquish Flex instrument by means of physical or simulated GDV adaption. This is 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."[5] Furthermore, critical chromatographic results were easily maintained during the transfer. The resolution of the critical pair of impurity B and C was 3.2 or better in all tested scenarios, and peak tailing factors ranged from 0.99 to 1.12. The relative standard deviation of peak heights was always far below 1% (Figure 5a). Thus, USP system suitability was accomplished by all three systems, both with and without GDV adaption. The relative areas of all impurity peaks were constant over the three instruments (Figure 5b).

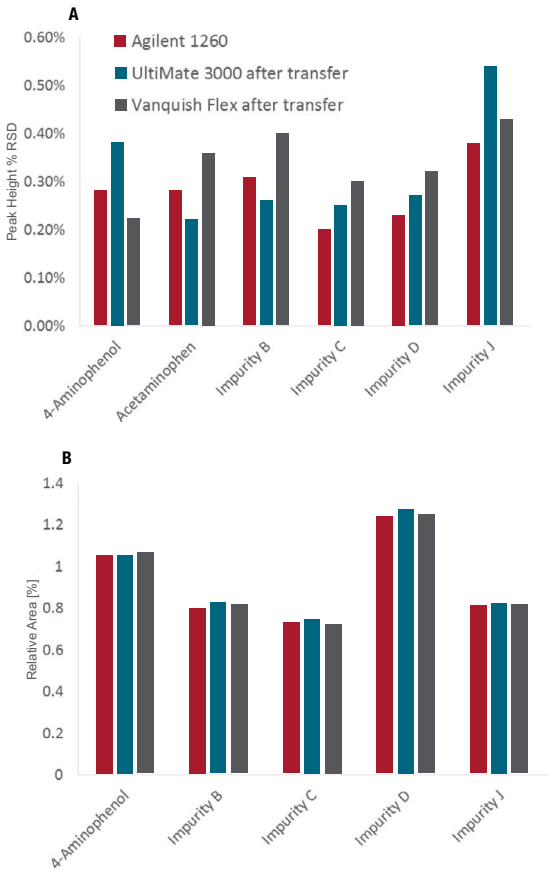

Figure 5. Averaged peak height precision (A) and relative areas of impurity peaks (B) for the originating system Agilent 1260 Infinity, UltiMate 3000 SD system after method transfer optimization (750 µL static mixer and gradient prestart), and Vanquish Flex system after method transfer optimization (100 µL sample loop and idle volume 68 µL)

In Figure 6, the signal-to-noise (S/N) ratios of the transferred method are summarized, illustrating a distinct improvement of S/N performance from the originating system to the Ultimate 3000 SD system and the Vanquish Flex system in the present configuration. As an alternative to the Thermo Scientific™ Vanquish™ DAD FG, the Thermo Scientific™ Vanquish™ DAD HL provides an outstanding S/N performance driven by Thermo Scientific™ LightPipe™ technology, which is demonstrated by the additional bars in that graph. These results were obtained with the same Vanquish system as before but with a swapped detector; with both the standard flow cell with equal light path length as the three previous systems of 10 mm and the high sensitivity flow cell with 60 mm light path. While the S/N enhancement by the standard LightPipe flow cell is mainly caused by further noise reduction, the enormous gain with the high sensitivity flow cell is particularly generated by the sensitivity gain due to the long light path. This cell is especially suited for analyses with columns of 4.6 mm inner diameter.[6] Thus, the DAD HL is very suitable for the analysis of low-abundant impurities, and if S/N performance or sensitivity are of critical concern, the utilization of that dedicated DAD technology is highly recommended.

Conclusions

- During method transfer of an acetaminophen assay from an Agilent 1260 Infinity system to an UltiMate 3000 SD system as well as to a Vanquish Flex system (all quaternary), straightforward retention time matches were achieved by true and emulated GDV adjustments by diverse tools provided by the Thermo Scientific platforms, like exchangeable eluent pre-heaters, pump mixers, sample loops, and adjustable autosampler idle volume.
- Further critical chromatographic results like resolution of critical peak pair, peak asymmetries, peak height precision, and relative peak areas were easily maintained during transfer. Signal-to-noise ratios improved distinctly during the transfer.
- If detection sensitivity of the method is of particular concern, the utilization of DAD LightPipe technology is recommended for LC-UV applications.

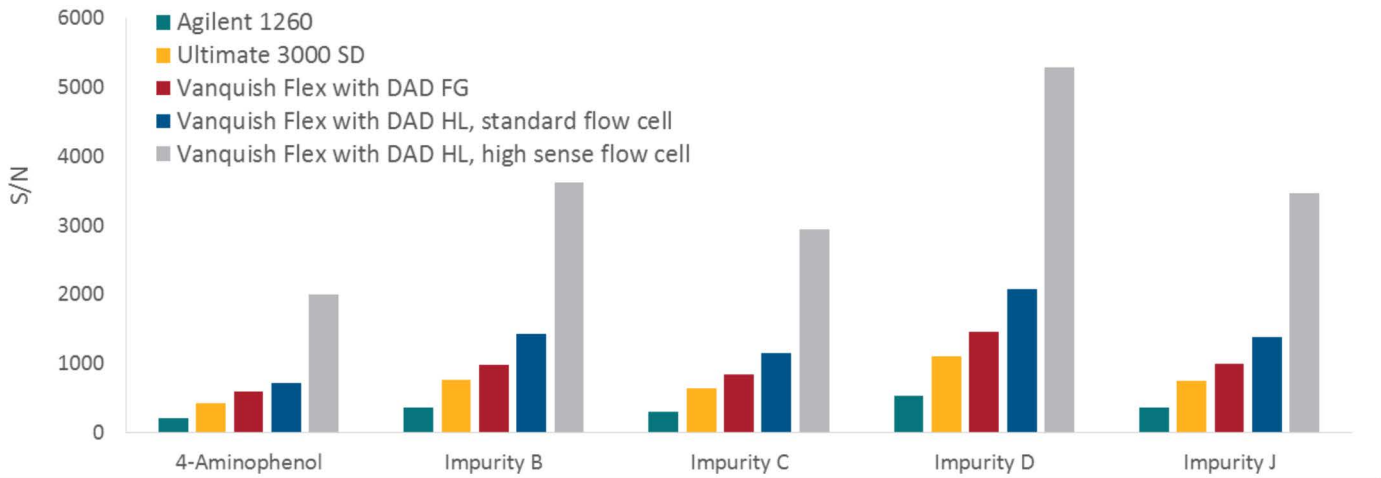

Figure 6. Signal-to-noise ratios (S/N) obtained with Agilent 1260 Infinity system, Ultimate 3000 SD system after method transfer optimization (750 µL static mixer and gradient prestart), Vanquish Flex system after method transfer optimization (100 µL sample loop and idle volume 68 µL) with DAD FG, DAD HL with 10 mm flow cell, and DAD HL with 60 mm high sense flow cell. Noise calculated from the current chromatogram 4.1–4.6 min.

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