

CASE STUDY:

## Marion Research

**Marion Research manufactures detectors for liquid chromatography (LC) and high-performance liquid chromatography (HPLC). The company specializes in affordable, high quality chromatography instruments to suit the needs of bench researchers in both academia and industry.**

### CHALLENGE

High pressure liquid chromatography (HPLC) has many uses including protein purification, routine process monitoring in pharmaceutical and beverage manufacturing, quality control, and biotech research. In HPLC, different compounds of the mixture pass through a column at varying rates due to differences in their partitioning behavior between the mobile phase and the stationary phase. Quantification of these components is done by UV-Vis spectroscopy, mass spectrometry or refractive index measurements. UV-Vis spectroscopy is the commonly preferred method of detection since many analytes absorb in the UV spectrum while solvents remain transparent at these wavelengths. Nearly 80 percent of all known chemicals and materials can be identified with HPLC.

Conventional bench top HPLC systems use deuterium lamps as their primary light source because of the high stability of light output through the duration of a measurement as well as the relatively high light output of the lamps at HPLC-relevant UV wavelengths. These units can measure a variety of parameters across all UV wavelengths. However, deuterium lamps require relatively significant electrical power (20-30 W), need expensive power supplies, and housing to store the lamp, adding significantly to the total system cost.

Several chromatography applications such as purification of proteins and food and beverage manufacturing require measurement at only one or two fixed wavelengths. Traditional HPLC systems offer more analysis than is needed for these applications. Marion Research turned to UVC LEDs because of their monochromaticity and small footprint to offer a tailored solution for these customers.

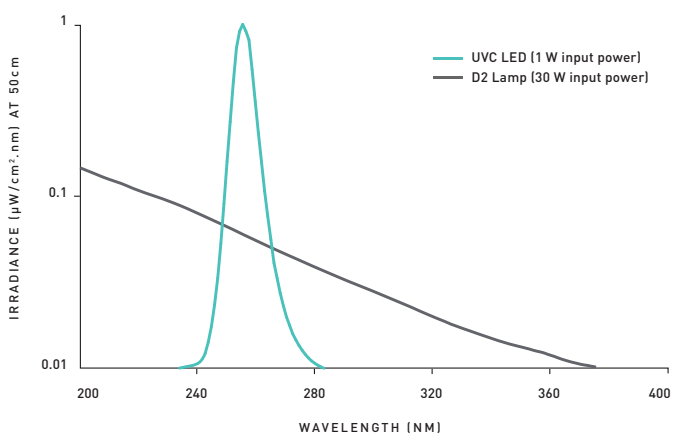
“USING A UVC LED ALLOWS US TO MAKE A MUCH SMALLER AND THEREFORE, PORTABLE FIXED WAVELENGTH HPLC DETECTOR”, SAID MIKE FARNET, PRESIDENT, MARION RESEARCH. “AT THE SAME TIME, THE HIGH STABILITY OF LIGHT OUTPUT OF CRYSTAL IS DEEP UV LEDs ENABLES US TO ACHIEVE LOW SYSTEM NOISE LEVELS, AND MONITOR LOWER COMPOUND CONCENTRATIONS”.



## SOLUTION

Optan UVC LEDs from Crystal IS helped Marion Research overcome the problems posed by deuterium lamps and develop a compact, less costly detector for fixed wavelength applications without sacrificing the quality of analysis. Because of the monochromaticity of LEDs, instrument designers can select the wavelength to match the absorption spectrum of the compound of interest. Compared to the broader spectrum of deuterium lamps, Optan LEDs offer more irradiance at a specific wavelength in the deep UV range (Figure 1).

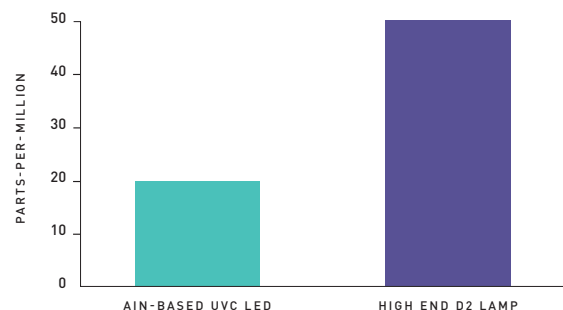
FIGURE 1



Irradiance comparison of a UVC LED with a peak at 255 nm versus a typical deuterium lamp.

Optan LEDs also exceed the stability of high-end deuterium lamps with a peak-to-peak fluctuation lower than 50 ppm (0.005%) (Figure 2). The superior stability (lower fluctuation) of UVC LEDs leads to lower detection limits in HPLC.

FIGURE 2: PEAK-TO-PEAK LIGHT OUTPUT VARIATION



Comparison of light output fluctuation between an AIN-based UVC LED and a high-end deuterium lamp. Peak-to-peak fluctuation within each 30-second interval is measured and averaged over a 15-minute time frame.



THE MARION RESEARCH SYSTEM  
EXCEEDS THE PERFORMANCE  
OF TRADITIONAL FIXED WAVELENGTH  
DETECTORS AT LESS THAN HALF  
THE COST, AND LESS THAN ONE-TENTH  
THE SIZE, WEIGHT, AND POWER  
CONSUMPTION.

UVC LEDs also allow designers to simplify the instrument optical design with fewer components and less bulky power supplies. Leveraging the small footprint of Optan LEDs, Marion Research was able to develop the world's most compact (44 mm diameter x 44 mm long), low cost detector.

Dramatically reducing the footprint allows for chromatography systems to be used both in the lab and in the field. Portable systems also enable more widespread use in environmental analysis and point-of-care diagnostics since sample decomposition during transport can be avoided while reducing time and cost of analysis.

The Marion Research system is based on shining UV light from a Crystal IS 255 nm Optan LED towards the detection window at the end of an HPLC flow cell. A photodiode placed on the other side of the detection window monitors the UV light passing through the sample or the signal. A set of photodiodes directly monitors off-axis light from the LED and acts as the reference. The log of the ratio of the signal and reference intensities is used to determine the UV absorbance of the analytes, and, using Beer-Lambert law, their concentration. To eliminate noise from the temperature sensitivity of photodiodes, the Marion Research detector modulates the light with a synchronous multiplexer. This allows the detector to measure differentially between the dark (zero) and light states. Systems using a UV lamp require the addition of a physical shutter for modulation, which is more expensive. LEDs, on the other hand, turn-on instantaneously and their output can be modulated by accurately modulating the current.

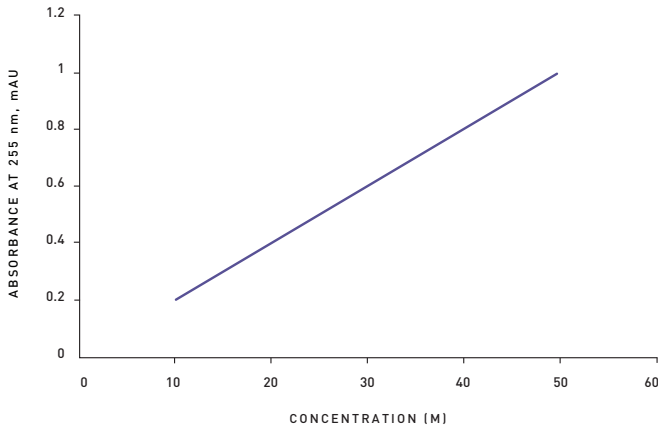
### Noise, linearity and separation performance

The baseline noise of the detector was evaluated by acquiring the signal with a data acquisition system and determining the peak-to-peak fluctuations over a period of 60 seconds. The noise from the UVC LED was lower than the noise value with a deuterium lamp in similar systems (see Table below). This is due to the improved stability of light output from the UVC LED, as shown in Figure 2. Note that the drift of the LED-based detector is also lower compared to the deuterium lamp system. This is due to the relatively lower sensitivity of light output with temperature for the Optan LED and the use of the synchronous multiplexer.

Detector System	Noise	Drift
Optan LED	8 $\mu$ AU	67.7 $\mu$ AU/hr
Deuterium Lamp	13.5 $\mu$ AU	111.3 $\mu$ AU/hr

Next, the linearity of the detector was examined by filling the flow cell with different concentrations of a standard solution. The data shows a linear response over a large range of concentrations (Figure 3). The good linearity indicates conformance to Beer-Lambert law as well as negligible levels of stray light and dark current on the photodiodes for the relevant absorbance range.

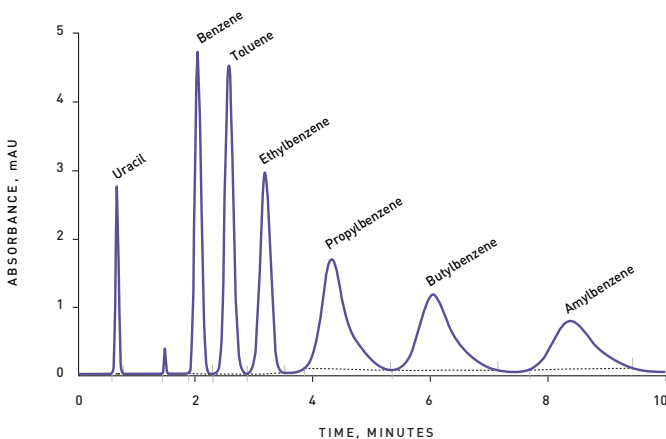
FIGURE 3



Plot of absorbance versus concentration for standard measured with Optan LED emitting at 255 nm

The detector was then coupled to an HPLC setup and the separation performance was evaluated using standard solutions with detection at 255 nm (Figure 4). The results show that at the optimum detection wavelength the system is able to provide an acceptably high sensitivity. In addition, there is no noticeable drift and the baseline noise is relatively low.

FIGURE 4



Chromatogram with detection at 255 nm

The Marion Research system exceeds the performance of traditional fixed wavelength detectors at less than half the cost, and less than one-tenth the size, weight, and power consumption.



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### Crystal IS **ADVANTAGE**

LEDs offer instantaneous response and generate less heat compared to traditional light sources. In addition, Crystal IS deep UV LEDs provide:

- >Simplification in the optical train and lower system cost
- >High spectral quality for measurement linearity over a wide concentration range
- >Long lifetimes and replacement cycles for low cost of ownership for end users