# Thermo Scientific Liquid Chromatography Systems

## The Latest LightPipe Technology for the UltiMate 3000 Platform

### Product Spotlight

Diode Array Detectors (DAD) with optical waveguides are highly-effective UV detectors for HPLC. The LightPipe™ technology of the Thermo Scientific™ Vanquish™ Diode Array Detector HL takes UV detection to the next level for unmatched detection performance for both, HPLC and UHPLC. Now, this detector is also available for the Thermo Scientific™ Dionex™ UltiMate™ 3000 system platform. With two different flow cell formats, the limit of detection can be significantly improved for any application.

### More Sensitivity

Sensitivity is one of the most important factors for any detection technology. The sensitivity of a UV detector is primarily influenced by:

- The light path length. According to Beer's law, the absorbance of light and thus the signal height is directly proportional to the length of the light path.
- Peak dispersion. The less the peaks are dispersed, the narrower and higher they get.
- Detector noise. The lower the baseline noise, the better the signal-to-noise ratio.

Improving one of these parameters works usually only at the expense of the others. Extending the light path, for example, results in a higher cell volume which, in turn, leads to more peak dispersion. LightPipe technology is designed to combine a long light path with minimum dispersion and noise.

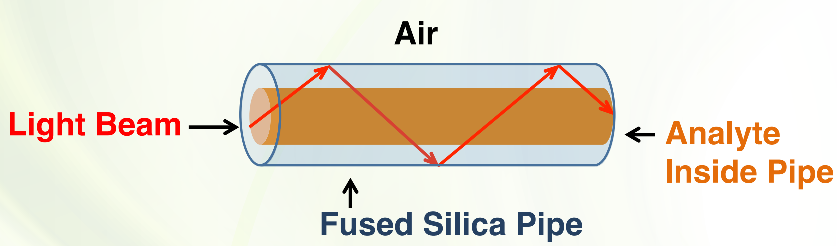


Figure 1: Simplified scheme and working principle of a LightPipe flow cell.

LightPipe technology uses a thin fused silica pipe as a waveguide that is surrounded by air. This pipe serves as the illuminated part of the flow cell. For best noise performance, fiber optics make sure that light is efficiently guided into and out of the pipe. The difference in refractive index between the fused silica LightPipe (n ~ 1.5) and the surrounding air (n ~ 1) leads to total internal reflection. All light falling on the interface between LightPipe and air is directed back into the fluidics of the flow cell. Thus, light passes through the flow cell not linearly but in a zig-zag-pattern (Figure 1). With this sophisticated design, this type of flow cell can be increased in length without hampering light throughput and impairing noise performance.

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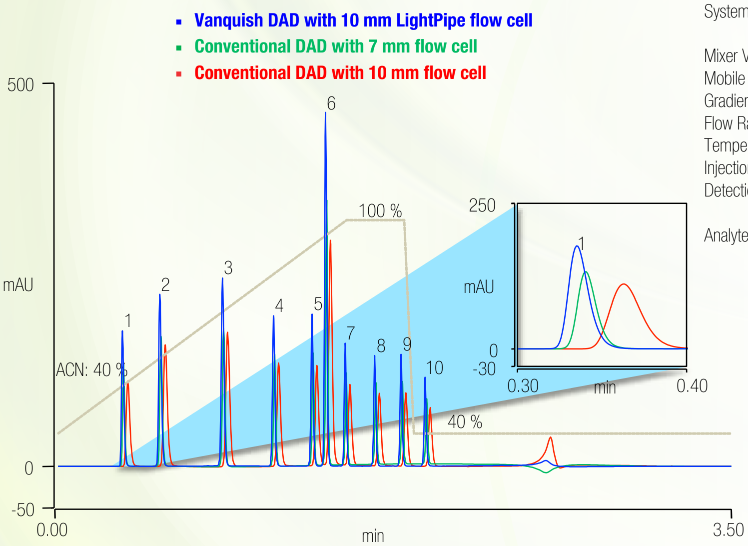
### Effects of Light Path and Dispersion

To show the effects of light path and dispersion, experiments were carried out with an UltiMate 3000 system coupled to two different DAD types with different flow cells. These were the Vanquish DAD HL (VDAD) with standard LightPipe flow cell with 10 mm light path and 2 µL volume, and a conventional DAD with two different flow cells; a 10 mm light path, 13 µL volume and a 7 mm light path, 2.5 µL volume flow cell. All volumes mentioned are illuminated volumes, that is, fluidic volumes of the light path. With the standard 10 mm LightPipe flow cell (blue chromatogram), peaks are higher and narrower than with the 10 mm conventional flow cell (red chromatogram, Figure 2). Compared to the 7 mm flow cell (green chromatogram) that is typically used in UHPLC applications, all peaks are 30-40% higher. This difference in height is mainly related to the extended light path and the optimized fluidic design of the 10 mm LightPipe flow cell.




| | |
|---|---|
| Column: | Thermo Scientific™ Hypersil GOLD™ C18, 2.1 x 100 mm, 1.9 µm, P/N 25002-102130 |
| System: | Binary UltiMate 3000 RS System with conventional DAD or Vanquish DAD |
| Mixer Vol.: | 200 µL |
| Mobile Phase: | A: water B: acetonitrile (ACN) |
| Gradient: | as shown in the chromatogram |
| Flow Rate: | 0.7 mL/min |
| Temperature: | 35 °C |
| Injection Volume: | 1 µL |
| Detection: | 4 nm slit, 254 nm, 4 nm bandwidth, 20 Hz, 0.2 s response time |
| Analytes: | 1. uracil<br>2. acetanilid<br>3. acetophenone<br>4. propiophenone<br>5. butyrophenone<br>6. benzophenone<br>7. valerophenone<br>8. hexanophenone<br>9. heptanophenone<br>10. octanophenone<br>50 µg/mL each,<br>except for uracil: 25 µg/mL |

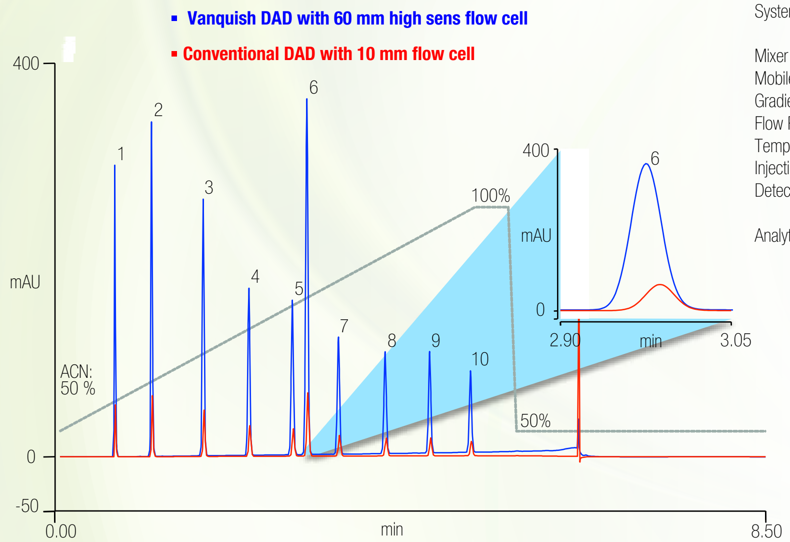
Figure 2: Chromatogram overlay of the same application, either detected by the Vanquish DAD with 10 mm LightPipe flow cell (blue), conventional DAD with 10 mm (red) flow cell, or conventional DAD with 7 mm (green) flow cell.

For the Vanquish 10 mm LightPipe and the conventional 10 mm, 13 µL flow cell, the physical light path is the same but the volumes differ. In LC, the flow cell volume should ideally be 10 times smaller than the peak volume to preserve column efficiency and chromatographic resolution. The UHPLC column used in this example creates low volume peaks of less than 20 µL. With the 10 mm conventional flow cell, the flow cell volume is close to the peak volumes of this separation example. In such a scenario, a pronounced dispersion can be expected, generating wider peaks compared with a smaller volume flow cell. The illuminated volume of the 10 mm LightPipe flow cell is 2 µL, suiting the peak volumes much better, leading to higher and narrower peaks.

In summary, the combination of long light path and small dispersion makes the VDAD with 10 mm LightPipe flow cell the best choice for fast UHPLC applications.

## Boosting Trace Detection in Conventional HPLC

Compared to a typical analytical flow cell with 10 mm light path, the 60 mm high sensitivity flow cell offers a six times longer light path. Figure 3 shows an overlay of two chromatograms, one obtained with the same system as before but with the VDAD using a 60 mm LightPipe flow cell (blue), the other one with a conventional DAD using a 10 mm flow cell as aforementioned. Due to the increased light path, peaks measured with the 60 mm flow cell are nearly six times higher, but also slightly wider.




| | |
|---|---|
| Column: | Thermo Scientific™ Acclaim C18, 4.6 x 150 mm, 3 µm, P/N 059133 |
| System: | Binary UltiMate 3000 RS System with conventional DAD or Vanquish DAD |
| Mixer Vol.: | 200 µL |
| Mobile Phase: | A: water B: acetonitrile (ACN) |
| Gradient: | as shown in the chromatogram |
| Flow Rate: | 2.2 mL/min |
| Temperature: | 35 °C |
| Injection: | 1 µL |
| Detection: | 4 nm slit, 254 nm, 4 nm bandwidth, 20 Hz, 0.2 s response time |
| Analytes: | 1. uracil<br>2. acetanilid<br>3. acetophenone<br>4. propiophenone<br>5. butyrophenone<br>6. benzophenone<br>7. valerophenone<br>8. hexanophenone<br>9. heptanophenone<br>10. octanophenone<br>50 µg/mL each,<br>except for uracil: 25 µg/mL |

Figure 3: Overlaid chromatograms, measured with Vanquish DAD and 60 mm high sensitivity LightPipe flow cell (blue) or conventional DAD with 10 mm flow cell (red).

Physically increasing the length of the light path of any flow cell type always increases the illuminated volume and results in higher peak dispersion. By increasing the length of the LightPipe from 10 to 60 mm, the illuminated volume changes from 2 to 13 µL. Although the nominal volumes of the 60 mm LightPipe and the 10 mm conventional flow cells are now the same, the dispersion effect is slightly different. The reason for this is that not only the volume influences dispersion but also differences in the geometry of the flow cell fluidics.

In general, peak dispersion caused by the flow cell becomes apparent a) with smaller inner column diameters and b) with early-eluting peaks, as both factors lead to smaller peak volumes. Analytical flow cells > 10 µL of conventional or optical waveguide design should typically only be used with column IDs ≥ 3 mm. With these columns, peak volumes are large enough for a good peak-to-flow-cell-volume compatibility.

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### Enhanced Signal-to-Noise Performance

One of the most important parameters for detection is the signal-to-noise ratio (S/N). This can be enhanced in two ways: by improving signal height or reducing noise. The first point has already been discussed. So, does the LightPipe flow cell contribute to a reduced noise?

In general, the Vanquish DAD HL is an all new detector, using state-of-the-art technology in optics and electronics. By default, the noise level is superior. Figure 4 shows the benefits of LightPipe technology with gradient separations. The left part of the figure shows three blank injections, corresponding to the chromatograms shown in Figure 2. For the conventional DAD with both the 10 mm, 13 µL (red) and 7 mm, 2.5 µL (green) flow cell, the course of the gradient is clearly visible in the baseline. Further, the gradient step from 100% to 40% acetonitrile (ACN) causes a positive or negative deflection. These baseline influences are caused by refractive index (RI) effects. With the VDAD and LightPipe flow cell (blue), the baseline is smooth and the deflection is less pronounced. This ruggedness against RI effects leads to an improved noise performance.

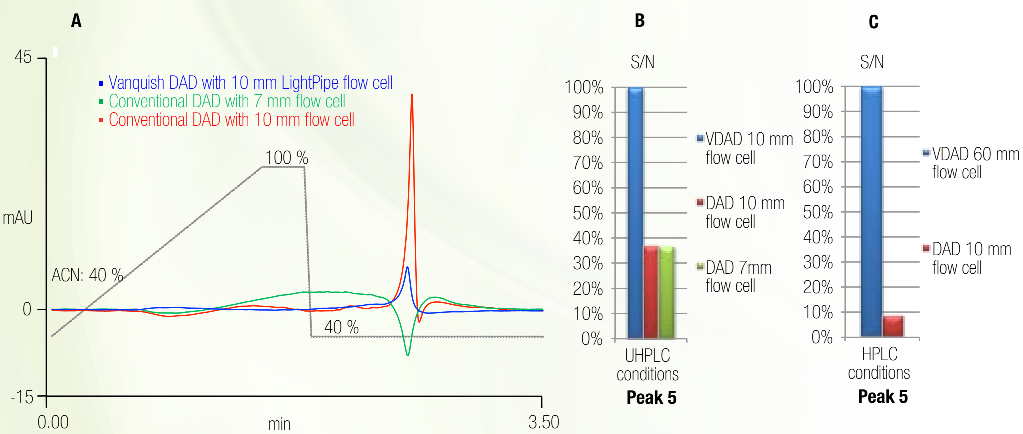


Figure 4: (A) Blank injections measured with the Vanquish DAD and 10 mm LightPipe flow cell (blue) and conventional DAD with both 10 mm (red) and 7 mm (green) flow cells. (B) Signal-to-noise ratios (S/N) for peak 5 from Figure 2 measured with the same setup. The highest S/N value was normalized to 100%. Noise value was acquired in a window of 5 times peak width at half height around the expected peak's retention time from respective blank run shown on the left. (C) Signal-to-noise ratios (S/N) for peak 5 from Figure 3 measured with the VDAD and 60 mm LightPipe flow cell (blue) and conventional DAD with 10 mm flow cell (red). The highest S/N value was set to 100%. Respective blank runs were used to obtain the noise value.

The superior noise performance of the VDAD in combination with increased signal heights result in a S/N improvement by a factor of 2-4 for the 10 mm LightPipe flow cell (see example in Figure 4B) and by a factor of 5-14 for the 60 mm high sensitivity LightPipe flow cell (see example in Figure 4C).

This outstanding S/N performance enables highest sensitivity and leads to a new level of trace detection, making the Vanquish DAD HL the detector of choice for highest detection sensitivity within the Vanquish and the UltiMate 3000 system platforms.

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