Wide Polarity Range Chromatography

Using Combi*Flash*® Systems

TELEDYNE ISCO
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Chromatography Application Note AN77

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 Combi*Flash*® system equipped with a Teledyne ISCO UV-Vis detector, a 43 g Redi*Sep* Gold® C18 column (PN 69-2203-334), and 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). 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 Combi*Flash* system (Figure 1).

Normal Phase Diol

Crude tea extract purification was performed on a Combi*Flash* system. One gram of extract was dissolved in methanol and adsorbed onto Celite 545 (Acros Organics) in a 5 g 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* Gold diol column. The multiple solvent gradient was programmed to automatically switch on the Combi*Flash* 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

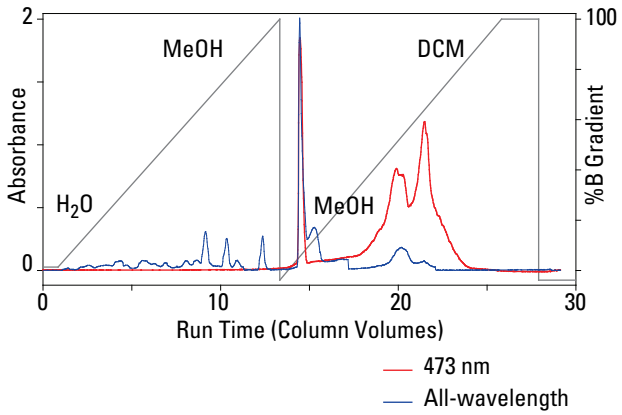

Figure 1: Wide polarity purification of tomato extract A1: H_2O; B1: MeOH; A2: MeOH; B2: DCM

Figure 1 demonstrates the ability of the Combi*Flash* 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

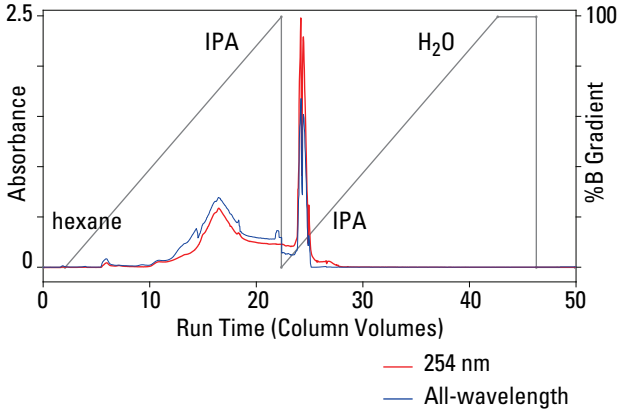

Figure 2: Initial purification of green tea extract
A1: Hexane; B1: IPA; A2: IPA; B2: H_2O

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.

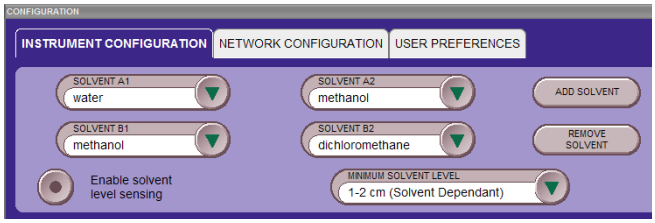

Figure 3: One arrangement of solvents for wide polarity chromatography on C18

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

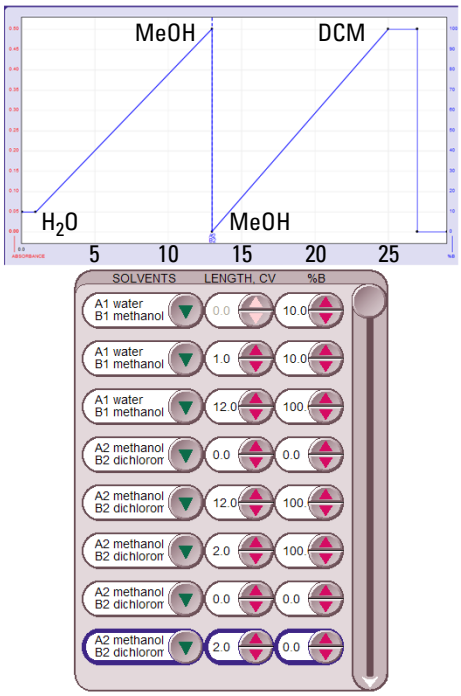

Figure 4: Programming three solvents for C18 wide polarity range chromatography running from water through methanol and finishing with dichloromethane.

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.

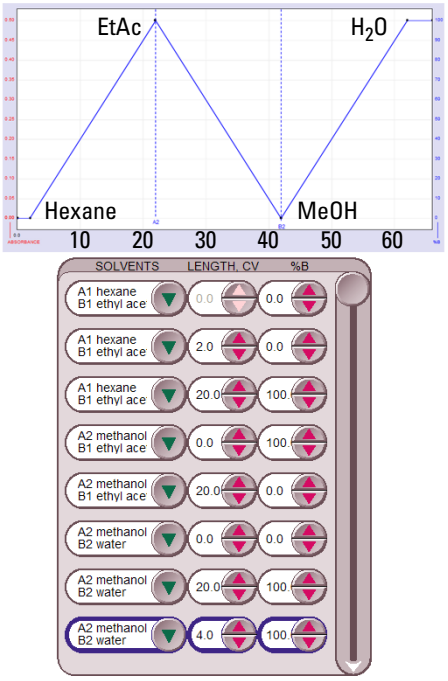

Figure 5: Four-solvent gradient for wide polarity range chromatography on a diol column starting with hexane, going through 100% ethyl acetate to 100% methanol and finishing with water.

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 Combi*Flash* system. 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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