Raising the sensitivity benchmark in diode array detection with optical improvements

IN RECENT years, the diode array detector (DAD) has become the industry-standard HPLC detector for method development in research laboratories. While early DAD models lacked sensitivity, continual design upgrades—the use of dual lamps, high-intensity light sources, reference wavelength compensation, and diode bunching—steadily improved sensitivity performance.[1-3] Today, sensitivity levels of many DADs rival those of conventional absorbance detectors. A benchmark noise level of $\pm 1 \times 10^{-5}$ AU/cm pathlength (10 μAU) is generally thought to be the physical limit in UV-VIS detection, a limit imposed by short-term fluctuations of the source, thermal flow noise caused by flowing liquid in the flow cell, and noise from the signal processing electronics.

If reduction of background noise is impractical with current technologies, then why not enhance sensitivity (signal-to-noise ratio, or S/N) by increasing signal? To do this, one obvious approach extends the pathlength of the flow cell, thus increasing the absorbance (or signal) as governed by Beer's Law (where absorbance [A] = molar absorptivity (ε) × pathlength [b] × concentration [c]). Yet, for many years this approach for increasing sensitivity proved intractable. Flow cells of long pathlengths (>20 mm) and large volumes (>10 μL) cause deleterious band broadening and unacceptable loss of peak resolution. Meanwhile, flow cells with smaller diameters (<1 mm) result in significant light energy loss through absorption or scatter by the internal cell wall. The light energy emerging from the flow cell is further reduced by the exit optical slit.

This paper describes two key optical developments that overcome this difficult dilemma in flow cell design. The development of LightPipe and BeamShaper technologies (**Thermo Separation Products**, San Jose, CA) allows the extension of pathlength with minimal energy loss. Their successful implementation in the SpectraSYSTEM® UV6000LP (**Thermo Separation Products**) results in a fivefold increase in sensitivity and a two- to fivefold improvement in detection limits. Additional improvements that enhance accuracy, linearity, and ease of system validation are also discussed. Finally, specifications and performance evaluation data are summarized and compared with those of competitive models.

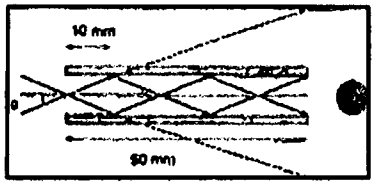

Figure 1 *Schematic diagram showing total internal reflectance at the coated, internal cell wall of the LightPipe flow cell with 50-mm pathlength.*

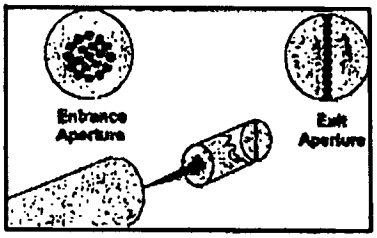

Figure 2 *Schematic diagram illustrating the use of BeamShaper technology consisting of 19 tightly packed optical fibers. The fibers transform the 0.5-mm circular optical beam exiting the flow cell into the rectangular shape required for diode array detection.*

Flow cell pathlength extension with minimal energy loss

LightPipe technology is responsible for the system's high sensitivity.[4,5] *Figure 1* illustrates the patented PEEK™ flow cell design (50 mm length, 0.5 mm i.d., and 10 μL volume) (patent 5, 608, 517, **Thermo Separation Products**, 1997). Total internal reflectance is achieved with a proprietary reflective inner wall coating. Compared with a conventional 15-μL, 10-mm flow cell, the LightPipe flow cell enhances the S/N by two- to fivefold with similar peak dispersion. PEEK, a biocompatible polymer as

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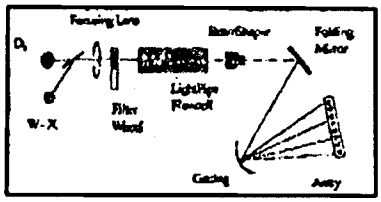

Figure 3 *Diagram of the optical system in the UV6000LP showing the implementation of LightPipe, BeamShaper, and validation filter wheel technologies.*

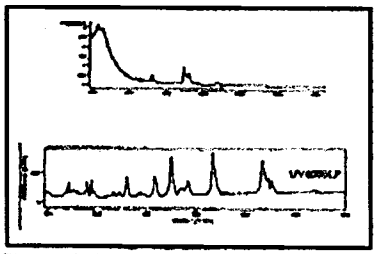

Figure 4 *Comparative UV-VIS spectra of conventional holmium oxide filter (a) vs. holmium oxide/perchloric acid cuvette (b). The liquid cuvette allows automated wavelength calibration and verification in both UV and visible regions.*

Table 1

Summary of system's technological features

Design	Benefits
LightPipe with 50-mm pathlength and total internal reflectance	Noise at $\pm 3 \times 10^{-6}$ AU/cm (6 μAU), a sensitivity increase of fivefold
BeamShaper with 19 bundled optical fibers to eliminate the use of energy-wasting external slit	Preserves both sensitivity and spectral resolution
Dual deuterium/tungsten-halogen lamps	Wavelength range of 190–800 nm
High-speed 512-element diode array at 20-Hz scan rate	Provides 1.2-nm spectral resolution and compatibility to Fast LC
Encapsulated electronics for signal processing; Intel I960 processor with 20-bit A/D converter	Reduces background electronic noise; provides high signal resolution for data precision
Savitzky-Golay smoothing algorithm	Produces high-quality spectra for spectral matching of trace peaks
Built-in holmium-oxide liquid cuvette; optional filter wheel for linearity verification	Easier and more accurate wavelength calibration and linearity verification
Use of optical filters, internal baffles, and low-scatter grating to reduce stray light	Extends linearity range to >2AU
PC1000 data management software	Used for data handling, single-point system control, and all spectral manipulation tasks

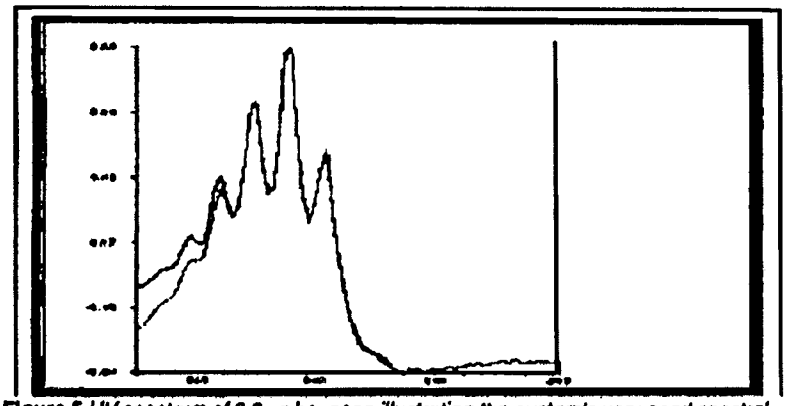

Figure 5 *UV spectrum of 8.8 ng benzene illustrating the system's very good spectral resolution and sensitivity performance.*

well as a low thermal conductor, is used as the cell end cap to eliminate the need for troublesome inlet heat-exchanger tubing (for the reduction of thermal flow noise).

The flow cell employs the same optical technology as the more familiar fiber-optic transmission lines used in communication and image viewing applications, in which light is conducted over long distances with minimum energy loss. Snell's Law defines the lateral reflection of light energy in an optical fiber. Briefly, it requires that a conduit be lined or coated with a material with lower refractive index than that of the core material. In the case of the LightPipe flow cell, the core material is the column effluent (usually a mixture of water and organic solvent), while the cell wall is coated with an amorphous polymer with a lower refractive index.[4,5] The condition required for total internal reflection is defined by the following equation:

$$\sin \theta < (n_1^2 - n_2^2)^{0.5} \qquad (1)$$

where θ is the angle of the incident light and n_1 and n_2 are the refractive indexes of the mobile phase and the polymer coating, respectively.

High sensitivity and spectral resolution

The ideal shape for the light beam from the source through the flow cell is circular in cross-section. Since the natural shape of the deuterium arc and the ideal flow cell geometry are circular, the maximum energy is transmitted through a circular optical beam. In a conventional DAD, the circular beam is trimmed to a rectangular shape by a mask-type slit for optimum dispersion by the grating onto the diode array elements. While a narrow slit is necessary for high spectral resolution, a major fraction of the light intensity (up to 80%) is thereby discarded, resulting in significant sensitivity loss. To alleviate this problem, some DAD models use a programmable slit system allowing operation in either high-sensitivity (wide slit width) or high-resolution (narrow slit width) mode.[3]

Table 2

Specifications for UV6000LP versus competitive models

Specification	SpectraSYSTEM UV6000LP	Competitive models' typical range
Detector type	512-element PDA, 20 scans/sec	Similar
Light source	Deuterium and tungsten/ halogen lamps	Deuterium only, or deuterium and tungsten lamps
Wavelength range	190–800 nm	190–600 (800) nm
Spectral resolution	1.2 nm, digital	1–2 nm
Noise*	$\pm 3 \times 10^{-4}$ AU/cm (6 μAU) @ 254 nm	± 10–50×10^{-4} AU/cm (20–100 μAU)
Drift*	1×10^{-3} AU/hr @ 254 nm	1–2×10^{-3} AU/hr
Linearity*	>2 AU <±5%	1–2 AU
Wavelength accuracy	±1 nm	±1–5 nm
Flow cell	LightPipe 50-mm pathlength, 10-μL volume, pressure rating 1000 psi	Pathlengths of 3–10 mm, cell volumes 2–13 μL
Validation features	• Automated calibration and verification of UV-VIS wavelengths with holmium oxide cuvette	• Verification with holmium oxide filter in the visible region only
	• Optional filter wheel for linearity verification; automated evaluation of noise and drift	• None

*Measurement according to ASTM E1657-94.

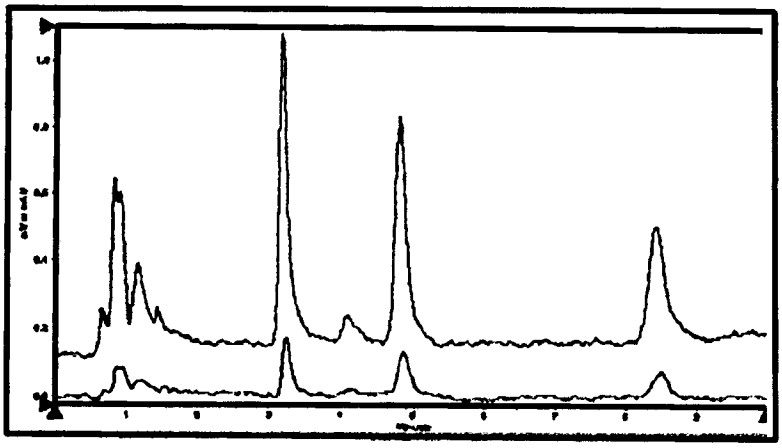

Figure 6 *Comparative chromatograms illustrating the detection sensitivity performance of the UV6000LP (top chromatogram) vs. that of a standard, single-wavelength UV/VIS detector with conventional 10-mm flow cell (bottom chromatogram). A significant S/N enhancement was found using identical sample and chromatographic conditions.*

Figure 2 illustrates the function of a BeamShaper apparatus that uses 19 bundled optical fibers to reshape the circular light beam exiting the flow cell into a narrow rectangular shape. A focusing grating spectrally disperses this rectangular beam onto the photodiode array (PDA) element. This tightly packed fiber bundle has minimal interstitial space, resulting in only minor energy loss.

The combination of LightPipe flow cell and BeamShaper technologies provides maximum energy transmission and high spectral resolution to yield high-sensitivity LC analyses with quality spectra. In contrast to the use of a programmable slit or diode bunching,[3] high detection sensitivity and spectral resolution can be achieved simultaneously in a single chromatographic run.

Performance and accuracy improvements

Figure 3 shows the optical system in the UV6000LP. Two high-intensity lamps (deuterium and tungsten-halogen) provide increased light levels across the full UV/VIS range of 190–800 nm. The source lens focuses the energy combined from both sources into the flow cell. The exiting circular beam is transformed by the BeamShaper to a slit-shaped beam and directed by a folding mirror to a concave grating. The dispersed rectangular beam then impinges on a high-speed 512-element diode array, which provides a fast scan rate (20 scans/sec) with 1.2-nm digital resolution.

Electronic components for signal processing are thermally encapsulated and located on the optical bench to reduce noise generated by thermal or electromagnetic interference. A powerful Intel i960 microprocessor (Intel Corp., San Jose, CA) with 20-bit A/D converter provides high signal resolution for better data precision. A sophisticated Savitzky-Golay smoothing algorithm[6] is used to produce low-noise spectra for more accurate matching of trace peaks.

Instead of solid holmium oxide filters, a sealed cuvette filled with 6% holmium oxide in perchloric acid solution (located in a standard filter wheel) is used for automated wavelength calibration and verification. *Figure 4* compares the spectra of these two holmium oxide references. The liquid-filled cuvette provides nine sharp absorption bands in the UV-VIS region (241–800 nm); the solid filter provides only visible reference wavelengths (361, 412, and 536 nm).

The system also features an optional validation filter wheel, which includes five cuvettes filled with

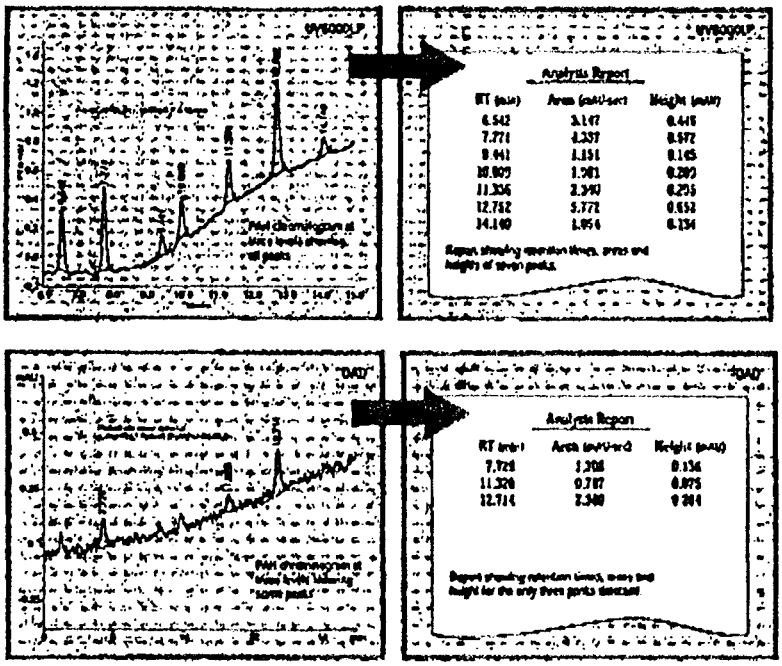

Figure 7 *a) Chromatogram of a trace-level PAH sample run on a conventional photodiode array detector. b) Chromatogram of the same trace-level PAH sample run on the UV6000LP in series after the photodiode array detector and under identical conditions.*

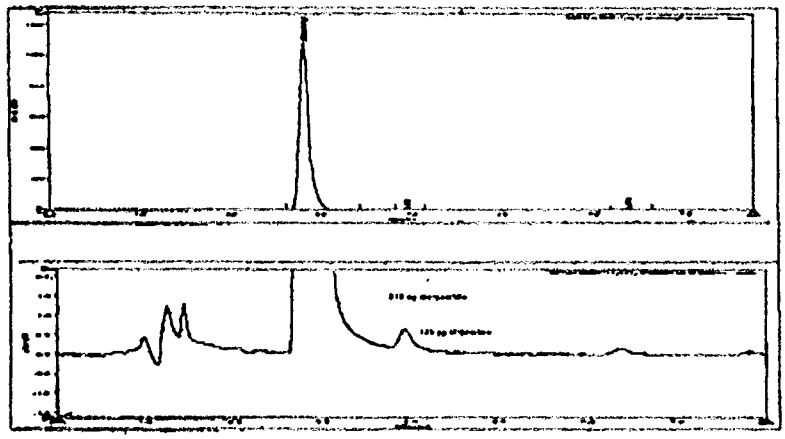

Figure 8 *Analysis of trace impurities (0.05%) of a methyl paraben sample, an antimicrobial used in pharmaceutical products.*

NIST-traceable K_2CrO_7 solutions in 0.001 N perchloric acid (including a blank cuvette). This device facilitates rapid verification of detector linearity and absorbance accuracy.[7] Source and order selecting filters, internal baffles, and a low-scatter holographic grating reduce stray light in the optical system (which degrades linearity performance). PC1000 software (**Thermo Separation Products**) is used for data handling, single-point control of the entire system, and all spectral manipulation including peak purity evaluation and spectral library matching. *Table 1* summarizes the technological features responsible for performance and accuracy enhancement in the system.

The specifications of the system are listed in *Table 2* with compara-

tive data of competitive models. The system exceeds the noise and drift specifications of most competitive models, while excelling in linearity performance and validation ease.

Performance evaluation data

Figure 5 shows a UV spectrum for a sample of 8.8 ng benzene and illustrates the very good spectral resolution and sensitivity performance of the system. The 1.2-nm digital resolution allows easy resolution of the five-fingers pattern of the benzene spectrum. The low-noise spectrometer generates high-quality spectra at trace levels for more accurate spectral matching and identification. *Figure 6* compares the detection sensitivity of the system with LightPipe flow cell with that of a high-sensitivity, single-wavelength UV-VIS detector with a conventional 10-mm flow cell. *Figure 7* shows two chromatic hydrocarbon (PAH) sample run on the UV6000LP and a conventional DAD simultaneously, under identical conditions. A S/N enhancement of five times was found. The sample in *Figure 8*, run on the UV6000LP, shows a trace impurity peak (0.05%) eluting at the tail of the major component. Both compounds can be quantitated in a single run.

Summary and conclusion

This paper describes several technological improvements that allow fivefold sensitivity enhancement in PDA detection. A noise benchmark of $\pm 3 \times 10^{-6}$ AU/cm (6 µAU) is achieved. Very good performance in drift, spectral resolution, linearity, and user convenience for validation are maintained. Clearly, the system is most useful for pharmaceutical assays in which determinations of less than 0.1% impurity levels are often

References

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