

Wide Polarity Range Chromatography

Using CombiFlash® Rf Systems

Abstract

In most reactions and subsequent purifications, compounds exhibit polarity and solubility properties near enough to allow purification with a single pair of solvents in the gradient. There are times when compounds of interest span a large polarity range, such as pharmacognosy applications. By adroit use of gradients and solvents, chromatograms can be run that span the polarity range from hexane through water in a single run for a normal phase column. Reverse phase columns can be run that span the range from water through methylene chloride in a single run. The ability to automatically change solvents and gradients saves time and can extend column life. Solid load cartridges avoid solubility issues since all compounds are released from the cartridge at the appropriate time in the purification.

Examples using a normal phase diol and reverse phase C18 columns are presented.

Materials and Methods

Reverse Phase C18

A crude tomato paste extract was run with a CombiFlash Rf-200 system equipped with a UV-vis detector (PN 68-5230-008, Teledyne Isco, Lincoln, NE) with a 43 g RediSep Rf Gold® C18 column (PN 69-2203-334, Teledyne Isco) using a water to methanol gradient followed by a methanol to methylene chloride gradient with automatic solvent switching. A sample was adsorbed onto Celite® and placed into empty 25 g Solid Load Cartridges (PN 69-3873-240, Teledyne Isco). Lycopene compounds were detected at 473 nm; other compounds were fractionated with All Wavelength Collection (200–360 nm range). The column was initially run with a water/methanol gradient which eluted polar compounds then automatically switched to a methanol/dichloromethane gradient to purify the lycopenes. This gradient program, including the solvent changes, was programmed into the CombiFlash Rf-200 (Figure 1).

Normal Phase Diol

Crude tea extract purification was performed on a CombiFlash Rf-200 system (Teledyne Isco, Lincoln, NE, part number 68-5230-006). One gram of extract was dissolved in methanol and adsorbed onto Celite 545 (Acros Organics) in a 5 g 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 15.5 g RediSep Rf Gold diol column (Teledyne Isco part number 69-2203-515). The multiple solvent gradient was programmed to automatically switch on the CombiFlash Rf system. The alkaloids and catechin compounds eluted

together while the tannins eluted early in the water gradient (Figure 2). Fractions were collected using the All-Wavelength Collection algorithm. Detection was 254 nm (peak width=1 min) and All-Wavelength (200–360 nm, peak width=8 min).

Results and Discussion

Reverse Phase C18

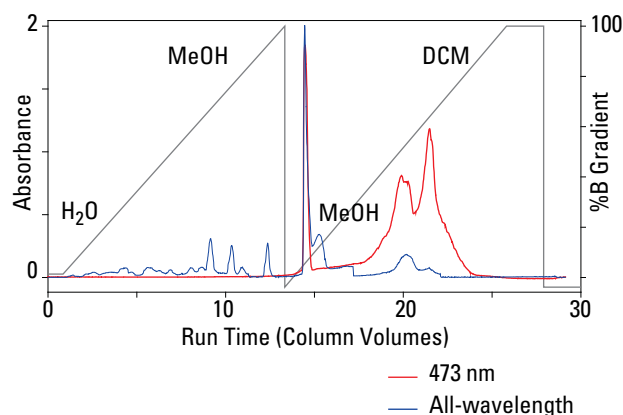


Figure 1: Wide polarity purification of tomato extract A1: H₂O; B1: MeOH; A2: MeOH; B2: DCM

Figure 1 demonstrates the ability of the CombiFlash Rf-200 to automatically switch solvents during purification and isolate a wide range of compounds in a single run. The compounds range from essentially water-soluble to completely water insoluble.

Normal Phase Diol

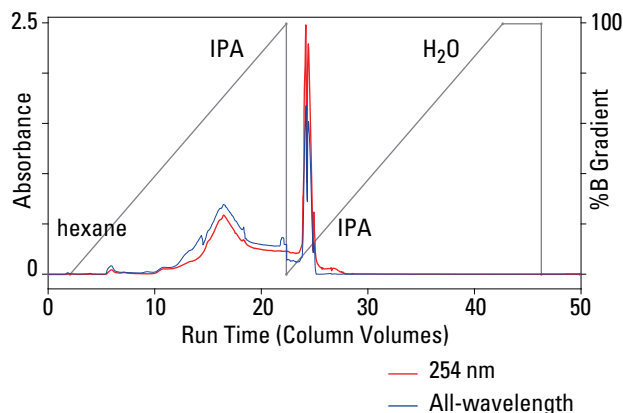


Figure 2: Initial purification of green tea extract A1: Hexane; B1: IPA; A2: IPA; B2: H₂O

Wide Polarity Range Chromatography works well in normal phase as well (Figure 2). In this case, the porphyrin compounds elute first, followed by catechols and xanthine alkaloids, and finally the tannins at the start of the water gradient.

For these experiments, one solvent was common to all portions of the gradient. This solvent was defined as B1 and A2 (Figure 3); solvent lines A2 and B1 were placed in this solvent container.

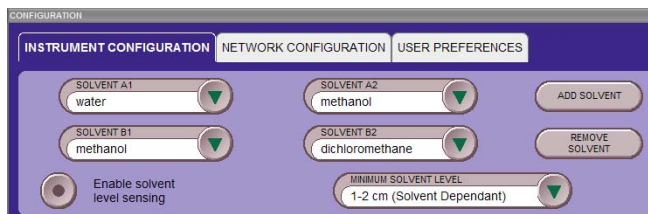


Figure 3: One arrangement of solvents for wide polarity chromatography on C18

After the solvents are configured, the gradient table can be generated (Figure 4) in the method editor.

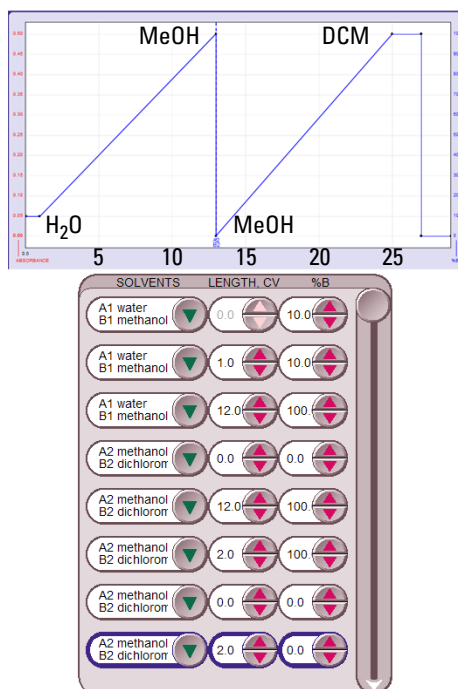


Figure 4: Programming three solvents for C18 wide polarity range chromatography running from water through methanol and finishing with dichloromethane.

Generally, the B solvent is considered to be the “strong” solvent in chromatography. Keeping the strong solvent as “B” allows easier conception of the gradient.

Figure 5 demonstrates the use of four solvents for wide polarity chromatography. The diol column is initially run with a hexane/ethyl acetate gradient. The second gradient is ethyl acetate/methanol; the final gradient is methanol/water. This is a gradient useful for scouting both polar and non-polar compounds for natural products.

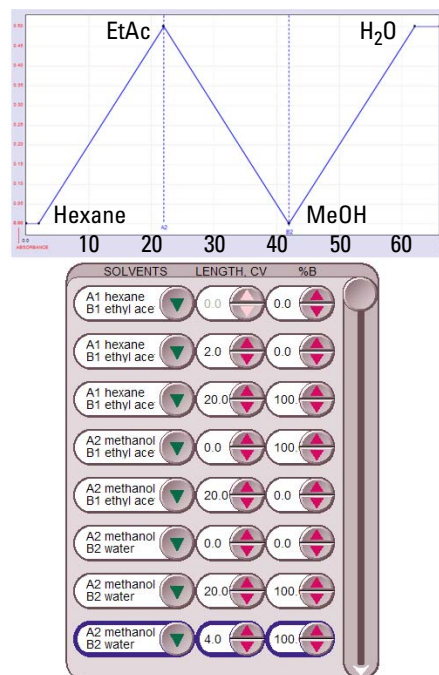


Figure 5: Four-solvent gradient for wide polarity range chromatography on a diol column starting with hexane, going through 100% ethyl acetate to 100% methanol and finishing with water.

Conclusion

Wide Polarity Range chromatography is an easy method to purify compounds of widely differing solubility with a single run. The use of solid load cartridges avoid issues of solubility, and avoids compounds precipitating and clogging the column. The solid load cartridge allows the full method to be run automatically; the solvent changes are programmed into the gradient table of the CombiFlash Rf-200. The adroit use of solvent switching allows the use of immiscible solvents, such as hexane through ethyl acetate, and then to some concentration of methanol on a silica column.

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