The UV Detector for HPLC —
An Ongoing Success Story

Bruno E. Lendi\textsuperscript{a} and Veronika R. Meyer\textsuperscript{b}
\textsuperscript{a}OmniLab Ltd, Mettmenstetten, Switzerland,
\textsuperscript{b}EMPA St. Gallen, Swiss Federal Laboratories for Materials Testing and Research, St. Gallen, Switzerland.

UV detection is the most common detection principle for HPLC, and as such users pay little attention to its physical and technical background. This article presents the contemporary features of a UV detector and mentions its improvements during the last 30 years. Recommendations concerning some technical details that may be relevant to detector purchase are also given.

UV detection is the most usual and widespread detection principle in high performance liquid chromatography (HPLC). The instrument is simple and is available from numerous manufacturers; many (or even most) analytes suitable for HPLC absorb in the UV, whereas many solvents, including the ones useful for reversed-phase separations, are transparent in the UV. If necessary, the wavelength range can be easily expanded to the region of visible light (vis) for the detection of coloured analytes.

UV/vis detection is more rugged than many other detection systems. This is why it is so popular and the technique has come a long way since the days when chemists had to build their own instruments, which was common practice in 1958.\textsuperscript{1} All textbooks on HPLC include a section about the various detection principles, including UV/vis instruments, but they often do not cover areas such as stray light or the properties of the various light sources. One book that does is \textit{Analytical Instrumentation} by Currell, which discusses many aspects of theory, physical background and practical realization.\textsuperscript{2}

This article describes the UV detector from an engineer’s perspective, including tips on proper care and maintenance, and some points to consider when buying a new instrument.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{unnamed.png}
\caption{The principle of photometry or UV/vis detection. \(I_0\): incident light intensity, \(I\): emergent light intensity.}
\end{figure}
Lambert–Beer’s law: UV/vis spectrometry is based on the comparison of incident light intensity \( I_0 \) and emergent intensity \( I \) after passing through the solution of interest (Figure 1). The ratio \( I/I_0 \) is the so-called transmittance (T). A logarithmic transformation is needed to obtain the absorbance (A = \( \log I/I_0 \)) because A is directly proportional to \( c \), the concentration of the absorbing analyte in the solution, if the path length d of the measuring cell is held constant.

This relationship is expressed by the Lambert–Beer law, a combination of two laws. Lambert’s law states that \( \log I/I_0 \) is proportional to \( d \), and Beer’s law says that \( \log I_0/I \) is proportional to \( c \). In combination we obtain:

\[
A = \epsilon c d \tag{1}
\]

with \( \epsilon \) is molar absorptivity (L/mol cm) if \( c \) is in mol/L and \( d \) in cm. The higher is A, the less light is passing through the cell. If \( I \) is 10% of \( I_0 \), then \( A \) is 1.0; if only 1% of \( I_0 \) is detected after the cell then \( A \) is 2.0.

Lambert–Beer’s law makes UV/vis spectrometry more complicated than it would be with multiple comparisons of \( I_0 \) and \( I \). The necessity of the log transformation was a challenge for the electronics thirty years ago.

The favourable range of absorbance: The repeated measurement of transmittance has a certain degree of standard deviation \( \sigma_T \) (as any other measurement). This is linked to the relative standard deviation of a concentration determination, \( s_c/c \), as follows:

\[
s_c/c = 0.434 \sigma_T / T \text{log} I \tag{2}
\]

Irrespective of the value of \( \sigma_T \), the minimum of this function is at 0.36 T, which is equivalent to 0.44 A (Figure 2). In photometry, this is the absorbance that should be preferred. It can often be matched by appropriate sample preparation. The value \( s_c/c \) is low between 0.2 and 1 A. In HPLC the situation is more complicated because a peak stretches a certain range of absorbance, namely from a theoretical A = 0 on the baseline up to its maximum. But it is no question that a moderate range of absorbance, namely from a theoretical A = 0 on the baseline up to 1 A, would be best.

Bandwidth: Lambert–Beer’s law is only valid for monochromatic light because the molar absorptivity \( \epsilon \) depends on the wavelength (remember the shape of a typical UV spectrum). If two wavelengths, \( \lambda_1 \) and \( \lambda_2 \), are involved, then also two absorptivities, \( \epsilon_1 \) and \( \epsilon_2 \), need to be considered as well as the respective light intensities, \( I_{1,0} \) and \( I_{2,0} \). This leads to:

\[
A_{\text{observed}} = \log (I_{1,0} + I_{2,0}) - \log (I_{1,0}10^{-\epsilon_1 dc} + I_{2,0}10^{-\epsilon_2 dc}) \tag{3}
\]

(simplifying to Equation 1 if \( \epsilon_1 = \epsilon_2 \)). Thus, polychromatic light decreases the linearity of quantitative analysis and should be avoided. Obviously the effect is less pronounced if \( \epsilon_1 \) and \( \epsilon_2 \) are similar. For HPLC detection a compromise is used and bandwidths are typically 10 nm because too small a bandwidth results in low light intensity \( I_0 \).

UV/vis detection is more rugged than many other detection systems. This is why it is so popular and the technique has come a long way since the days when chemists had to build their own instruments.
was the prism. It is rather cheap, it covers the whole wavelength range from UV to near IR and is not a source of stray light. Its main drawback is the non-linear dispersion resulting in much poorer wavelength resolution in the visible than in the low UV visible range. Therefore, the mechanical construction of a photometer was complicated. Moreover, the dispersion depends on temperature.

Prisms have been replaced by gratings. Today it is possible to manufacture high-quality gratings at a moderate price. Their main disadvantage is the fact that not only the desired wavelength \( \lambda \) is reflected in a well-defined direction (where it is allowed to pass through a slit) but also the higher orders \( \lambda/2, \lambda/3 \) and so on, (i.e., stray light). This is not a problem with deuterium lamps because the higher order wavelengths are absorbed by oxygen and all quartz parts in the instruments. For a deuterium lamp is used at 340 nm, \( \lambda/2 \) is 170 nm, which is below the range that can be used for detection. The situation is different in the visible region. If a tungsten-halogen lamp is used it does not emit below 340 nm, as described above for deuterium. Therefore, if detection is performed at 600 nm, the wavelength \( \lambda/2 = 300 \ nm \) does not exist anywhere in the detector. If, however, a deuterium lamp is used near its high end of radiation, the 300 nm emission is prominent even if the grating is constructed in such a way (by "blazed", asymmetric grooves) that the higher orders are as weak as possible. In these instances, the 300 nm emission must be removed by appropriate filters. Another drawback of a prism is that it reflects high energy light only within a limited range of wavelengths from 2/3 to 7/4 of the blazing wavelength (e.g., 200 nm to 525 nm if constructed for 300 nm).

**Detector cell:** The cell should be as long as possible because the absorbance is proportional to the optical path length. (An exception are cells for preparative chromatography with short length.) But its volume should be small, preferably one half of the maximum allowed injection volume, which itself depends on the elution volume of the peak of interest and the permitted degree of band broadening. In practice, the cell construction represents a compromise with typical dimensions of 10 mm length and 8 \( \mu L \) volume in a Z-shape design, although 8 \( \mu L \) may be too large for an early eluted peak.

*Figure 2:* The relative standard deviation (RSD) of concentration determination as a function of absorbance. The y axis scale depends on the standard deviation of the transmittance measurement \( s_t \) but the shape of the curve does not. Data given in the plot are for \( s_t = 0.002 \) (0.2 %).

*Figure 3:* Contemporary design of a UV detector (simplified). If a vis lamp is also built in, a selector mirror is necessary.
was perhaps even filled with mobile phase. A design from the early 1970s is shown in Figure 6.

**The Usable Wavelength Range**

The lower wavelength range of a UV detector is limited by the UV cut-off of the mobile phase, usually defined as the wavelength where the absorbance is 1.0 in a cell of 10 mm length, with air as the reference. A cut-off below 190 nm is only possible with water or acetonitrile of highest purity. It is difficult to work below 200 nm and ghost peaks may appear in the chromatogram because the eluate has a certain absorbance even under the best possible circumstances. The oxygen present in air also absorbs below 197 nm (or 195 nm depending on the tolerated absorbance). A possible loop is flushing the detector with nitrogen. The ultimate limit is given by the absorbance of the quartz lens and windows, again depending on quartz quality and tolerated absorbance. 

The reasons for the upper wavelength limits have been mentioned above. The upper UV range is set by the energy of the deuterium lamp which drops markedly at 360 nm. The lower vis range is set by the tungsten–halogen lamp, but by its energy, by its UV cut-off filter. The upper vis range comes in principle from the photodiode, but in reality from the fact that detection in the near UV is not sharp thanks to the blaze of the possible analytes. A certain limitation comes also from the blazing wavelength of the grating. Detectors without this possibility are usually limited to 880 nm.

**What do you Need to do?**

The UV or UV/vis detector is a simple device and the user should not have any trouble with it. The lamp must be replaced after the interval recommended by the manufacturer; in many instruments, the lamp hours are counted and a replacement demand appears on the display when the time has come. If detection is performed in the low UV region an earlier lamp replacement may be necessary. The replacement is usually easy and no adjustment is needed; however, it is good advice to check this before buying an instrument.

Cell windows can become dirty, especially when analytes with poor solubility in the mobile phase are injected at high concentration. Even under normal working conditions their transparency will decrease with time. Cleaning should be done by pumping through pure solvents (water and/or organic solvents depending on the suspected kind of contamination) after the column has been removed. Rinsing with semi-concentrated nitric acid is a last resort. It can be very effective but afterwards plenty of water (or hours of pumping) is needed to remove the last traces of this UV-absorbing compound.

Irrespective of any contamination problems, it is recommended to remove the cell windows every two years or so in order to clean or to replace them. Again, it is best to look for an instrument with easy handling and no need for cell adjustment when it is built in again.

It should be well-known that buffer solutions must not remain in the HPLC system, including the detector, when the instrument is not in use. Otherwise the salts could crystallize, leading to clogging, high pressure and destruction of the cell.

A detector test should be performed at regular intervals.14

**Frequently Asked Questions**

Below are a collection of possible scenarios to take into account before buying a new detector.

**Only UV-active analytes:** In this instance, a tungsten–halogen lamp, its power supply and the detector mirror are not needed and the detector is cheaper. Note that it can be impossible to install the parts for vis detection later.

**Only vis-active analytes:** Most (or all) detectors are equipped with a deuterium lamp. This lamp is sometimes also recommended for vis detection. But as discussed above, this approach is inadvisable because of the low energy of the deuterium lamp in the visible region and the possible stray light problem. Therefore it is necessary to buy a detector with both a deuterium and a tungsten–halogen lamp.

**Detection in the low UV (< 220 nm):** You need an excellent detector, high-quality solvents and reagents and the best possible working techniques and cleanliness. Note also the remarks given above under “usable wavelength range.”

**Trace analysis:** Only the best instrument is good enough. What is needed is electronics with low noise, a data processing unit with modern algorithms that can suppress noise without eliminating the peaks, and a small-volume cell with as high a length as possible. Note that such a cell may be the cause of a distinct baseline drift in gradient elution. If the sample amount is limited, it is necessary to work with capillary HPLC and the appropriate micro detectors, maybe even with on-column detection.15

**No trace analysis:** Look for a simple but easy-to-handle detector. Lamp and cell exchange should be possible without higher-than-average manual skills and special instruments.

**Capillary HPLC:** Depending on the capillary diameter, it may be possible to use a common detector, equipped with a low-volume flow-cell. The considerations of Martin et al.8 allow the maximum permitted cell volume to be calculated. In most instances it will become obvious that a special detector constructed for capillary chromatography is necessary, furnished with a carefully designed capillary flow cell. The smallest volume available is approximately 3 nL.

**Occasional UV spectra:** A diode-array detector is not needed but many UV/vis detectors offer the possibility to register spectra.
without a need to stop the flow. Note that the bandwidth of a
detector is larger than the one of a spectrophotometer, so the
spectral resolution is poorer (in many instances this is a minor
drawback because the spectra of analytes in solution are broad).
In addition, a peak cannot be used for quantitative analysis
when its spectrum is registered.

Gradient separations: A flow-cell with low refractive index
dependency is needed. This is easier to realize the shorter the
cell is, which means that it is less suited for trace analysis. It
must be of tapered shape.

Only isocratic separations: Even in this instance it is
advantageous to use a cell with low refractive index dependency
in order to keep the possible “injection peaks” small (signals at
t0 which do not represent an analyte).

Indirect UV detection: When working in this mode, the mobile
phase has a distinct absorptivity and the peaks are negative
(lower absorptivity). The detector and its data processing unit
must be able to handle positive and negative signals. An analysis
system with high energy and a large dynamic measurement range are necessary.

Preparative separations: This is an approach with high analyte
concentrations and high flow-rates. The flow-cell must have a
short path length of 0.1 to 1 mm, preferably adjustable by the
use of appropriate exchangeable spacers. Negative signals may
occur, so the detector should be able to process them.

High pressure after the detector: A pressure-resistant flow-cell
is a must. Many (but not all) instruments come with cells which
tolerate up to 50 bar. Cells designed for supercritical fluid
chromatography have a pressure limit of 300 bar or more but
they are expensive because their design and manufacture is
much more demanding.

No money: There are many detectors on the market with
similar specifications and price. Therefore, it is more important
to check the prices of the accessories and the consumables.
Your own work is perhaps cheaper than the one of a service
engineer, therefore it is best to buy an instrument which allows
an easy exchange of the lamp, cell and/or cell windows. If a
detector is to be purchased that must fit into existing
instrumentation, its compatibility (mainly of the signal ports) is
of utmost importance.

Old detector, is there a need for a new one? It is possible that
the technical support for an old instrument is no longer
guaranteed and that the consumables are out of stock. The
optical system of the detector can be contaminated, resulting in
lower energy and increased noise. In addition, a new
instrument has a real added value thanks to its improved
specifications.

Working under EN 17025 or GLP: It is up to the instrument
manufacturers to guarantee the performance and reliability of a
detector (and of all other parts of the HPLC system including
the data processing). You as a user need to act your part in
understanding both the analytical method and the instrument,
therefore in ensuring proper use, maintenance and
calibration. The instrument's logbooks must be updated after every service
operation and they must be stored in the laboratory, not in
your office.

Conclusions
The dependability of UV/vis detectors for HPLC has
improved greatly during the last ten to twenty years, although
the users do not really pay attention to this ongoing process.
Every part of the instrument has better performance, smaller
size (e.g., the monochromator) and/or higher reliability. This
results in less noise (thanks to better lamps, improved flow-cell
design, better photodiodes), thus lower detection limits; better
linearity (thanks to improved cell design and less stray light);
and higher accuracy (thanks to the digital lin-log signal
conversion and generally better electronics).
Acknowledgments
We want to thank Mr Urs Kämpfer of the Department of Chemistry and Biochemistry, University of Bern; Mr Oliver Gützow of Herbert Knauer Berlin; and Dr Wolf-Dieter Beinert of VWR International, Darmstadt, Germany. They all provided us with technical information on old detectors after investigating archives and old files.

References

Bruno E. Lendi was a product and business manager for analytical instruments. Today he runs OmniLab Ltd in Switzerland, supporting chromatography, spectroscopy and labware. Veronika R. Meyer, PhD, is a specialist in HPLC and measurement uncertainty. She works as a research scientist at EMPA St. Gallen, Switzerland, and teaches at the University of Bern.