All-Wavelength Collection

on Combi*Flash*® Systems

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Chromatography Application Note AN81

Abstract

All-wavelength Collection is a technique to purify compounds by measuring the absorbance across a user-defined range of a UV-vis detector. The data is presented as a single trace that allows easy fractionation of peaks. Signal processing techniques eliminate baseline drift from solvent absorbance.

Overview

All-Wavelength Collection in Combi*Flash* systems measure the average absorbance on all wavelengths detected on a photodiode array. The signal is processed to remove baseline drift caused by solvent absorbance. This creates a single voltage that allows the fraction collection program in the MPLC or Flash chromatography system to properly cut the peak. All-wavelength detection is useful when:

- The UV-vis spectrum is unknown, such as compounds purified from natural products.
- There is a mixture of compounds with various absorbances such that a single wavelength cannot "see" all the compounds in the mixture.
- The elution solvent spectrum overlaps that of the desired compound.
- Compounds with similar spectra overload the detector, making it difficult to properly fractionate compounds.

All-wavelength detection improves the ability for a Combi*Flash* system to purify compounds in an automated fashion. These improvements are illustrated in the following examples.

Example with a compound mixture

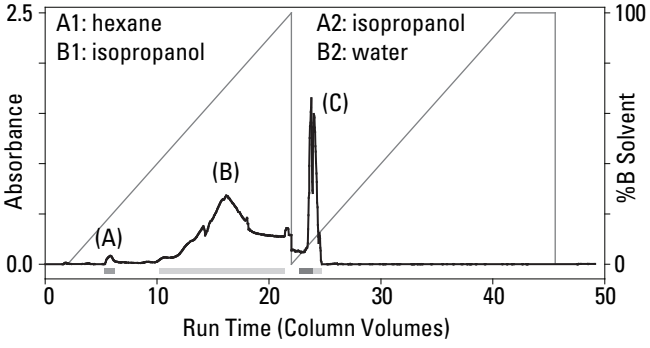

Figure 1: Chromatogram shows the purification of chlorophyll (A), caffeine and catechins (B), and tannins (C) using All-Wavelength Collection with a diol column. All of these compounds have differing spectra yet all were detected with All-Wavelength Collection.

Example of unknown spectrum

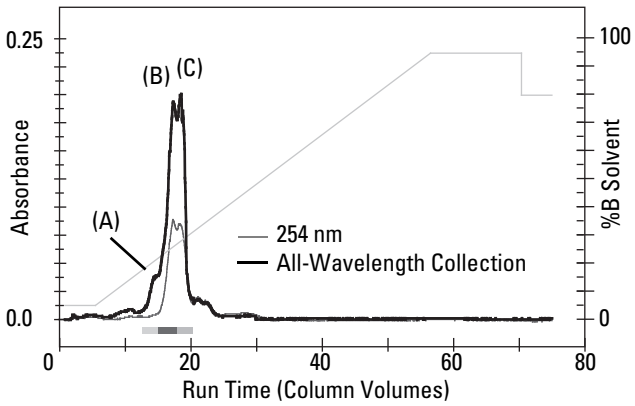

Figure 2: Detection of catechin (A) along with caffeine (B) and other catechin compounds (C) using All-Wavelength Collection

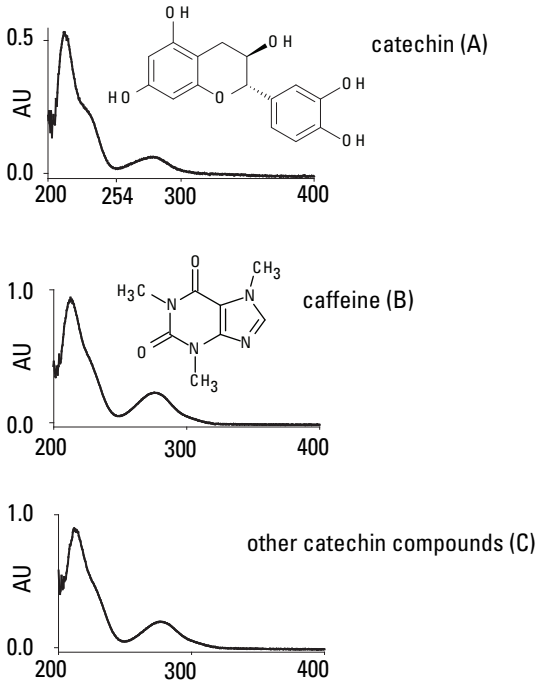

Figure 3: Diagram showing UV absorption of compounds separated in Figure 2.

In Figure 2, catechin is not detected with the commonly used 254 nm wavelength since this is a minimum in the spectrum, but the All-Wavelength Collection was able to detect and fractionate the compound. This is an especially useful technique for natural products where the absorbance of the desired compound generally is not known until after the final purification.

Solvent spectrum overlaps compound

Ethyl acetate and dichloromethane are two of the most commonly used solvents for Flash chromatography. Both of these solvents absorb UV light below 250 nm which interferes with detection of compounds that also absorb in this wavelength range when gradients are used. The constantly changing baseline interferes with the ability of the fraction collector to properly cut fractions.

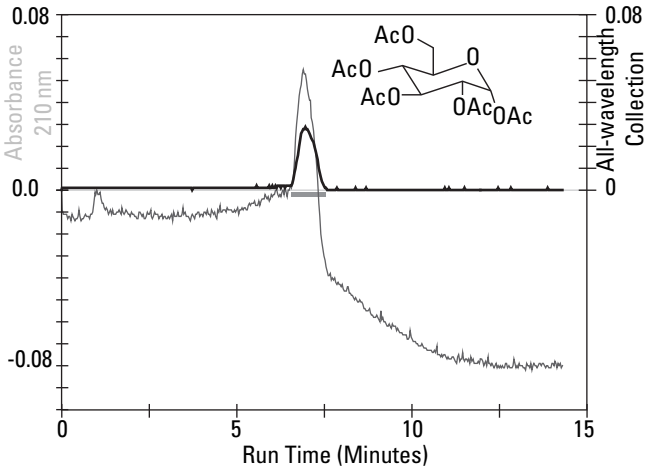

Figure 4: Purification of glucose pentaacetate with All-Wavelength Collection using a dichloromethane/methanol gradient.

Glucose pentaacetate shows only weak end adsorption which is further suppressed by the absorption of the dichloromethane (Figure 4). As the dichloromethane concentration is decreased, the baseline drifts downwards. This drift tends to confuse common fraction collection programs but is not an issue with All-Wavelength Collection. All-wavelength collection filters out the baseline drift to create a baseline usable by the fraction collector in the Combi*Flash* system.

Sample overloads detector

High sample loads that cause the absorbance to saturate the detector are common in Flash chromatography. If compounds are closely eluting, the saturation prevents the fraction collector from separating the compounds properly since the saturated peak is seen as a single large peak.

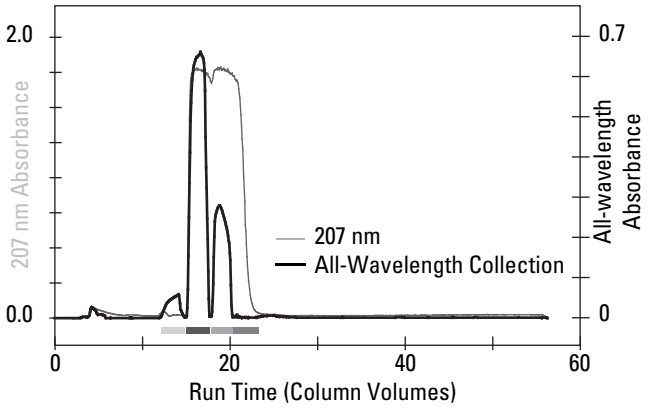

Figure 5: Purification of closely eluting, saturated peaks with All-Wavelength Collection

All-Wavelength Collection measure all absorbance within the range selected by the user and can cut the peaks since the program detects an absorbance change at non-saturating wavelengths. In Figure 5 catechol and resorcinol are purified from an overloaded, overlapping peak with All-Wavelength Collection.

Collect related compounds within a UV-vis range

Since All-Wavelength Collection wavelength range can be changed, it is possible to purify compounds with a range of wavelengths different from others. This permits only the collection of compounds of interest. Although one could choose a single wavelength to perform this task, All-Wavelength Collection can be set to a range that permits collection of a family of related compounds.

Figure 6 shows three compounds collected at 254 nm. Figure 7 demonstrates selective purification using All-Wavelength Collection where peaks 1 and 3 absorb between 295 and 325 nm.

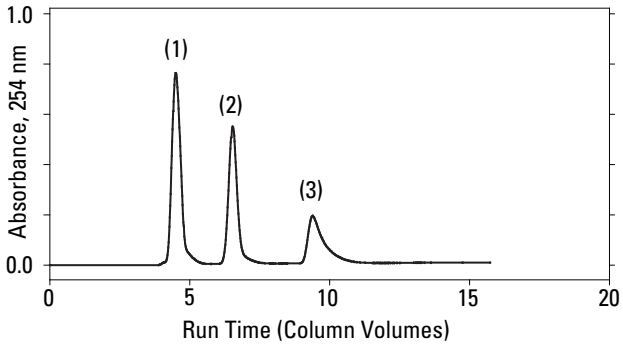

Figure 6: Purification of compounds at 254 nm

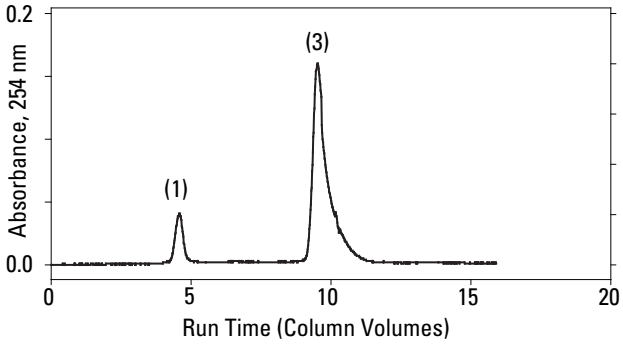

Figure 7: Selective purification of compounds with All-Wavelength Collection between 295 and 325 nm

Suggested parameters for All-Wavelength Collection

The All-Wavelength Collection parameters are set from the Method Editor screen on the Combi*Flash* system. After enabling All-Wavelength Collection, the detector options can be set (Figure 8). Parameters that can be set include the wavelength range (to exclude solvent or undesired compounds), peak width, slope, and threshold. Setting the wavelength range to include values where there is no absorbance will reduce the sensitivity of All-Wavelength Collection but will still allow collection of compounds.

A single detector wavelength should also be chosen at a wavelength at the absorbance of most of the peaks.

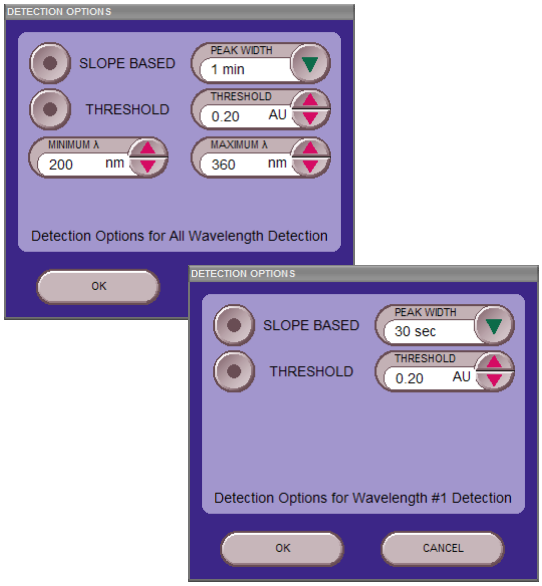

Figure 8: Detection parameters for All-Wavelength Collection and single wavelength

All-Wavelength Collection Parameter Examples

Example 1: Solvent does not absorb in the defined wavelength range

If the solvent does not absorb in the selected wavelength range, set the peak width to the maximum of eight minutes. This setting can be used for isocratic runs as well, even if the solvent absorbance is within the range chosen for All-Wavelength Collection since the baseline is not changing. Figure 1 shows an example of a purification under these parameters.

Example 2: Solvent absorbs within the wavelength range

If the solvent absorbs within the chosen detector range (Figure 4), the All-Wavelength Collection peak width should be set to twice the peak width of the single wavelength detector. See Figure 8 for an example. This parameter minimizes solvent artifacts (Figures 9 and 10).

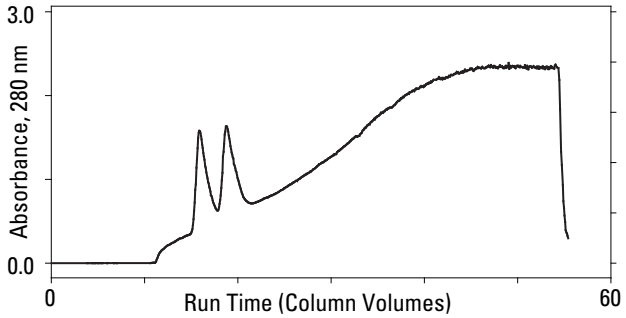

Figure 9: 280 nm collection of catechol and resorcinol with a hexane:acetone gradient. The baseline drifts with the increasing percentage of acetone.

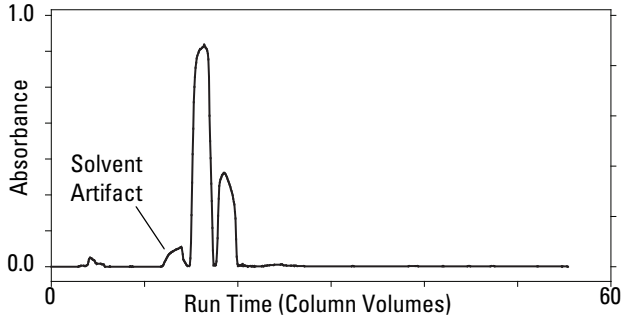

Figure 10: All-Wavelength Collection of catechol and resorcinol with a hexane:acetone gradient. All-wavelength collection filters out most of the baseline drift.

Conclusion

All-Wavelength Collection is a useful technique for purifying compounds with unknown absorbance, or absorbance masked by the solvent. By properly fractionating peaks whose absorbance overloads the detector, All-Wavelength Collection enhances the automated, unattended operation of Combi*Flash* systems.

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