Boosting Trace Detection Performance with the Vanquish Diode Array Detector and High-Sensitivity LightPipe Flow Cell

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Technical Note 165

Key Words
Column ID, Dispersion, HPLC, LightPipe Flow Cell, Noise, Sensitivity, Signal-to-Noise Ratio

Goal
To demonstrate how the Thermo Scientific™ Vanquish™ Diode Array Detector (DAD) HL with a 60 mm high-sensitivity LightPipe™ flow cell boosts UV trace detection performance of HPLC applications. Detection side effects such as refractive index effects and peak dispersion are explained and used to evaluate the data from this flow cell. The peak dispersion data further leads to recommendations which column formats are best suited for this type of flow cell. The Vanquish DAD is available for the Vanquish system platform and the Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC system platform.

Introduction
One of the most important parameters for any detection technology is sensitivity. More sensitivity enables the detection of analyte traces, and therefore, in many cases a successful analysis. Different generations of UV-Vis absorption detectors for high-performance liquid chromatography (HPLC) have made evolutionary improvements in trace detection performance, through optimized optics, electronics, and dispersion characteristics. Although UV-Vis absorption detector technology is well established and mature, the Vanquish Diode Array Detector elevates absorption detection performance to a new industry-leading level. It is based on a completely new design, improving all aspects relevant for best trace detection performance. Most importantly, it uses recently developed LightPipe technology in the flow cell.

According to Beer's law, the absorbance of light, and thus the signal height, is directly proportional to the interaction length between light and sample. LightPipe technology is designed to combine a long flow cell light path with minimum peak dispersion and noise. To achieve this, it relies on total internal reflections at the interface between the pipe and air on the outside[1], as shown in Figure 1.

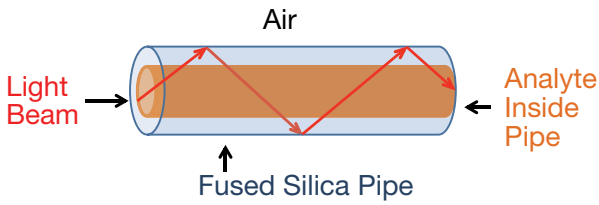

Figure 1. Simplified scheme and operating principle of a LightPipe flow cell.

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The Vanquish DAD can be equipped with a high-sensitivity flow cell of 60 mm length for maximum interaction between light and analyte.

This technical note focuses on the use of this flow cell. It demonstrates how much the sensitivity can be improved, discusses side effects, and leads to recommended column formats.

Experimental

Instrumentation

First setup for detector and flow cell comparison

UltiMate 3000 RS UHPLC system equipped with:

- HPG-3400RS Pump (P/N 5040.0046)
- WPS-3000TRS Thermostatted Autosampler (P/N 5840.0020)
- TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
- SRD-3400 Solvent Rack with Degasser (P/N 5035.9245)
- Either a Vanquish Diode Array Detector HL (P/N VH-D10-A) equipped with a LightPipe Flow Cell, high sensitivity, 13 µL volume, 60 mm path length (P/N 6083.0200), or conventional Diode Array Detector equipped with a 13 µL flow cell of 10 mm path length

Chromatographic Conditions

Column:	Thermo Scientific™ Acclaim™, C18, 4.6 × 150 mm, 3 µm (P/N 059133)	
Mixer volume:	200 µL	
Solvent A:	Water	
Solvent B:	Acetonitrile (ACN)	
Gradient:	Time (min)	%B
	0	50
	5.0	100
	5.4	100
	5.5	50
	8.5	50
Flow rate:	2.2 mL/min	
Temperature:	35 °C	
Injection volume:	1 µL	
Detection:	Slit width:	4 nm slit
(both detectors)	Wavelength:	254 nm
	Bandwidth:	4 nm
	Data collection rate:	20 Hz
	Response time:	0.2 s
Analytes:	1. Uracil	
	2. Acetanilide	
	3. Acetophenone	
	4. Propiophenone	
	5. Butyrophenone	
	6. Benzophenone	
	7. Valerophenone	
	8. Hexanophenone	
	9. Heptanophenone	
	10. Octanophenone	
	50 µg/mL each, except for uracil: 25 µg/mL	

Second setup to measure the influence of peak volume on dispersion

Vanquish UHPLC system equipped with:

- Vanquish System Base (P/N VH-S01-A)
- Vanquish Binary Pump H (P/N VH-P10-A)
- Vanquish Split Sampler HT (P/N VH-A10-A)
- Vanquish Column Compartment H (P/N VH-C10-A)
- UltiMate 3000 VWD-3400RS Variable Wavelength Detector (P/N 5074.0010) equipped with Ultra-Low Dispersion UV-Monitor, 45 nL volume (P/N 6074.0285) and
- Vanquish Diode Array Detector HL (P/N VH-D10-A) equipped with high-sensitivity LightPipe Flow Cell, 13 µL volume, 60 mm pathlength (P/N 6083.0200)

Chromatographic conditions

Column:	Thermo Scientific™ Hypersil GOLD™, 1.9 µm, 1.0 x 100 mm (P/N 25002-101030)	Hypersil GOLD, 1.9 µm, 2.1 x 100 mm (P/N 25002-102130)	Hypersil GOLD, 1.9 µm, 3.0 x 100 mm (P/N 25002-103030)
Flow rate:	0.12 mL/min	0.53 mL/min	1.08 mL/min
Injection volume:	1.0 µL	4.4 µL	9.0 µL
Mobile phase:	50 % acetonitrile in water, premixed		
Mixer volume:	35 µL		
Temperature:	35 °C		
Detection:	VWD-3400RS	Vanquish DAD HL	
	Wavelength: 254 nm	Wavelength: 254 nm	
	Data collection rate: 50 Hz	Slit width: 4 nm slit	
		Bandwidth: 4 nm	
		Data collection rate: 50 Hz	
		Response time: 0.1 s	
Analytes	1. Uracil		
	2. Acetanilide		
	3. Acetophenone		
	4. Propiophenone		
	5. Butyrophenone		
	6. Benzophenone		
	7. Valerophenone		
	8. Hexanophenone		
	50 µg/mL each, except for uracil: 25 µg/mL		

Data Processing

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2 SR 2 or 6.8 SR 14 (or later)

Results and Discussion

Comparison of Signal Response

To show the effect of the extra-long light path of the high-sensitivity LightPipe flow cell, experiments were conducted with an UltiMate 3000 system coupled either to a conventional DAD with a conventional 10 mm flow cell, or to the Vanquish DAD HL with a 60 mm high-sensitivity LightPipe flow cell (see first experimental setup). The illuminated channels of both flow cells have the same volume of 13 µL.

Compared to the conventional 10 mm flow cell, the 60 mm high-sensitivity LightPipe flow cell offers a six-fold increased light path. According to Beer's law, the absorbance of light, and thus the signal height, is proportional to the length of the light path. An overlay of chromatograms obtained from measurements with both flow cell types is shown in Figure 2. As expected, peaks measured with the 60 mm flow cell are almost six times higher.

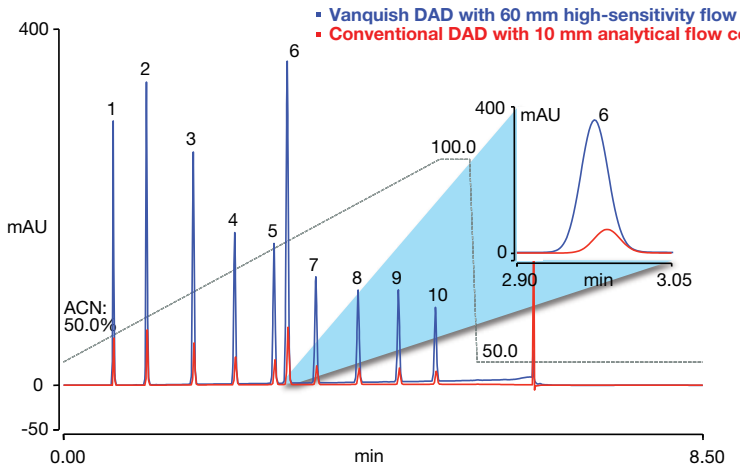

Figure 2. Overlay of chromatograms measured with Vanquish DAD and 60 mm high-sensitivity LightPipe flow cell (blue) and conventional DAD with 10 mm analytical flow cell (red).

Although the nominal volumes of the 60 mm LightPipe and the 10 mm conventional flow cells are the same, peaks measured with the long LightPipe flow cell seem to be wider. In fact, peak widths (both peak width at baseline and peak width at half height) measured with these flow cells only differ by about 4% as a result of the different geometries of the cells´ flow paths.

Noise Performance Comparison

The noise is as important for the signal-to-noise (S/N) performance as the signal height. Figure 3 shows blank injections corresponding to the chromatograms shown in Figure 2 with two zooms. Zoom A shows a part of the linear gradient directly influencing the peaks eluting in this region. Zoom B magnifies a peak-like positive deflection caused by the sudden 100% to 50% acetonitrile step. When changing quickly between different eluents or eluent-mixtures with different refractive indexes, light is focused or dispersed by the boundary layer between these eluents/eluent-mixtures. This gradient step causes a so-called dynamic refractive index (dynamic RI) effect. Depending on the characteristics of the flow cell, this can result in positive or negative deflections (focusing or dispersion of light within the optics) of varying extent, as shown in Figure 4. Zoom B of Figure 3 demonstrates that the dynamic RI effect is pronounced for the conventional flow cell. Although analytes typically elute prior to this baseline artifact, with more complex gradient profiles such a dynamic RI effect may adversely affect the separation or may be misinterpreted as an analyte peak. It is therefore beneficial to reduce dynamic RI effects as much as possible.

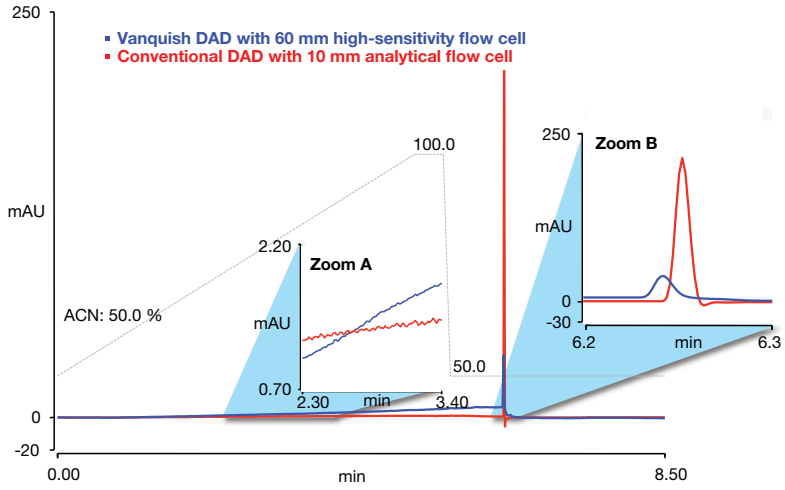

Figure 3. Overlay of blank injections corresponding to the chromatograms in Figure 2 measured with the Vanquish DAD and 60 mm LightPipe flow cell (blue) and conventional DAD with 10 mm analytical flow cells (red). Zoom A shows the baseline in the area of analyte peak elution and Zoom B magnifies the dynamic RI effect caused by a quick gradient change.

Zoom A of Figure 3 shows differences in noise and drift of the two flow cell types. The drift of the baseline correlating with the gradient profile can be caused by absorption of the mobile phase or by static refractive index (static RI) effects. The mobile phase composition in this test is non-absorbing at the measuring wavelength of 254 nm, thus the drift is caused by static RI effects. These are the results of the optical characteristics of the flow cell changing with the optical density of the mobile phase. Figure 4 shows dynamic and static RI effects as result of two gradient steps (from 0% to 10% acetonitrile, and back to 0%) measured in a conventional DAD flow cell. The dynamic RI effects are a consequence of the sudden gradient step, leading to a negative deflection at the first gradient step and a positive deflection at the second step. The direction and appearance of these deflections depend on the flow cell design and are characteristic for each flow cell. The static RI effect between the two gradient steps, emerging as baseline shift corresponding to the gradient step, is a result of reflections at different interfaces inside the flow cell. Depending on the mobile phase's refractive index, light is reflected differently at the interfaces between mobile phase and flow cell, for example at the interface between the flow cell windows and the sample. Thus, with varying mobile phase composition, this reflection loss changes, leading to a baseline shift. With a linear gradient instead of gradient steps, the baseline shift of the static RI effect translates into a baseline drift.

Zoom A of Figure 3 also exhibits a different noise performance for both flow cell types. The baseline is less noisy with the LightPipe flow cell. This can be attributed to the latest optical and electronic technology used in the Vanquish DAD and LightPipe flow cells. The noise difference may vary depending on isocratic or gradient elution, the type of gradient, and the efficiency of the mobile phase mixing.

Signal-to-Noise Performance

To achieve the best signal-to-noise ratios, the signal height should be maximized and the baseline noise minimized simultaneously. Over the course of a gradient, the baseline noise may vary. The Vanquish DAD with the high sensitivity LightPipe flow cell demonstrates increased signal heights (Figure 2) and excellent noise behavior (Figure 3). Depending on the peak and the corresponding baseline noise, this results in a S/N enhancement of factor 5-14 compared to a conventional DAD with 10 mm non-LightPipe flow cell. Figure 5 shows the S/N improvement as a ratio of the S/N value for the Vanquish DAD with high sensitivity LightPipe flow cell to the S/N value for the conventional DAD with 10 mm flow cell for all peaks of Figure 2. Noise values were obtained from respective blanks (as shown in Figure 3) in a window of five times peak width at half height around each peak.

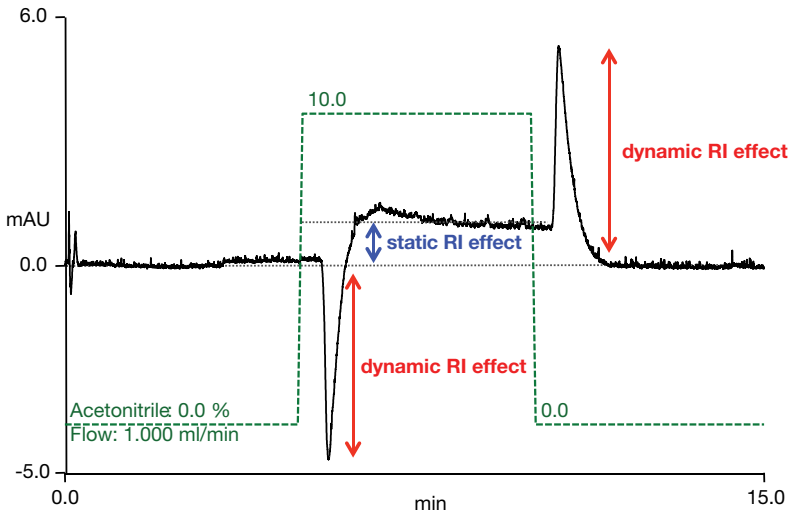

Figure 4. Dynamic and static refractive index (RI) effects as consequence of two non-absorbing gradient steps.

Zoom A of Figure 3 demonstrates that the static RI effect (under given test conditions) is more distinct with the 60 mm LightPipe flow cell. However, this needs to be put in relation to the increased signal obtained with the long light path of this cell. The baseline slope is approximately four times steeper, but signals are typically six times higher. In proportion to the peak height, the baseline drift is therefore less distinct.

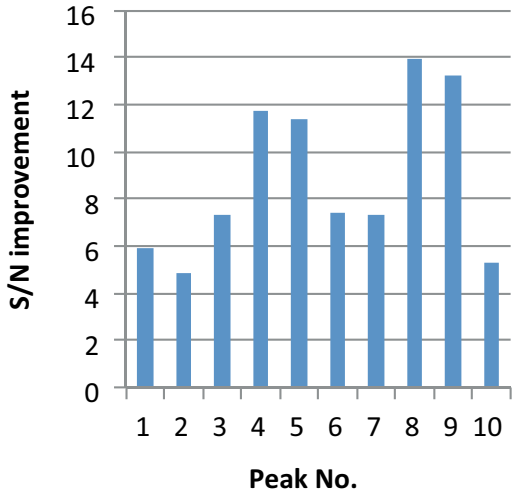

Figure 5. S/N improvement for the Vanquish DAD with 60 mm LightPipe flow cell compared to a conventional DAD with 10 mm flow cell.

The S/N improvement with the Vanquish DAD is not the same for all peaks. The increase in signal height is not causing this difference as it is very similar for all analyte bands. Instead, the reason for the variations of the S/N is that the noise changes over the course of the gradient. The noise of the Vanquish DAD is always equal or better than for the conventional DAD resulting in a S/N improvement as high as a factor of 14.

Limitations: Choosing the right column

In general, extra column band broadening will be insignificant if the flow cell volume is no larger than approximately 10% of the (smallest) peak volume.[2,3] Although the volume of the high-sensitivity LightPipe flow cell is quite small compared to the length of its light path, a 13 µL volume is not ideal for UHPLC applications with peak volumes down to 20 µL or less. The smaller the ratio between peak volume and flow cell volume, the more extensive is the dispersion leading to more and more loss in chromatographic efficiency, smaller peaks, and pronounced peak tailing.

The peak broadening effects on narrow peaks in the 13 µL flowcell were determined by two sequentially connected detectors with different flow cell volumes (also see second experimental setup). The first detector was a variable wavelength detector (VWD) with a 45 nL flow cell, creating only negligible dispersion. In the evaluation of this experiment, it is therefore treated as reference for narrowest peaks (original peak volume). Downstream of this detector, the 60 mm high sensitivity LightPipe flow cell was connected. To vary the peak volume, columns with identical packing material but different inner diameters were used. Different peak volumes were obtained as a consequence of the inner diameter (ID) change of the column: The smaller the ID, the smaller the peak volume. Furthermore, different peak volumes were obtained through isocratic separations increasing peak volumes with retention time. The ratio of Vanquish DAD peak volumes to VWD peak volumes is called peak broadening factor.

Figure 6 shows the peak broadening in the high sensitivity LightPipe flow cell as a function of the original peak volume. Under isocratic conditions, within one chromatographic separation, the peak volume increases with increasing retention time or retention factors k, respectively.

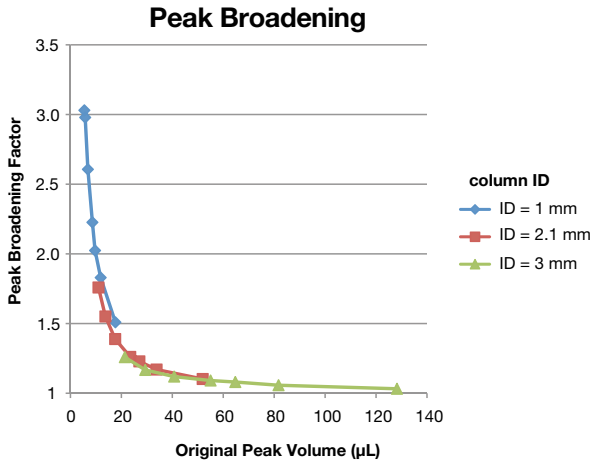

Figure 6. Peak broadening caused by the 60 mm high-sensitivity LightPipe flow cell compared to peak volumes obtained from a VWD with 45 nL flow cell as a function of original peak volume.

Furthermore, Figure 6 demonstrates that larger column IDs lead to larger peak volumes and that with increasing peak volumes peak broadening decreases dramatically. In this set of experiments, acceptable peak broadening factors between 1.25 and 1.03 over the entire chromatographic separation were only obtained with the 3 mm ID column.

The influence of the column geometry on the peak volume can be explained with the following equations. The peak volume V_{Peak} is defined as

$$V_{Peak} = 4\sigma_{column}, \quad \text{(Eq. 1)}$$

where σ_{column} is the dispersion generated by the column. The column dispersion can be also described as

$$\sigma^2_{column} = V_o^2(1 + k)^2/N, \quad \text{(Eq. 2)}$$

with V_o being the column void volume, k the retention factor and N the number of theoretical plates. Combining these two equations leads to

$$V_{Peak} = \frac{4V_o}{\sqrt{N}}(1 + k). \quad \text{(Eq. 3)}$$

Equation 3 expresses how the column geometry and the retention factor influence the peak volume. A larger column geometry increases its volume and consequently also its void volume, leading to increased peak volumes. A higher retention factor also leads to higher peak dispersion and consequently increased peak volumes.

In general, column and flow cell have to be chosen carefully to prevent disproportional peak broadening caused by a mismatch between peak volume and flow cell volume. As a general guideline, flow cells with illuminated volumes > 10 µL, no matter if of conventional or optical waveguide type, are recommended to be used with column IDs ≥ 3 mm. Using them with column IDs ≤ 2 mm may lead to pronounced loss of chromatographic efficiency.

Conclusion

The Vanquish DAD HL with the high-sensitivity LightPipe flow cell is a powerful instrument to improve detection performance. The data of this technical note demonstrates that this technology can increase the peak height by a factor of up to six and the signal-to-noise ratio by a factor of up to 14 compared to a conventional DAD. It also demonstrates and explains why the high-sensitivity LightPipe flow cell is best suited for column IDs of ≥ 3 mm.

References

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