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PERSPECTIVES
IN MODERN HPLC

Ultraviolet Detectors: Perspectives, Principles, and Practices

This installment is the third of a series of four installments on HPLC modules, focusing on pumps, autosamplers, ultraviolet (UV) detectors, and chromatography data systems. This installment provides a technical overview of the design and operating principles of variable wavelength and photodiode array detectors, and includes historical perspectives and common practices in operation and maintenance.

Michael W. Dong and Jedrzej Wysocki

A typical high performance liquid chromatography (HPLC) system consists of these modules: a pump, an injector (autosampler), one or more detector(s), and a chromatography data system (CDS). The detector measures the concentrations or mass flows of the separated analytes, and converts them into electronic signals. The availability of reliable and sensitive detectors is mostly responsible for the success of HPLC as a pervasive analytical technique in scientific discovery and quality control applications.

There are many types of HPLC detectors, which have been extensively reviewed in books (1–4) and review articles (5–9). Three broad categories of HPLC detectors have become most important in recent years: Ultraviolet (UV) detectors for chromophoric compounds; evaporative light scattering detectors (ELSD) or charged aerosol detectors (CAD) for nonchromophoric compounds; and mass spectrometers (MS) for scientific research and multiresidue analysis. In addition, several detectors are common for specific applications, such as refractive index detectors for polymer and sugar analysis, fluorescence detectors for environmental, food, and tagged protein applications, and electrochemical detectors for neuroscience applications (3).

The UV detector is the most common detector in use today because of its reliability, ease of use, and universal response to chromophoric compounds, including most pharmaceuticals. While the prominence of the UV detector has been overshadowed by MS, it remains the undisputed workhorse in quality control laboratories. For instance, in the pharmaceutical and chemical industries, the normalized area-under-the-curve (AUC) values with UV detection are often equated with purity percentages by weight. The International Council of Harmonization (ICH) guidelines, followed by all pharmaceutical laboratories in production and late-stage development, require sensitivity in the range of 0.05–0.10% for the stability-indicating HPLC methods of drug substances and drug products (3). The use of UV detection is implicitly assumed in the ICH Q3A guidelines for these methods. For pharmaceutical testing, the higher precision achievable with UV detection (<0.2% RSD) is pivotal and necessary in this regulatory testing because a typical potency specification for drug substances is 98.0 to 102.0% (3).

This installment provides a technical overview of the UV detector and its operating principles, recent developments, and common operation and maintenance procedures.

Glossary of Key Terms and Definitions

- **UV Detector:** A UV detector is an in-line device that measures the UV absorbance of the HPLC eluent and provides a continuous signal that can be used to quantify the amount of chromophoric compounds emerging from the HPLC column. There are three types of UV detectors: fixed wavelength, variable wavelength, and photodiode array detectors.
- **Variable wavelength detector (VWD) or UV-visible (UV-vis) absorbance detector:** This device uses a deuterium source and a monochromator to allow the selection of a particular wavelength in the UV-vis region for selective detection.
- **Photodiode array detector (PDA) or diode array detector (DAD):** This is a common UV detector that monitors the entire UV-vis spectrum of material passing through the flow cell using a photodiode imaging sensor, typically consisting of 512 or 1024 pixels or elements. The detector yields both absorbance and spectral data that can be used for quantitation, identification, and peak purity assessments. Note that some manufacturers offer a DAD as a multiple wavelength detector at a lower cost without the spectral scanning capability.
- **Monochromator:** An optical system in a spectrometer that allows the selection of light of a specified wavelength. It consists of a movable diffraction grating (or prism) for light dispersion that can be rotated to select a wavelength through an exit slit.

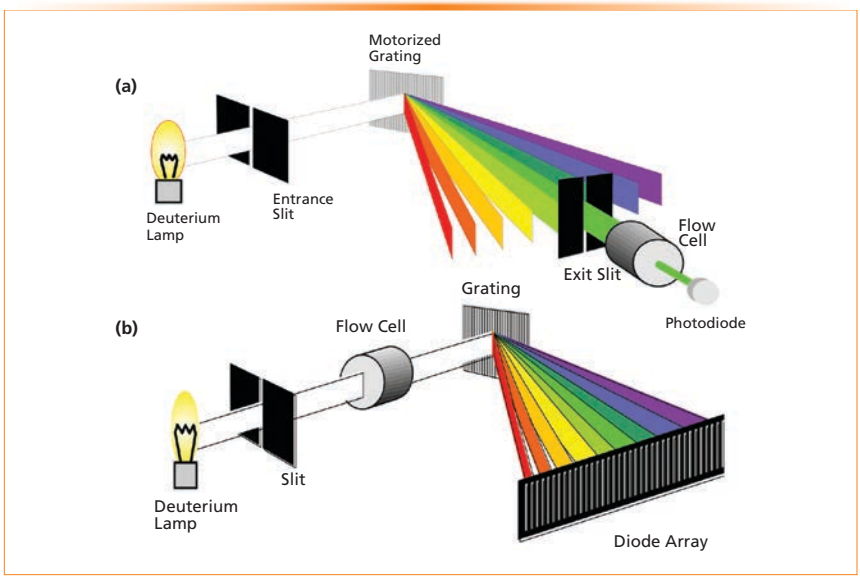

FIGURE 1: A schematic of the optical systems in: (a) A UV-vis absorbance detector showing the monochromator and the flow cell illuminated by the selected wavelength after the exit slit, (b) A diode array (DAD) detector with a fixed grating which dispersed the light onto a diode array imaging element. Note that the entire spectrum passes through the flow cell. Figure adapted from reference 3.

TABLE I: Advantages and limitations of UV detectors

Advantages	Limitations
Nondestructive, very reliable, and easy to use	Analytes must have chromophoric activitiy
Near-universal detection of organics at low UV wavelength; compatible with gradient analysis	The mobile phase must be transparent to UV to have acceptable sensitivity and linearity
Signal relatively immune to changes of mobile phases (refractive indexes and flow rates) and temperature fluctuations	Detector responses depend on ε and can be highly variable for different compounds
Medium- to high-sensitivity to compounds with high molar absorptivity (ε)	Closely related compounds with unaltered chromophore moieties can have very similar UV-vis spectra; for this reason, the usefulness for peak identification and purity assessment is much lower than that of MS
Selective detection of most active pharmaceutical ingredients (APIs) and their related substances using detection at λ_{max}	-----
High-precision (<0.2% RSD) making it the preferred detector in regulatory analysis	-----
Five orders of magnitude of linear dynamic range allowing the use of single-point calibration for most assays	-----
Provides information on peak identity and purity with a DAD	-----

- **Source:** The light source or lamp that provide light in a spectrometer. The typical source in a UV detector is a low-pressure arc discharge deuterium (D2) lamp with light energy in the 190 to 600 nm range. It can be augmented by a supplemental tungsten source to provide more visible light energy >400 nm (to 950 nm) if required.
- **Flow cell:** The flow cell is a small flow-through device in the UV detector that is connected physically to the outlet of the column. It has two quartz windows or lenses at each end of the cell that defines the optical pathlength. Typical flow cell volumes are 8–18 µL and 0.5–1 µL for HPLC and ultrahigh-pressure liquid chromatography (UHPLC), respectively, with a pathlength of 10 mm.
- **Absorbance:** Absorbance is defined as the negative logarithm of transmittance, which is the ratio of the final and initial intensity of light passing through the flow cell at a specific wavelength. The units are absorbance unit (AU) and milli-absorbance unit (mAU).
- **Pathlength:** The pathlength is the length of the flow cell, which is important for the sensitivity of the detector, because absorbance is proportional to pathlength.
- **Molar absorptivity (ε):** Molar absortivity is also known as the extinction coefficient, and is the constant specific to a chromophoric compound that defines how strongly the compound absorbs light at a specific wavelength.
- **Beer's law:** Beer's law, often known as the Beer-Lambert law, states that absorbance is equal to the products of molar absorptivity (ε), pathlength (b), and the concentration (c) of the analyte.
- **Maximum absorbance wavelength, or λ_{max}:** The maximum absorbance wavelength is the characteristic wavelength of the absorption peak of a UV spectrum of a chromophoric molecule, which is often used as the monitoring wavelength in HPLC and for peak identification.
- **Dispersion:** Dispersion, or the band broadening effect of the UV detector, is dependent mostly on the volume of the flow cell and its flow characteristics.
- **Spectral bandwidth:** Although the user may select a particular wavelength, the actual selection consists of a range of wavelengths that passes through the flow cell and the exit slit. The typical spectral bandwidth is 5–8 nm for a UV-vis detector. Because the DAD sees the entire spectrum, the spectral bandwidth can be specified from a single nanometer to any segment of the entire spectrum via the control software.
- **Peak purity:** Peak purity, or peak homogeneity, is typically expressed as a peak

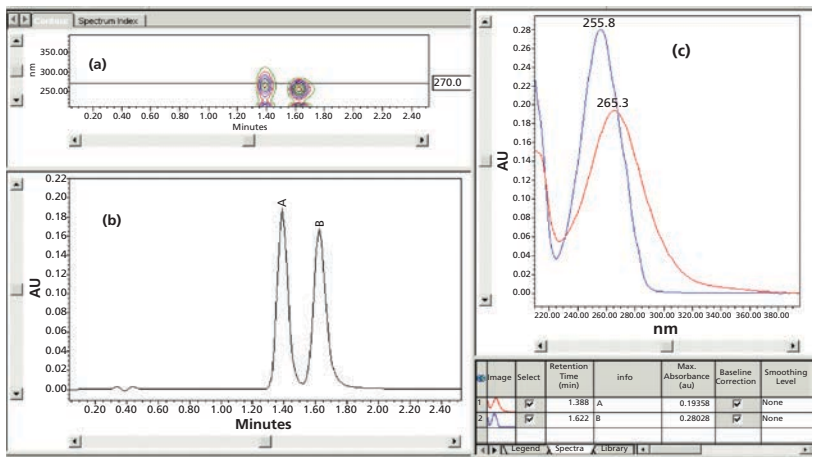

FIGURE 2: (Waters Empower) chromatography data system screenshots showing several windows of display of both chromatographic and spectral data from an injected sample. (a) A UV spectral contour map that allows the display of chromatogram in any wavelength from 200–400 nm; (b) a chromatogram at 270 nm showing the separation of nitrobenzene (A) and propylparaben (B); (c) UV spectra of these two components annotated with their respective λ_{max}. values. Figure adapted from reference 3.

TABLE II: Requirements and desirable characteristics of modern UV detectors

Requirements
• Provides precise absorbance measurements of HPLC effluence with high-sensitivity (noise <±1 x 10^{-5} AU) and specificity (spectral bandwidth <5–8 nm) in UV-vis range (190 to 600 nm)
• Equipped with a small flow cell for intended applications (8–15 µL for HPLC or 0.5–1 µL for UHPLC) and adequate pathlength (10 mm) to maintain sensitivity and reduce extracolumn dispersion; constructed from materials compatible to common mobile phases
• The linearity of the response of ~5 orders of magnitudes up to 1.5 absorbance units (AU); adjustable response times and sampling rates to accommodate both slow or fast eluting peaks
• Controlled by chromatography data system (CDS) with software for spectral data displays from DAD
Desirable Characteristics
• General: Compact dimensions compatible with other HPLC modules (stackable to minimize footprint); Easy to operate and maintain (front-panel access to source and flow cell); self-aligned source and flow cell; built-in diagnostics, leak detection, and wavelength calibration (holmium oxide filter or mercury lamp).
• Performance: Low noise (<±0.4 x 10^{-5} AU); low drift (noise <1 x 10^{-4} AU/h); High linear dynamic response range up to 1.5–2.0 AU; relatively immune to changes of refractive index, flow rate, and temperature of mobile phases; wavelength accuracy <±1 nm; built-in temperature control to reduce drift.
• DAD: Ability to select spectral bandwidth by bundling pixel responses; programmable slit to increase spectral resolution; multiple wavelengths outputs (4–8 signals); wavelength programming; 2D and 3D spectral displays; automatic annotation of λ_{max}; peak purity assessments; library searches.
• Source: Long-life deuterium source to 2000 hours; available tungsten source to increase sensitivity in visible region >400 nm.
• Flow cell: availability of optional flow cells for lower dispersion or extended (higher sensitivity) pathlengths; high-pressure ratings for LC–MS; bioinert or micro or nano LC applications.

purity index or purity angle obtained by comparing the UV spectra of the upslope to the downslope of the chromatographic peak.

- **Chromophore:** A chromophore is a part of a compound (its structural moiety) that absorbs UV or visible light.
- **Noise:** Noise in a UV detector is he stability or fluctuation of the light intensity as seen by the detector, and is often expressed as peak-to-peak or root-mean-square noise. The historical benchmark of noise specifications from a UV detector is ±1 x 10^{-5} absorbance unit (AU) which is exceeded by most modern UV detectors.

Advantages and Limitations of UV Detectors

Table I summarizes the advantages and limitations of UV detectors. The overwhelming advantages of the UV detector, such as reliability, ease of use, high precision, and linearity make it an ideal detector for quality control applications of any chromophoric compounds (for example, pharmaceuticals). Detection limitations, such as the requirements for the mobile phase optical transparency and the variable response of the UV detector to different analytes, is dependent on the analyte molar absorptivity; these limitations are generally less serious, and can be mitigated using an appropriate selection of mobile phases and calibration techniques (3). For nonchromophoric compounds of no or low molar absorptivity, the use of universal detectors such as refractive index (RID), evaporative light scattering (ELSD), or charged aerosol detector (CAD) is recommended (3).

Requirements and Desirable Characteristics of UV Detectors

Table II summarizes the requirements and desirable characteristics of a modern UV detector (VWD or DAD), followed by a discussion of historical perspectives, optical designs, operating principles, and common operation and maintenance procedures. Our goal is to increase the understanding of the UV detector for the laboratory scientist, thus allowing the implementation of better operating practices.

Chromophore	λ_{max}(nm)	ε_{max}
alkyne	225	160
carbonyl	280	16
carboxyl	204	41
amido	214	41
azo	339	5
nitro	280	22
nitroso	300	100
nitrate	270	12
olefin conjugated	217-250	>20,000
ketone	282	27
aklylbenzenes	250-260	200-300
phenol	270	1,450
aniline	280	1,430
naphthalene	286	9,300
styrene	244	12,000

FIGURE 3: A summary of UV absorption characteristics of common organic chromophoric groups with their λ_{max} and molar absorptivity. Data extracted from reference 14.

Types, Principles, and Historical Developments

The historical developments of HPLC instrumentation are documented in books (1–4) and journal articles (5–9). The availability of sensitive and reliable UV detectors has been a pivotal factor in the success of HPLC in pharmaceutical applications (3). Here are brief highlights of the historical developments of different types of UV detectors and their operating principles, leading to the modern renditions in use today.

Early Fixed Wavelength UV Detectors

Fixed wavelength UV detectors with low-pressure mercury lamps (having a strong 254 nm emission line) were first available in the late 1960s (6,10). A cutoff filter was used to eliminate other high-order wavelengths from the source. Other wavelengths such as 280 or 265 nm can be obtained by adding phosphor to the source (6). For low wavelength analyses, a zinc lamp can be used for detection at 214 nm. One fixed wavelength UV detector introduced in 1968 had a reported noise of ±0.2 mAU (11), which was ~50 times less sensitive than today's detectors. Currently, fixed wavelength UV detectors are found mostly in low-cost or portable systems (12).

Variable Wavelength Detectors

Early variable wavelength detectors (VWDs), also called *UV-visible absorbance* (UV-vis) detectors, are adaptations of existing spectrophotometers by replacing the cuvette with a small flow cell. Dedicated UV-vis detectors for HPLC were designed to improve performance and became popularized in the 1980s. Figure 1a shows a schematic of the optical system, which uses a low-pressure deuterium arc discharge lamp to provide continuous emission in the 190–600 nm UV-vis region. The polychromatic light spectrum is directed into a monochromator, consisting of an entrance slit, a diffraction grating (or a prism), and an exit slit. The motorized grating disperses the light spectrum and can be rotated to select a specific wavelength through the exit slit to the flow cell. The transmitted light from the flow cell then impinges on a single photodiode that transforms the light energy into electrical signals. A beam splitter is placed before the flow cell to direct a portion of the source energy into a reference photodiode. The entire optical system is placed inside a sealed cabinet which is painted black to reduce stray light that will limit detector linearity. Numerous design improvements of the optics and electronics were implemented in the ensuing years to increase detector performance to be discussed later. One of the highest sensitivity VWD that set a sensitivity benchmark (noise $<\pm 1.0 \times 10^{-5}$ AU) in the 1990s was the Kratos 757 Spectroflow HPLC UV detector.

Diode Array (DAD) Detectors

In recent years, the prominence of the variable wavelength detector has been superseded by the diode array (DAD) detector, also known as a *photodiode array detector* (PDA), which offers substantially more flexibility and capability at an incremental cost. One of the first DAD detectors for HPLC (HP 1040A) was introduced by Hewlett Packard (Agilent) in 1982 (13).

A DAD detector provides UV spectra of eluting peaks while functioning as a multiwavelength UV-vis detector. The DAD facilitates peak identification, and is the preferred detector in pharmaceutical laboratories and for HPLC method development.

Figure 1b shows the schematic of a DAD detector where the entire spectrum of the deuterium lamp passes through the flow cell, and the transmitted light is dispersed by a fixed grating onto a diode array element that monitors the intensity of light at each wavelength. Most DADs use a charge-coupled diode array with 512 to 1024 diodes (or pixels), capable of a spectral resolution of about 1 nm. Spectral evaluation software allows the display of both chromatographic and spectral data of all the peaks in the sample (an example is shown in Figure 2). These software features are integrated into the CDS, and can include automated spectral annotations of λ_{max} and display of UV spectra; 2D contour maps, which allow the display of chromatograms at different detection wavelengths; UV spectral library searches; and peak purity evaluation. Peak purity evaluation works by comparing the upslope, apex, and downslope spectra, and can detect a co-eluted impurity with different spectral characteristics (3).

Principle of UV Detection and Performance Characteristics of a UV Detector

The principle for UV detection is Beer's law, also called the Beer-Lambert law, where

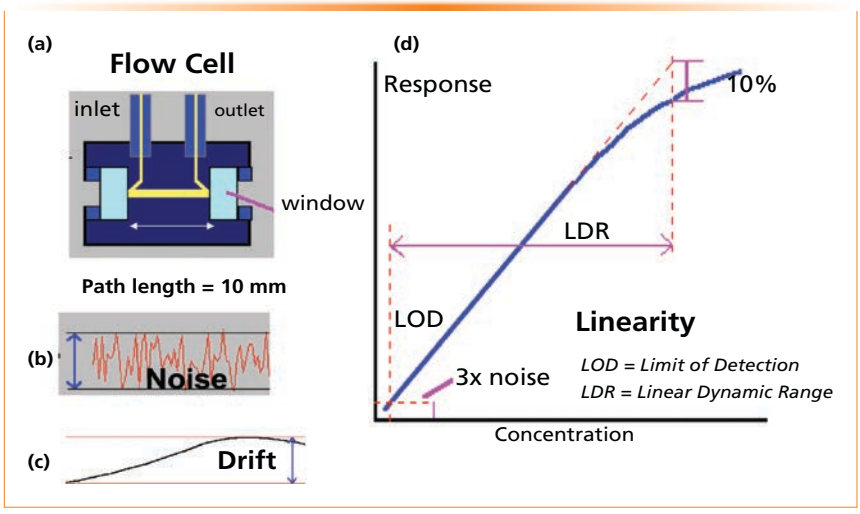

FIGURE 4: Schematic diagrams of (a) an HPLC flow cell with two quartz windows and a pathlength of 10 mm; (b) baseline chromatogram showing noise (magnified, peak-to-peak); (c). baseline chromatogram showing drift; (d) Chart of UV response versus concentration of the analyte injected. Linearity range is generally recognized from the limit of detection (LOD) to the point of the response curve deviating 10% from a linear correlation. Diagrams adapted from Savant Academy and other sources.

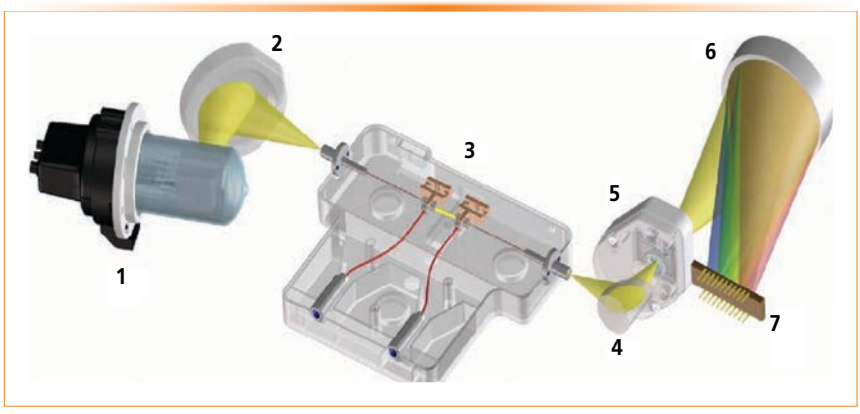

FIGURE 5: A schematic diagram of the key components in the optical system of a modern DAD detector: (1) deuterium lamp, (2) lamp mirror, (3) cartridge flow cell with capillary made from fused silica for total internal reflectance, (4) fold mirror, (5) programmable or fixed slit, (6) holographic grating, (7) diode array. Figure courtesy of Agilent Technologies.

Absorbance (A) = molar absorptivity (ε) x pathlength (*b*) x concentration (*c*)

Absorbance is defined as the negative logarithm of transmittance, which is the ratio of intensities of transmitted light and the incident light. Note that absorbance is equal to 1.0 if 90% of the light is absorbed, and 2.0 if 99% of incident light is absorbed. At absorbance above 2, very low light intensity is transmitted in the sample beam, so the amount of stray light (background light detected) becomes a limiting factor for the upper end of the linearity range.

Most UV absorption bands correspond to transitions of electrons in the analyte molecules from $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$, or $n \rightarrow \sigma^*$ molecular orbitals (3). Figure 3 lists the λ_{max} and ε of some common organic functional groups with chromophoric (light-absorbing) properties (14).

Performance Characteristics

The UV-vis detector monitors the absorption of UV or visible light in the HPLC eluent by measuring the energy ratio of the sample beam against that of a reference beam. An HPLC flow cell (Figure 4a) has typical volumes of ~8 µL (that is, 1 mm-i.d. and a pathlength of 10 mm) with quartz lenses or windows at both ends of the flow cell.

The primary performance characteristics of UV-vis detectors are sensitivity (low noise), drift, and linear dynamic range (see illustrations shown in Figure 4). These characteristics are primarily controlled by the design of the flow cell, the optics, and its associated electronics. Sensitivity is specified by baseline noise (such as peak-to-peak, root mean square [RMS] noise, or using procedures described in ASTM E685-93 [15]). For years, noise specification for UV detectors has been benchmarked at $\pm 1.0 \times 10^{-5}$ absorbance unit (AU) (3).

Note that when a single wavelength is selected, a typical spectral bandwidth of 5 to 8 nm passes through the flow cell. Increasing the spectral bandwidth by widening the exit slits, due to more energy reaching the detector, improves detection sensitivity somewhat but reduces the linear dynamic range (LDR).

Flow cell design is important for increasing sensitivity because signals are proportional to the flow cell pathlength. Increasing pathlengths often leads to higher system dispersion or extracolumn band broadening. One of the biggest challenges in the design of a UV detector for UHPLC is the construction of a very small UV flow cell in terms of volume but maintaining the pathlength at 10 mm for sensitivity. For instance, by reducing the diameters of the flow cells to 0.5 mm and keeping the 10 mm pathlength, the volume is reduced to 2 µL. Similarly, a 0.25 mm-i.d. flow cell has a volume of 0.5 µL. This is accomplished by reducing the size of the light aperture and the use of a new material such as Teflon AF with high refractive index than most common mobile phases where the entire incident light would experience total internal reflectance in the narrow path of the flow cell without signal attenuation (3,5).

Drift is defined as the change of baseline absorbance with time and is measured in AU (Figure 4c). Drift performance is typically 1.0×10^{-4} AU/h in modern UV detectors. It is important for UV detectors to have a wide LDR from 10^{-5} to ~2 AU or five orders of magnitude. This linearity range allows for the use of normalized peak area percentages for the quantitative determinations of trace impuri-

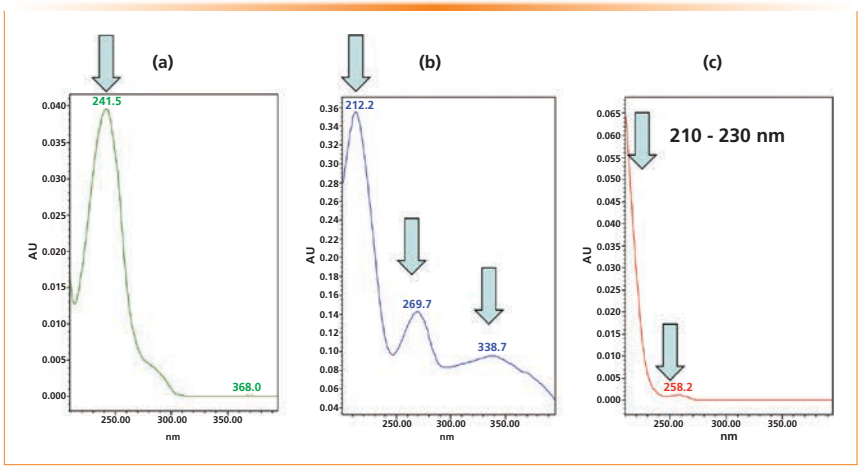

FIGURE 6: UV spectra of three analytes illustrating the selection process in HPLC monitoring wavelength. (a) The λ_{max} at 241 nm is a clear choice; (b) The three λ_{max} at 212 nm, 269 nm, and 339 nm give rise to different options; 269 nm is the obvious choice, although 339 nm may be selected for higher selectivity if interference from other matrix components is an issue. (c) The selection of λ_{max} at 258 nm can be problematic due to low sensitivity. Far UV (210–230 nm) offers better sensitivity and a more universal detection. Figure adapted from reference 3.

ties and the use of single-point calibration in most pharmaceutical analysis (3).

Recent Developments in VWD and DAD Detectors

Figure 5 shows the key components of the optical system implemented in a modern HPLC DAD detector, illustrating refinements such as an interchangeable cartridge-type flow cell that allow the use of an extended pathlength flow cell, and an exit slit with programmable slit-width (software selectable at 1, 2, 4, 8, and 16 nm) (16,17). Current UV detectors represent the use of mature technologies where basic designs remain fundamentally unchanged for two decades. Nevertheless, they have undergone incremental performance improvements, particularly in recent adaptations to UHPLC adaptations.

The high end of the linear dynamic range has been extended from a typical level of 1–1.5 AU to 2–2.5 AU by lowering stray-light levels and the use of electronic compensation techniques (12). The typical lifetime of the deuterium lamp is now ~2000 hours. Most UV detectors have features such as self-aligned sources and flow cells, leak sensors, and built-in holmium oxide filters for wavelength accuracy verification.

As mentioned earlier, one important innovation was the design of small-volume flow cell for UHPLC application using light pipe (fiber optics) technology to extend the pathlength without increasing noise or chromatographic dispersion. By constructing the light-pipe with a reflective polymer to allow total internal reflection, small flow cells with normal or even extended pathlengths were possible without sacrificing sensitivity (0.5 µL with 10 mm pathlength or 2.5 µL with 25 mm pathlength) (9,18–19).

Best Practices in UV Detector Operation and Maintenance

Best practices in HPLC operation, maintenance, and troubleshooting have been described in books (20), journal articles (21), and manual or publications from manufacturers (17,22). A summary of the general operating practice for UV detectors is included here as a brief reference guide. The reader is referred to information from the manufacturers' manuals or resources on the specific models.

UV Detector Operation

The following operating guidelines are for UV-vis or DAD detectors. Consult vendor's manuals for detailed procedure specific to your model.

1. Turn the lamp on for at least 15 min to warm up the lamp before analysis.
2. Monitoring wavelength: Set to the appropriate monitoring wavelength dependent on the analyte's UV spectrum. For most applications, setting the monitoring wavelength to the analyte's λ_{max} offer the best sensitivity and selectivity to its related substances (3). For analytes with multiple λ_{max}, the selection of a higher wavelength may offer more selective detection and eliminate some interference in a complex sample (see examples in Figure 6). The selection of a far UV detection wavelength (200–230 nm) offers universal detection through the use of phosphate buffer, or alternatively, phosphoric acid may be required for acceptable sensitivity. However, sensitivity and linearity performance may be highly compromised at low UV detection if MS-compatible mobile phases with less UV transparency are used. In addition, at low UV wavelengths, specificity could become challenging due to sample matrix components and purity of solvents.
3. Spectral bandwidth: The spectral bandwidth of a VWD is dependent on the slit width and is typically fixed at about 5 to 8 nm. The spectral bandwidth of a DAD is also dependent on its slit width and the number of diodes that are combined for averaging the output signal. For routine analysis, this should be set at 2–8 nm (4 nm is the default) for the best selectivity and sensitivity performance (3). Widening the spectral bandwidth can yield more universal detection, but can also impact linearity, because Beer's law is only valid for monochromatic light. Setting a narrower bandwidth to 1 nm (using a DAD with programmable slit width) may increase the spectral resolution of collected UV spectra at the expense of sensitivity performance.
4. Detector response time (that is, settings of 0.5–2 s for typical HPLC or 0.1–0.5 s for fast analyses). Similarly, data or sampling rates should be fast enough to provide 10-20 points across the narrowest peaks. The effects of data collection rate and detector filter response on peak width and measured column efficiencies of fast eluting peaks can be found elsewhere (23).
5. Set wavelength range of DAD (for example, 200–400 nm) for general method development to collect all UV spectra during initial HPLC method development. A

spectral resolution of 2 to 4 nm is a standard setting for sample analyses while collecting UV spectra.

6. Reference wavelength: Some DAD instruments allow the setting of a reference wavelength typically at a higher wavelength (360 nm with a bandwidth of 100 nm) to reduce gradient baseline shifts (18). Care should be exercised when performing stability-indicating assays because impurity(ies) may absorb at this reference wavelength, causing erroneous results for this analyte.
7. Extended flow cell pathlengths of 25–80 mm are available for some UHPLC-compatible UV detectors, and can increase UV sensitivity by 2–8 fold for analyses using external standardization (for example, cleaning verification, analysis of potentially genotoxicity impurities, and extractable and leachable studies). However, these are less compatible with small columns (for example, 2.1 mm-i.d.) due to extracolumn band broadening, and to stability-indicating analysis using normalized area percent calculation, since the main peak can easily saturate the detector (exceeds the linearity of the detector) (18).
8. Deuterium lamps typically last 12 months with limited use or 1000–2000 hours. Replace the lamp if sensitivity loss is observed. Aged lamps typically yield higher baseline noise. To increase lamp lifetime, the user can shut off lamps after sample analysis. The detector power can be left on without compromising the lifetime of the lamp.
9. Column connections to the UV detector. Note the inner diameter of the column outlet connection tube to the detector flow cell is typically very small (0.003 to 0.005 in., or 0.08-0.125 mm) in order to reduce extra-column band broadening. Low-cost finger-tight PEEK fittings rated to 5000 psi or 35 bar are sufficient for the column outlet connections (3). A back pressure device (for example, 300 psi or 20 bar) is recommended for some UHPLC detectors to reduce mobile phase outgassing due to longitudinal viscous heating when operating at high flow rates and column temperatures.

General Comments on UV Detector Maintenance and Troubleshooting

Modern UV absorbance detectors are designed for easy maintenance and often have front panel access to the lamp and the flow cell (3). Procedures for replacing or servicing the lamp and flow cell are summarized below. Both units are self-aligning and do not require any user adjustment upon replacement.

- UV Lamp: Turn off the detector and unplug the power cord as a safety precaution. Let the lamp cool for 5 min. Disconnect the high-voltage power connector to the old lamp and loosen to remove the securing screws. Replace with the new lamp and reattach the high-voltage connector. Reset the lamp usage hour setting for the detector. Gloves should be worn for this operation to avoid soiling or touching the lamp surfaces with bare fingers. An aged lamp with weakened light emission is the primary root cause for higher than expected baseline noise.
- Flow cell: Disconnect the inlet and outlet solvent tubes, and then loosen to remove the securing screws to the flow cell. Remove the flow cell assembly from the instrument and inspect the flow cell windows for dirt, particles, contaminants, or window cracks by viewing it against a bright light source. Most flow cells can be disassembled for cleaning or window replacement.
- Alternately, the damaged or contaminated flow cell may be returned to the manufacturer for repair or reconditioning. Some of the optical components (windows, lenses, and mirrors) inside the detector might require cleaning or replacement after several years of use. Indicators for the need to service these optical items are given by low source energy or low sensitivity performance even after a new lamp has been installed; other indicators are baseline wandering and higher drift. Occasionally, the monochromator might need adjustment to restore wavelength accuracy. These procedures are best performed by a factory-trained specialist.

Other common symptoms relating to UV detector issues are:

- Signal spiking caused by bubbles from outgassing of dissolved gases or mobile phases (remedied by adding a back-pressure device of 50 to 300 psi, or 4 to 20 bar).

- Erratic and cyclical stepping baseline perturbations caused by a trapped air bubble in the flow cell (remedied by purging the flow cell to dislodge the air bubble throughout the column with acetonitrile at high flow rates).

- Poor detector baseline stability caused by an aging lamp, or contaminated or leaking flow cell.

- High gradient baseline shifts caused by an imbalance of absorbance of the weak and strong mobile phases (remedied by using balanced absorbance mobile phases (24).

Summary and Conclusions

This installment provides an overview of the design and operating principles of modern UV detectors to the analyst. Technical details and specifications of several types of UV detectors are described, followed by a brief discussion of common procedure on their operation, maintenance, and troubleshooting.

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ABOUT THE AUTHOR

Jedrzej Wysocki is a Product Manager at Agilent Technologies, in Waldbronn, Germany.

ABOUT THE AUTHOR

Michael W. Dong is a principal of MWD Consulting, which provides training and consulting services in HPLC and UHPLC, method improvements, pharmaceutical analysis, and drug quality. He was formerly a Senior Scientist at Genentech, a Research Fellow at Purdue Pharma, and a Senior Staff Scientist at Applied Biosystems/PerkinElmer. He holds a PhD in Analytical Chemistry from City University of New York. He has more than 100 publications and a best-selling book in chromatography. He is an editorial advisory board member of *LCGC North America* and the Chinese American Chromatography Association. Direct correspondence to: LCGCedit@ubm.com