The UV Detector for HPLC — An Ongoing Success Story

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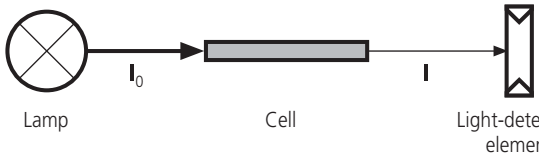
UV detection is the most common detection principle for HPLC, and as such users pay little attention to its physical and technical background. This article presents the contemporary features of a UV detector and mentions its improvements during the last 30 years. Recommendations concerning some technical 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.[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 *Analytical Instrumentation* by Currell, which discusses many aspects of theory, physical background and practical realization.[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.

Figure 1: The principle of photometry or UV/vis detection. I_0: incident light intensity, I: emergent light intensity.



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The Basics

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 ($A = \log 1/T = \log I_0/I$) 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:

$$A = \epsilon \cdot c \cdot d \qquad [1]$$

with ϵ 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 a simple comparison of I_0 and I. The necessary lin–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 s_T (as any other measurement). This is linked to the relative standard deviation of a concentration determination, s_c/c, as follows:[3]

$$\frac{s_c}{c} = \frac{0.434 \cdot s_T}{T \cdot \log T} \qquad [2]$$

Irrespective of the value of s_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, for example from 0 to 1, would be best.

Bandwidth: Lambert–Beer's law is only valid for monochromatic light because the molar absorptivity ϵ depends on the wavelength (remember the shape of a typical UV spectrum). If two wavelengths, λ_1 and λ_2, are involved, then also two absorptivities, ϵ_1 and ϵ_2 need to be considered as well as the respective light intensities, $I_{1,0}$ and $I_{2,0}$. This leads to:[4]

$$A_{observed} = \log (I_{1,0} + I_{2,0}) - \log (I_{1,0} \cdot 10^{-\epsilon 1 dc} + I_{2,0} \cdot 10^{-\epsilon 2 dc}) \qquad [3]$$

(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 ϵ_1 and ϵ_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.

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Stray light: Stray light is (a) light of incorrect (unwanted) wavelength, especially if not absorbed by the sample, and (b) light which does not pass through the sample but reaches the photodiode. The latter can be a most serious error of measurement because it decreases the apparent concentration in the sample cell: If, for example, the stray light is 0.1% of I_0 and the true absorbance is 1 (10% transmission), the light on the photodiode corresponds to 0.101 T which results in A = 0.9957 and c = 99.6% of the true value. Similarly, if the true absorbance is 2 (1% transmission) one finds c = 98% of the true value.

The reasons for stray light are defects in the diffraction grating (such as scratches), higher-order radiation reflected by the grating (see below) and fluorescence light emitted in the cell, besides such trivial faults as a detector housing, that is not optically sealed and closed.

The Parts of a UV Detector

The general design of a UV detector is shown in simplified form in Figure 3. The parts of most interest are the lamp, the monochromator, the cell, the light-detecting element and the electronics behind it.

Lamps: Instruments with fixed-wavelength lamps have almost completely disappeared from the market. Most were equipped with a mercury vapour lamp emitting at 254 nm (probably also at some other wavelengths, depending on the design of the lamp). Zinc (214 nm) and cadmium lamps (229 nm) added favourable extension to the wavelength range as did the phosphor screens used in some instruments. Nevertheless, the choice of wavelengths was very restricted even considering the fact that the UV spectra of most analytes in solution show broad maxima instead of sharp peaks.

Deuterium lamps have a continuous spectrum from less than 190 nm up to approximately 360 nm.[5] Their radiation even stretches up to 600 nm but the energy is low and discontinuous in the visible range (Figure 4). There are some instruments on the market that also use the deuterium lamp for detection in the visible light but this approach cannot be recommended for high demands. A deuterium lamp has a lifetime of 2000 to 5000 hours and a price of ∈ 500 to 800.

The completion in the vis range, if needed, is made by tungsten-halogen lamps. They emit continuously from around 340 nm up to the near infrared region (around 2000 nm). The lower limit comes from the UV blocking filter usually built in to such lamps because they are also used in home lighting. Tungsten-halogen lamps have a lifetime of 800 to 2000 hours and a price of ∈ 20 to 80.

Monochromators: The common monochromator of earlier days

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.[6] 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 dispersion is linear and independent of temperature, and wavelength resolution is higher than with prisms. Their main disadvantage is the fact that not only the desired wavelength λ is reflected in a well-defined direction (where it is allowed to pass through a slit) but also the higher orders λ/2, λ/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. If, for example, a deuterium lamp is used at 340 nm, λ/2 is 170 nm, well 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. Therefore if detection is performed at 600 nm, the wavelength λ/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)[7]

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 %).

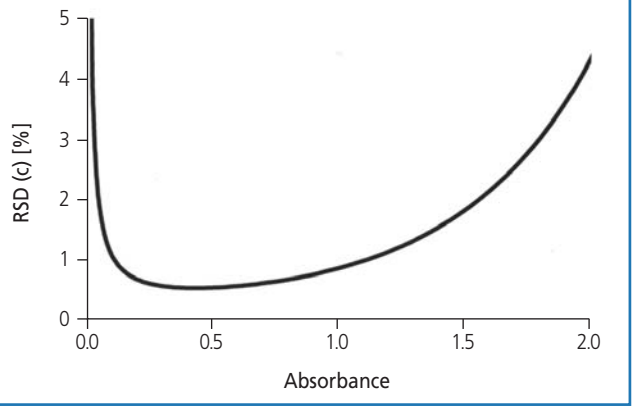
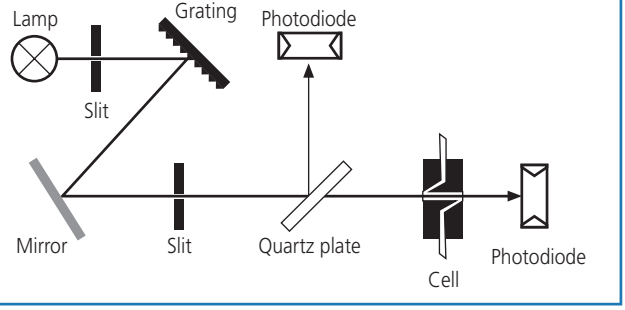


Figure 3: Contemporary design of a UV detector (simplified). If a vis lamp is also built in, a selector mirror is necessary.



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.[8] In practice, the cell construction represents a compromise with typical dimensions of 10 mm length and 8 µL volume in a Z-shape design, although 8 µL may be too large for an early eluted peak.[9]

Other requirements are poor carryover (or good flow characteristics), no bubble trapping effect (therefore the eluate must flow from bottom to top), a pressure resistance of at least 10 bar, and low refractive index dependency. The latter is more or less fulfilled if the cell is not a straight cylinder but is "tapered" (Figure 5).[10] In a straight cell some light may get lost because of the refraction of light rays towards the cell walls; the refraction occurs because the surrounding air, the quartz windows of the cell, the mobile phase and the analytes all have different refractive indices. In addition, the refractive index of the mobile phase depends on its pressure and temperature, which means that pulsations of the pump can result in fluctuations of the baseline and a change in temperature can be seen as drift. Despite a tapered design, the refractive index dependency of the detector increases with increasing cell length.

Light-detecting element: Photocells and photomultipliers were rather large component parts, namely vacuum tubes under high voltage. They are replaced now by photodiodes (semi-conductors with light-depending resistance) because they are small and cheap. Their other favourable properties are high quantum yield, fast response, good linearity, low noise and drift, a well-matching wavelength range from about 190 nm to 1100 nm and mechanical robustness. Two photodiodes are necessary, one for the light coming from the cell and the other responding to a small fraction of the lamp light, thus compensating for possible fluctuations of its radiation (Figure 3). A diode array detector has a multitude of photodiodes (up to 1000), all of them positioned side by side on a single chip.

Logarithmic conversion: Because of Lambert–Beer's law, the linear signal from the comparison of the photodiodes must be transformed into a logarithmic one. This is done the same way as in a pocket calculator, (i.e., by binary calculation in a microprocessor). Decades ago, the transformation was performed with electronic logarithmic operational amplifiers.[11] They were prone to ageing and low stability, therefore it was necessary to calibrate them from time to time. The usable span of such amplifiers is limited to two decades, resulting in the fact that the absorbance range above 2 A was problematic. In addition, their accuracy was poor.

Over all, today's UV detectors are simpler and more rugged than their ancestors. The noise of the photometric system could be decreased by two orders of magnitude (typical values are 2×10^{-4} and 3×10^{-6} absorption units, respectively). The old instruments were equipped with a reference cell which

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 loophole involves 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, coming into play at 180 nm or so.

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 not 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 IR is not needed thanks to the spectra of the possible analytes. A certain limitation comes also from the blazing wavelength of the grating. Detectors with vis possibility are usually limited to 850 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.[12,13] 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.

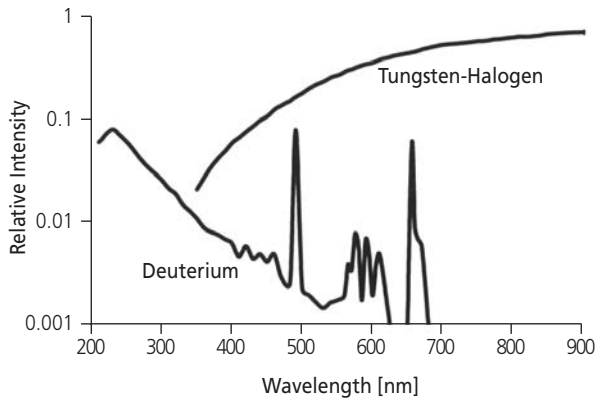
Irrespective of any contamination problems, it is recommended to remove the cell windows every two years or so in order to clean or to replace them. Again, it is best to look for an instrument with easy handling and no need for cell adjustment when it is built in again.

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

Figure 4: Spectra of deuterium and tungsten–halogen lamps (simplified). The relative position of the spectrum lines depends on the lamp power (wattage), (i.e., the form of the spectra is more important than its position in relation to the y axis). Note that the y axis has a logarithmic scale.



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 selector 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 t_0 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 optical 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.

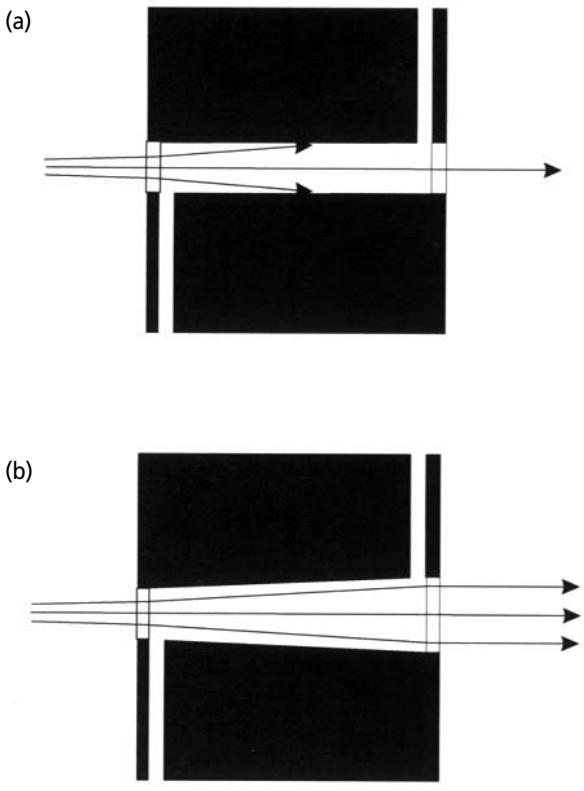
High pressure after the detector: A pressure-resistant flow-cell is a must. Many (but not all) instruments come with cells which tolerate up to 50 bar. Cells designed for supercritical fluid chromatography have a pressure limit of 300 bar or more but they are expensive because their design and manufacture 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, thereby ensuring proper use and maintenance. The instrument's logbook(s) must be updated after every service operation and they must be stored in the laboratory, not in your office.

Figure 5: (a) Straight detector cell with the risk of light intensity loss. (b) Tapered cell, guiding most of the incident light to the photodiode.

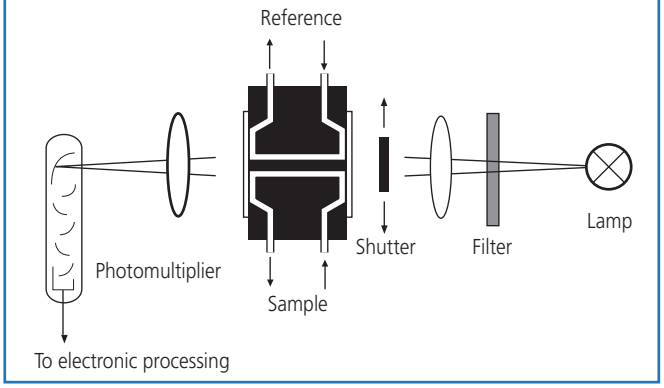


Conclusions

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

Figure 6: Design of a Chromatronix detector with mercury lamp in the early 1970s. Noise was declared as 2×10^{-4} absorption units. Electronic processing means: preamplifier, demultiplexer, lin–log converter, amplifier and output attenuator. Re-drawn after a brochure of Chromatronix Inc., Berkeley, USA (without date).



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