


All-Wavelength Detection: A New Detection Technique for Medium Pressure Liquid Chromatography Suitable for Natural Products

 **Jack E. Silver**
Teledyne Isco, Inc., 4700 Superior Street, Lincoln, Nebraska, USA, 68504

Abstract

During the purification of natural and synthetic compounds, there are often mixtures of compounds with widely different UV spectra. The varying absorption maxima cause the fraction collector's peak cutting program to miss peaks.

All-wavelength detection is a technique that monitors all detector wavelengths in a user-defined range. A change of absorbance within that range is treated as another peak and triggers collection or peak cutting by the fraction collector. Closely eluting peaks with similar spectra are collected in separate tubes while solvent absorbance is suppressed in the chromatogram.

The purification of catechins and xanthine alkaloids from *Camellia sinensis* is used as a model isolation for this technique.

Background

Flash chromatography makes use of UV detection to determine how to "cut" peaks based on changes in baseline and slope to collect fractions. Measurements based on UV absorbance can be subject to interference due to the absorbance of light by the solvent. Since fraction collection is often triggered by changes in baseline, solvent-induced baseline changes cause extra fractions to be collected that contain no compound. Detection is hindered if the compound doesn't absorb light at a wavelength chosen for the detector. Eluted peaks can also saturate the detector, appearing as a single peak.

All-wavelength detection monitors the absorbance across a user-specified wavelength range. The program applies a signal processing algorithm that suppresses interference from solvent absorbance that causes drifting baselines that can "hide" peaks or interfere with fraction collection.

Detecting all wavelengths ensures that no compound absorbing light in a single wavelength range is missed during collection. This feature, when combined with fractionation and the advanced peak-cutting algorithms of Teledyne Isco's CombiFlash® Rf system, is hereafter referred to as All-Wavelength Collection.

Experimental

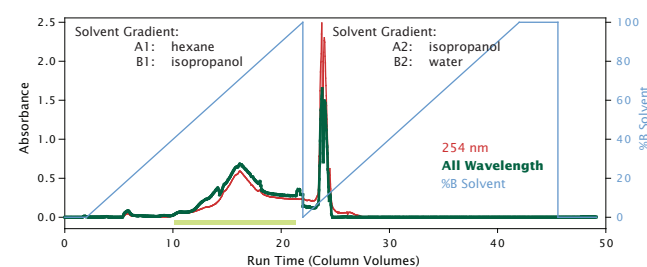
Green tea was purchased at a grocery store in Lincoln, NE. A mass of 150 g was extracted with methanol. The extract was evaporated under vacuum and used as described in the various examples. All purifications were performed on a CombiFlash Rf system (Teledyne Isco, Lincoln, NE, part number 68-5230-006). Fractions were evaluated using HPLC (C18, water-methanol gradient containing 0.1% TFA) and thin layer chromatography (RediSep® TLC plates, Teledyne Isco, part number 69-2203-400, 15% methanol in methylene chloride). Fractions were compared to authentic samples of caffeine and catechin (Sigma-Aldrich) on HPLC and TLC.

Example 1: Initial Purification

One gram of extract was dissolved in methanol and adsorbed onto Celite® 545 (Acros Organics) in a RediSep solid load sample cartridge (Teledyne Isco part number 69-3873-235). The compound was eluted with a hexane : isopropanol gradient followed by an isopropanol : water gradient on a RediSep Rf Gold™ diol column (Teledyne Isco part number 69-2203-371). The four solvent gradient was automatically programmed on the CombiFlash Rf system. The alkaloids and catechin compounds eluted together while the tannins eluted early in the water gradient. Fractions were collected using the all-wavelength collection algorithm. This purification was repeated twice to generate material for Examples 2 and 3.

A diol column was selected because a single gradient run captures nearly all the compounds ranging from very non-polar through water soluble. The diol column is functionalized and can be reused many times.

Initial Purification of Green Tea Extract Using a Diol Column
Single, automated run covering entire polarity range



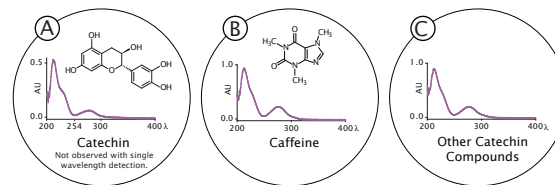
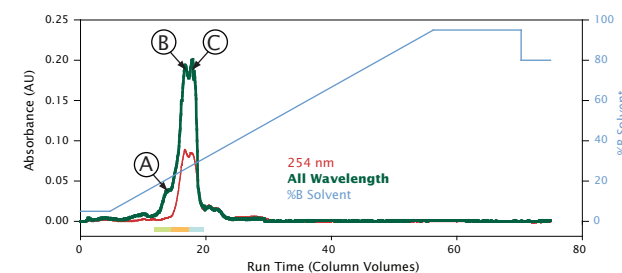
Fractions were collected and combined from 10 through 21 column volumes (CV) based on TLC and comparisons to authentic samples. The peaks at 22 CV are probably tannins. These were found to adsorb irreversibly onto polyamide.

The All-Wavelength Collection and trace compare favorably to that of detection at 254 nm.

Example 2: Detection of Compounds at Other Wavelengths

One gram of the extract partially purified on the diol column in Example 1 was adsorbed onto Celite 545 as described for the purification on diol. The compound was eluted using a methanol/ water gradient containing 0.1% TFA (5% methanol to 95% methanol) on a RediSep Rf Gold C18 column (Teledyne Isco, 69-2203-334). Fractions were collected with the All-Wavelength Collection program.

All-Wavelength Collection of "Hidden" Peaks
Purification on C18

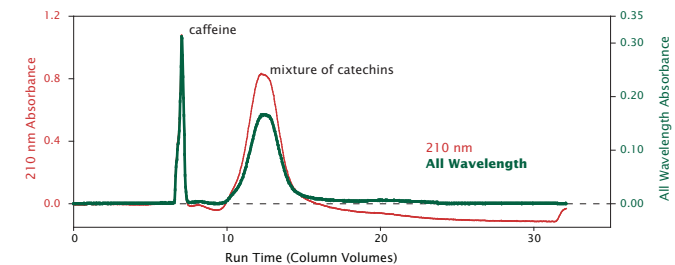


The All-Wavelength Collection program was able to detect all compounds visible at the single wavelength. Despite closely eluting compounds on the C18 column, the All-Wavelength Collection software was able to detect catechin that was missed at the single wavelength due to its relatively low concentration and minimal absorption at 254 nm. No compounds were missed by All-Wavelength Collection.

Example 3: Solvent Baseline Suppression

An extract mass of 0.25 g partially purified on the diol column in Example 1 was dissolved in methanol and injected onto a 12 g RediSep Rf silica column (Teledyne Isco part number 69-2203-312). The compound was eluted with a methylene chloride : methanol gradient to 30% methanol. Fractions were collected with the All-Wavelength Collection program. UV detection was at 210 nm.

All-Wavelength Detection with Drifting Baseline
Purification on silica gel

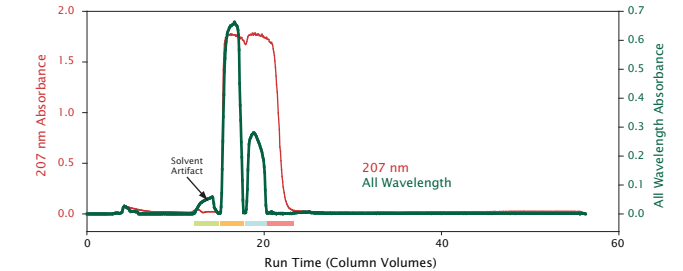


The All-Wavelength Collection algorithm detected all peaks while maintaining a flat baseline. The ability to suppress solvent absorbance is important because solvent absorbance disrupts peak collection based on a threshold level.

Example 4: Saturated Peaks

A 1:1 mixture of catechol and resorcinol (Sigma-Aldrich) was dissolved in ethyl acetate and adsorbed onto silica (Teledyne Isco, part number 60-5394-478) at 20.0% load. Of this mixture, 2.38 g (0.55 g combined phenols) was run on a RediSep Rf 4 g silica column (14% column loading). The mixture was run with a hexane : acetone gradient. The UV detector was set to 207 nm.

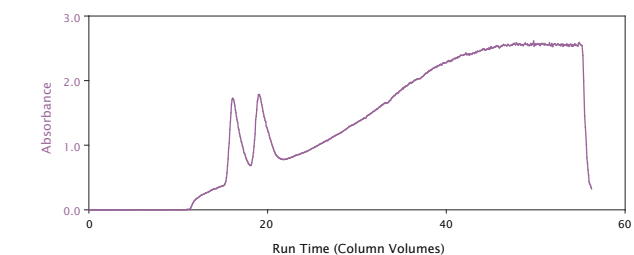
All-Wavelength Collection with Saturated Peaks
Purification on silica gel



Although the single 207 nm trace was saturated, the All-Wavelength Collection program is able to trigger accurate fraction collection of both compounds minimizing cross contamination between fractions. The single wavelength detection treats the large peak as if it were a single compound causing mixing of the eluted fractions.

The solvent artifact is a result of the initial solvent baseline change until it was filtered by the All-Wavelength Collection program. The trace below shows another example of baseline drift caused by solvent absorbance in the same run.

Solvent Baseline Drift at 254 nm



Conclusion

All-Wavelength Collection is a useful function on the CombiFlash Rf system for detecting compounds with unknown UV-vis absorbance. The algorithm also compensates for drifting baselines due to the solvent absorbance. All-Wavelength Collection also allows proper fractionation of compounds that saturate the detector in the Flash system and are not visible as separate peaks at a single wavelength.

All-Wavelength Collection is a preferred method to purify compounds from natural products.