Information Rich Flash Chromatography II: All-Wavelength Collection and Purity Measurement

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Chromatography Application Note AN94

Overview

UV-visible detectors have been used on flash chromatography systems for several years to control the fraction collector. The purity measurements were limited to ratio measurements that required *a priori* knowledge of the spectra of the compound and impurities so that the correct wavelengths could be used both to collect peaks and determine purity.

All-wavelength collection 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. All-wavelength collection can also be employed to determine peak purity over the entire spectral range chosen by the chemist with real-time solvent background suppression. Examples from synthesized compounds and natural products will be presented.

Background

All-wavelength collection is a technique useful for purifying compounds when the UV spectrum is unknown, as is the case for natural products. When measuring the UV spectra of synthesized mixtures, the UV of the desired compound may be masked by the stronger spectra of impurities causing an inappropriate detection wavelength to be chosen. All-wavelength collection helps to ensure the desired compounds are collected, even in the presence of solvent-induced baseline changes. When compounds closely elute, it is useful to be able to resolve the mixture based on peak purity measurements. Measurement of the entire UV spectrum in all-wavelength collection allows the possibility of purifying compounds by purity index measurements.

Experimental and Results

All experiments were run on a Combi*Flash* Rf$^+$ PurIon system. Solvents were ACS grade from VWR Scientific (Radnor, PA). Other details are described in the following sections.

Example 1: Initial Purification of Green Tea Compounds

1 g extract was dissolved in methanol and adsorbed onto Celite 545 (Acros Organics) in a Redi*Sep*® solid load sample cartridge (PN 69-3873-235). The compound was eluted with a hexane: isopropanol gradient followed by an isopropanol: water gradient on a 15.5 g Redi*Sep* Rf Gold Diol column (PN 69-2203-515). 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 Example 2.

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

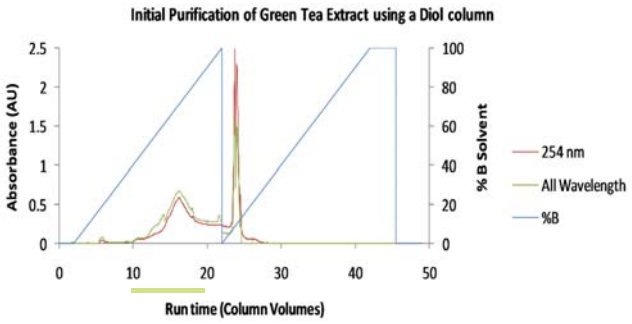

Figure 1: Initial purification of green tea extract with all-wavelength collection and a diol column. Color bar denotes fractions used for purification in Example 2.

Fractions were collected and combined from 10-21 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. Purification with C18

1 g 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) using a Redi*Sep* Rf Gold C18 column (PN 69-2203-334). Fractions were collected with the all-wavelength collection program.

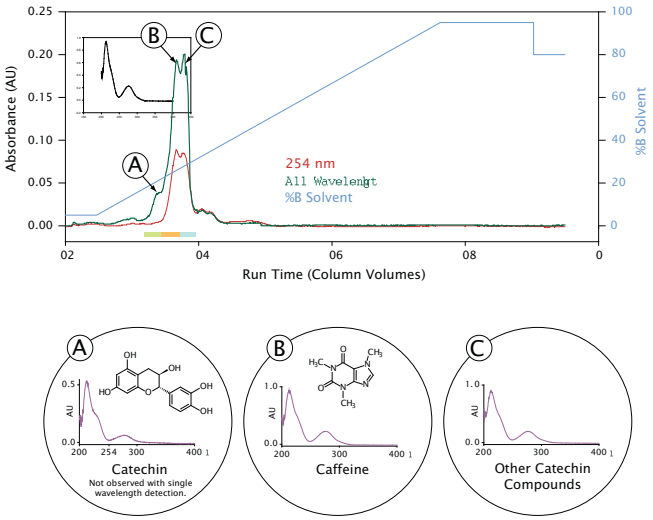

Figure 2: Detection of flavonoids and xanthine alkaloids with all-wavelength collection

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 using all-wavelength collection in this example.

Purity Determination

Peak*Trak*® software was used on the Combi*Flash* Rf⁺ PurIon system that stored spectral information. For the simulated chromatograms, the UV spectra were collected from the Combi*Flash* Rf⁺ PurIon system. The plate count at the λ–max was determined for each compound (run separately), along with the retention time for each compound. The chromatogram was then simulated using a partition chromatography model[1]. The simulations were run to generate a dataset without instrument noise to validate the models.

The chromatography sample was prepared by adsorbing 1:1 esculin monohydrate: 4-nitrophenyl ?-(D)-glocopyranoside on Celite (10% sample on Celite). The sample (285 mg) was run on a water/acetonitrile gradient adjusted to cause peak overlap on a 13 g RediSep C18 column (PN 69-2203-411). Another run with caffeine and theophylline was generated with 1:1 caffeine: theophylline on Celite (10% sample on Celite). This mixture (0.5 g) was eluted on a 12 g Redi*Sep* Rf silica column (PN 69-2203-312) with 100% acetonitrile.

Several algorithms were used to evaluate peak purity by spectral comparison including least-squares comparison, Similarity Index[2], Spectral Contrast Angle[2], and Pearson Similarity Index[3]. The Similarity Index was chosen because the real data best follows the theoretical predictions of the simulated chromatograms.

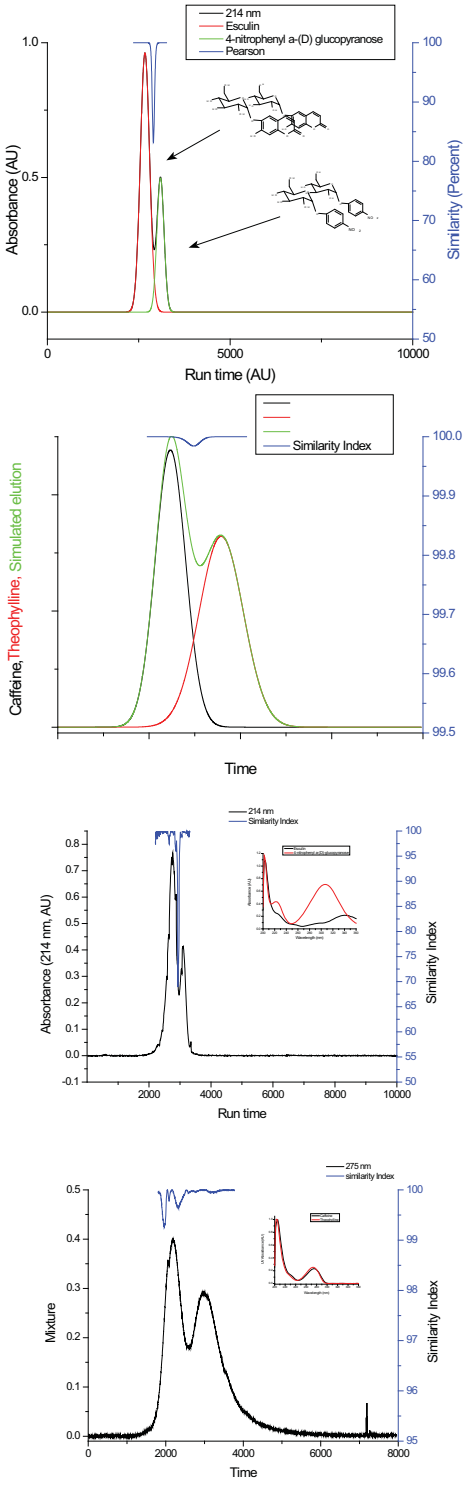

Figure 3: Simulated chromatograms (top two) demonstrate evaluation of peak purity using the Pearson method. The top two are simulated chromatograms; the bottom two are the Similarity index applied to the actual compound elution. The compound spectra are inset in the instrument run data. Compounds with spectra as similar as those of caffeine and theophylline appear to generate a similarity index on overlapping peaks, but it is difficult to state that the similarity index result in the actual sample run are not due to instrument noise.

Conclusion

All-wavelength collection can be used to detect and fractionate compounds when their absorbance isn't known. All-wavelength collection can compensate for a drifting baseline such as that induced by solvent absorbance[4]. Compounds and sample loadings that generate peaks that saturate a UV detector at a given wavelength can also be fractionated with All-wavelength collection[4]. An extension of all-wavelength collection can also be used to evaluate peak purity, even for closely related compounds. In addition to UV spectra, the purity measurements can be applied to other detection methods, such as mass spectrometry.

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2. Wan, K.X.; Vidavsky, I.; Gross, M.L. J. *Am. Soc. Soc. Mass Spectrom.* 2002, 13, 85-88
3. Papadoyannis, I.N.; Gika, H.G. Peak Purity Determination with a Diode Array Detector. In *Encyclopedia of Chromatography*, 2nd Edition; Cazes, J. ; Taylor & Francis: Boca Raton, FL, 2005; Vol 2, pp 1198-1202
4. Silver, J.E. All-Wavelength Detection:A New Detection Technique for Medium Pressure Liquid Chromatography Suitable for Natural Products. Presented at the American Society for Pharmacognosy meeting, July 2009

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