Get Insight into the SepaBean™ Machine with Engineer:

Diode Array Detector

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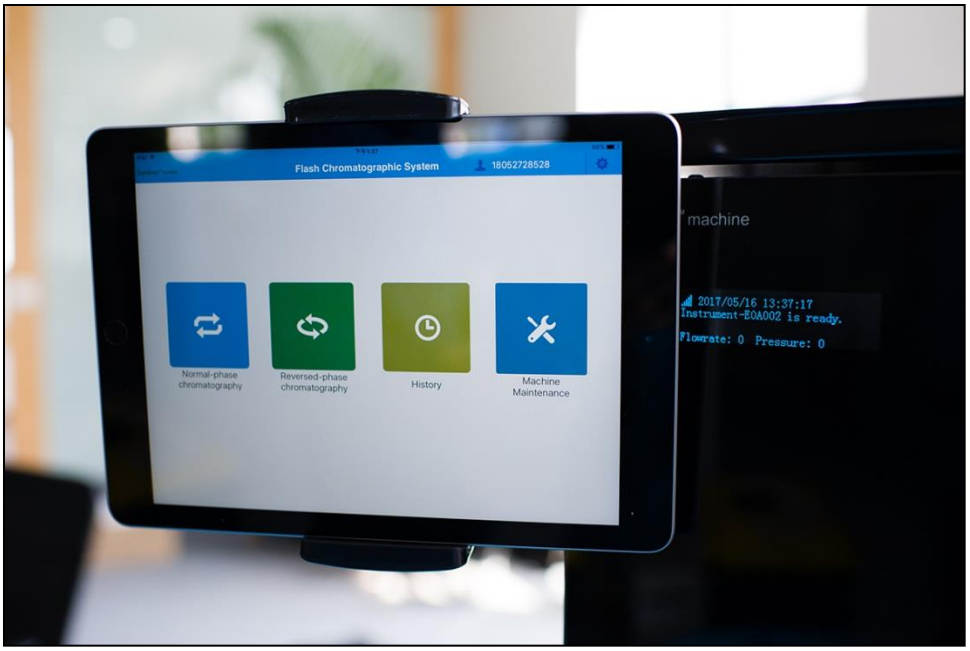

A typical flash preparative liquid chromatography system is usually consisting of system pump, injector, flash column, detector and controlling system. Among these components, the detector module is equivalent to the human eyes and is essential for the separation performance of the entire system. In this post, we will introduce the detector module used in the SepaBean™ machine, a flash preparative chromatography system from Santai Technologies.

In liquid chromatography system, the commonly used detectors include ultraviolet/visible detector (UV-Vis), diode array detector (DAD), fluorescence detector (FD), refractive index detector (RID), evaporative light scattering detector (ELSD), mass spectrometer (MS), and etc. The DAD was employed in the SepaBean™ machine from Santai Technologies. Compared with the traditional UV detector, the most important feature of DAD is that it can simultaneously detect the absorption of the sample in all wavelengths, thus obtaining more comprehensive spectral information. This unique feature can help

the user to discriminate the sample purity as well as to determine the proper detection wavelength.

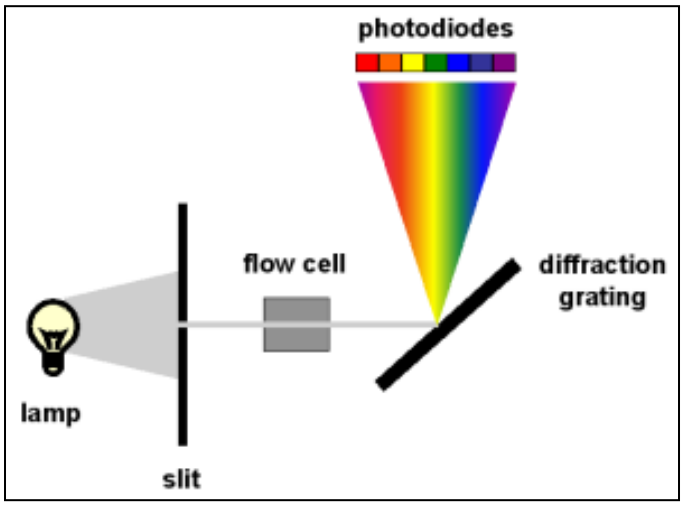

Figure 1. The detection principle of the diode array detector.

As shown in Figure 1, the continuous light from the light source pass through the slit and the flow cell, then dispersed into monochromatic lights by the holographic grating. Afterwards, the monochromatic lights are irradiated onto the photodiodes for detection by photoelectric conversion. Due to the different fractions eluted from the chromatography column which have their unique absorption of the incident light, the intensity of the transmitted light irradiated onto the photodiodes will be different in the full spectrum, therefore we could see the peaks with various sizes in the chromatogram.

So, what are the advantages of the DAD being applied in the purification of the samples? Several advantages are summarized as follows.

- Provides comprehensive spectral information of the compound – just like having an ultraviolet/visible spectrophotometer.

  If the user has sufficient understanding of the sample being purified, it is possible to determine whether the current eluting fraction is the target product based on the structural information of the target molecule.

- Provides the best detection wavelength – lowering the sample loss.

  When the purified products are collected by the automatic collector, the system will collect the eluted fractions by user-defined cut-off value of the absorption. In case of low absorption due to the sample's unique absorption spectrum or low amounts of the sample injected, we should select the maximum absorption wavelength of the sample for better detection sensitivity. In another case, the sample is overloaded

and thus produces a very strong response in the maximum absorption wavelength, we should select a smaller absorption wavelength for detection to avoid signal saturation.

- Provides the basis for sample purity determination – making the experimental results more accurate and reliable.

When peak overlapping occurs due to the similar retention behaviors of the eluting fractions in the sample, we could determine whether the eluted fraction is a single one or a mixed one according to the full wavelength spectrum scanning result.

In the following part, we will further introduce the full wavelength spectrum scanning of the DAD by an example of specific sample purification.

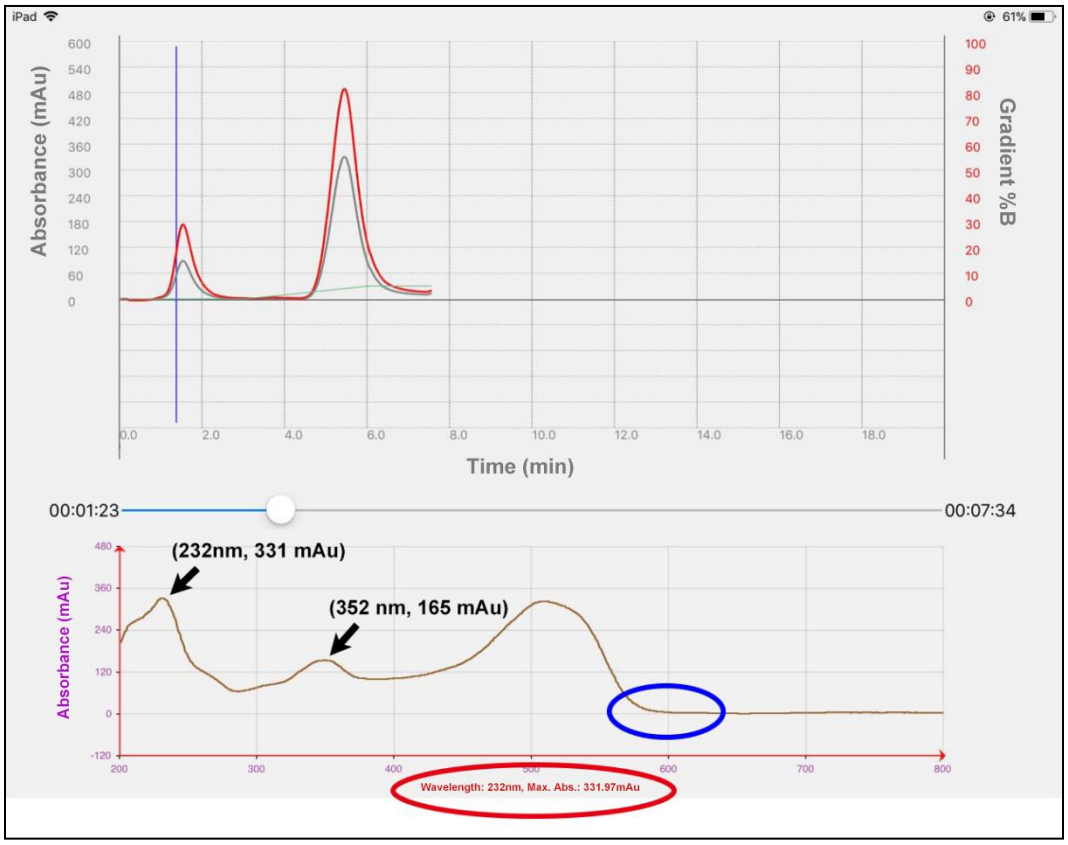

Figure 2. The chromatogram of a specific sample in the first run. The below part is the full wavelength spectrum of the first elution peak.

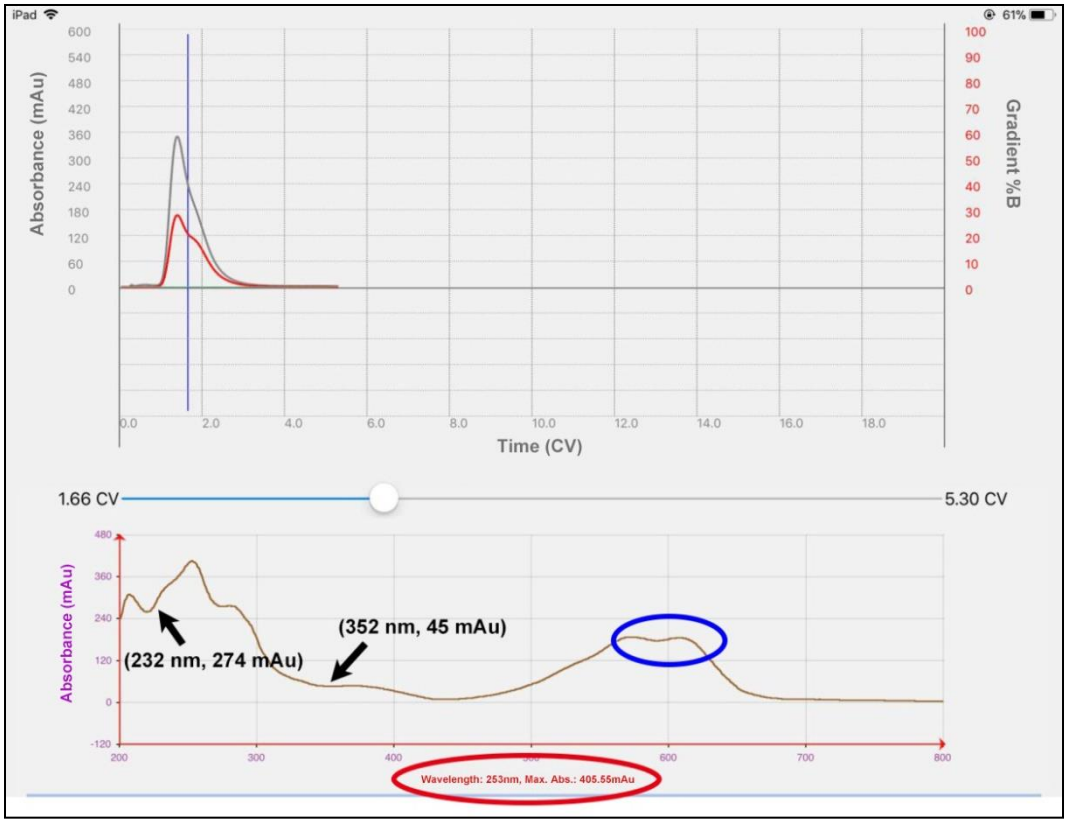

Figure 3. The chromatogram of a specific sample in the second run. The below part is the full wavelength spectrum of the elution peak.

A specific mixture was used as the sample to perform separation experiments for two runs with different elution gradient. The chromatograms of the sample were shown as Figure 2 and Figure 3. Since the sample used in the two runs was the same one, we can conclude that the two components of the sample were co-eluted in the second run based on the following points.

1. *The maximum absorption wavelength of the target compound*

General speaking, the maximum absorption wavelength of a specific compound is a constant. As shown in Figure 2, the maximum absorption wavelength of the target compound is 232 nm (as marked in red circle). As a comparison in Figure 3, the maximum absorption wavelength of the target compound is 253 nm (as marked by red circle). The variation of maximum absorption wavelength is considered to be due to the different components eluted from the column.

2. *The differences in characteristic absorption wavelength*

For a specific compound, its characteristic absorption wavelength is unique, just like a fingerprint. For different loading amount of the compound, the absorption may differ in

response value while the characteristic absorption wavelength will not change. As marked in blue circle in Figure 2 and Figure 3, the eluted fraction has obvious absorption peaks at 600 nm in Figure 3 while no peak at the same position was presented in Figure 2, indicating the eluted fraction in the second run has less purity.

3. The ratio of the characteristic absorption wavelength values

For a certain compound, the ratio of its absorption values at different characteristic wavelengths is a constant. As shown in Figure 2, the ratio of the first eluted fraction's absorption value at the two characteristic wavelengths (232 nm and 352 nm) is about 2.0. For comparison, this ratio of the same fraction in Figure 3 is approximately 6.1. Combining the chromatograms in Figure 2 and Figure 3, it can be determined that the first eluted fraction in the two runs is not the same. Furthermore, the purity of the first eluted fraction in the second run is relatively low.

In this post, we introduced how to determine the purity of the eluted fraction with the help of the full wavelength spectrum scanning from the DAD module in SepaBean™ machine. As always, the R&D stuff from Santai Technologies are developing more cool and practical functions, so stay tuned.

For further information on detailed specifications of SepaBean™ machine, or the ordering information on SepaFlash® series flash cartridges, please visit our website:

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