

# Diol columns – pretend they're normal phase

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## **Abstract**

Diol columns are useful as an intermediate polarity media between C18 and silica. Diol functionalized media are less polar, may have different selectivity, and are reusable compared to bare silica. Diol is particularly useful to chromatographers because it can be used with a wide range of solvents. This flexibility is confusing since phrases such as “normal phase mode” and “reverse phase mode” are commonly used. These terms cause confusion when developing methods for MPLC or Flash chromatography.

Treating the diol column as if it were normal phase for all solvents simplifies method development. A diol column is reusable and can be run with many solvent systems. Examples of method development using thin layer chromatography and columns are detailed.

## Background

Diol bonded phase columns are a useful substitute to bare silica columns. They can be reused many times while producing reproducible chromatograms. They are less polar than silica which allows them to be used for a wide range of compounds. They also are compatible with a wide range of solvents from hexane through water.

Users are often confused about how to develop methods for diol columns. This confusion stems from comments indicating that diol columns are “useful in normal or reverse phase<sup>1</sup>”. Although diol columns can be run in either fashion, a user is confused about which mode and solvents to use.

Treating the diol column entirely as normal phase greatly simplifies method development for most compounds. The column can be run in normal phase with solvents considered reversed phase, such as isopropanol and water. This allows methods to be rapidly developed on diol thin layer chromatography (TLC) plates.

## Experimental

### TLC Method Development

RediSep<sup>®</sup> diol TLC plates (P/N 69-2203-574, Teledyne Isco, Lincoln, NE, USA) were used to develop methods. Solvents were ACS grade from BDH (VWR, Brandywine, PA, USA) unless otherwise specified. Actual retentions were measured with a CombiFlash<sup>®</sup> Rf 200 system (P/N 68-5230-006) with the solvent mixtures specified in the tables. Test compounds were from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

### Initial purification of Green Tea Extract

Green tea was purchased at a local grocery store, of which 150 g was extracted with methanol. The extract was evaporated under vacuum. Next, 1.0 g of extract was dissolved in methanol and mixed with 4 g Celite 545 (Acros Organics, NJ, USA). The mixture was evaporated until dry and placed in a 5 g solid load cartridge (Teledyne Isco P/N 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 P/N 69-2203-515). The solvents for the gradient were automatically changed by the CombiFlash RF 200 system using the four solvent inlets. Compounds were detected with All-Wavelength Collection (200 – 360 nm).

## Purification of 3-(2-nitrophenyl amino) propionitrile

Crude 3-(2-nitrophenyl amino) propionitrile from another experiment was used. Methods were developed with *RediSep* silica TLC plates (P/N 69-2203-400, Teledyne Isco) and *RediSep* diol TLC plates. The *Rf*-to-Gradient feature in the *PeakTrak*™ software in the *CombiFlash* *Rf* system was used to develop a purification gradient. The compound was purified with a 12 g *RediSep* *Rf* Gold silica column (P/N 69-2203-345, Teledyne Isco) or 15.5 g *RediSep* *Rf* Gold diol column on a *CombiFlash* *Rf* 200 system with a hexane/ethyl acetate gradient.

## Column Reuse Experiments

Equal amounts (5.0 g each) of 2-aminobenzoic acid and 4-aminobenzoic acid were dissolved in ethyl acetate and mixed with 40.0 g of Celite 545 to make a slurry. A 0.5 g sample of this mixture was run in a 5 g solid load cartridge on a 12 g *RediSep* *Rf* Gold silica column or a 15.5 g *RediSep* *Rf* Gold diol column on a *CombiFlash* *Rf* 200 system in hexane and ethyl acetate. The same method was used for both columns with absorbance measured at 254 nm.

## Results and Discussion

### TLC Method Development

One premise to method development is that TLC plates are able to predict when the compound would elute from a column. A series of TLC experiments were run to confirm that the diol TLC plates both acted in a manner consistent with normal phase chromatography and were able to predict how compounds would be able to elute from columns.

With a minimal amount of compound, TLC plates can be used to rapidly scout solvent systems and predict chromatography. The relationship between the TLC retention factor (*Rf*) and the elution time for a compound is  $Rf=1/CV$ , where *CV*= the number of column volumes<sup>2</sup>.

Tables 1 and 2 demonstrate that diol TLC plates provide a reasonable prediction of how a variety of compounds will behave in a column. As the polarity of the solvent is increased, the compounds show higher retention factors demonstrating that the diol is running as normal phase. This predictability is useful because the *Rf*-to-Gradient feature in the *PeakTrak* software may be used to optimize the method for improved purification. Hexane/isopropanol is a useful solvent system for diol columns because it has a wide polarity range.

**Table 1:**

TLC of compounds on diol using hexane/ethyl acetate with comparisons of estimated and actual retention times

Compound	Hexane %	Ethyl Ac- etate %	Rf	Predicted Retention (CV)	Actual Retention (CV)
Dibutyl phthalate	75	25	0.79	1.3	1.3
	0	100	0.89	1.1	
Phenol	75	25	0.51	1.9	1.9
	0	100	0.81	1.2	
Hydroquinone	75	25	0.125	8	5.4
	0	100	0.73	1.4	
Caffeine	50	50	0.22	4.6	5.4
	0	100	0.59	1.7	
Theophylline	50	50	0.17	5.8	9.1
	0	100	0.48	2.1	

**Table 2:**

TLC of compounds on diol using hexane/isopropanol with comparisons of estimated and actual retention times

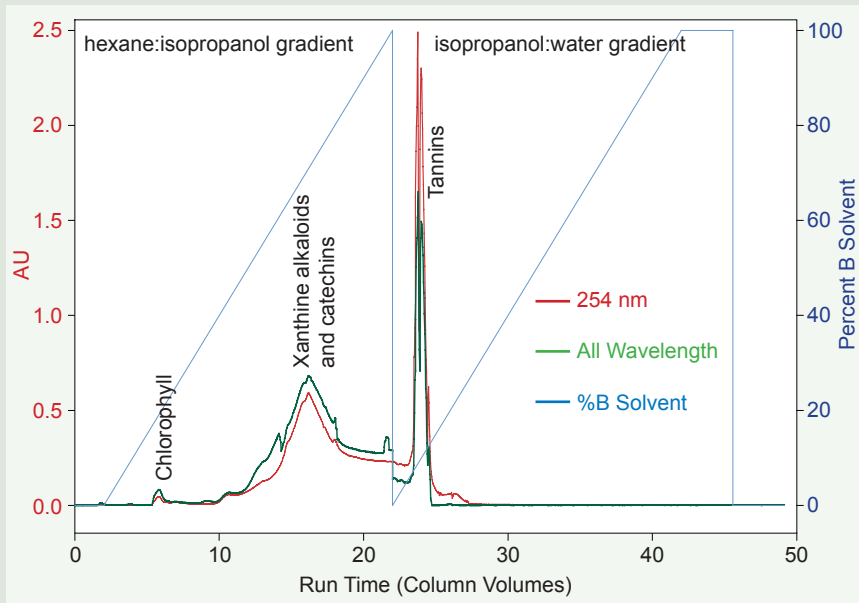
Compound	Hexane %	Isopropanol %	Rf	Predicted Retention (CV)	Actual Retention (CV)
Phenol	75	25	0.72	1.4	1.4
	50	50	0.83	1.2	
Hydroquinone	75	25	0.42	2.4	2.4
	50	50	0.83	1.2	
4-nitrophenyl- $\alpha$ -D-glucopyranoside	75	25	0.18	5.4	6.8
	50	50	0.5	2	
Caffeine	75	25	0.26	3.8	4.8
	50	50	0.48	2.1	

## Initial Purification of Green Tea Extract

The diol column was useful for a quick initial purification of compounds extracted from green tea. The wide polarity range of the diol column was exploited to purify different families of compounds. Note that the column was run as normal phase from 100% hexane through 100% water as a normal phase column in a single run. The tannins would normally be difficult to remove from a silica column, but they are readily washed from the diol column in the isopropanol/water gradient. Note that during this part of the purification, the column is still being run as normal phase although “reverse phase” solvents are employed.

All-Wavelength Collection was used to detect the compounds regardless of their absorbance spectrum.

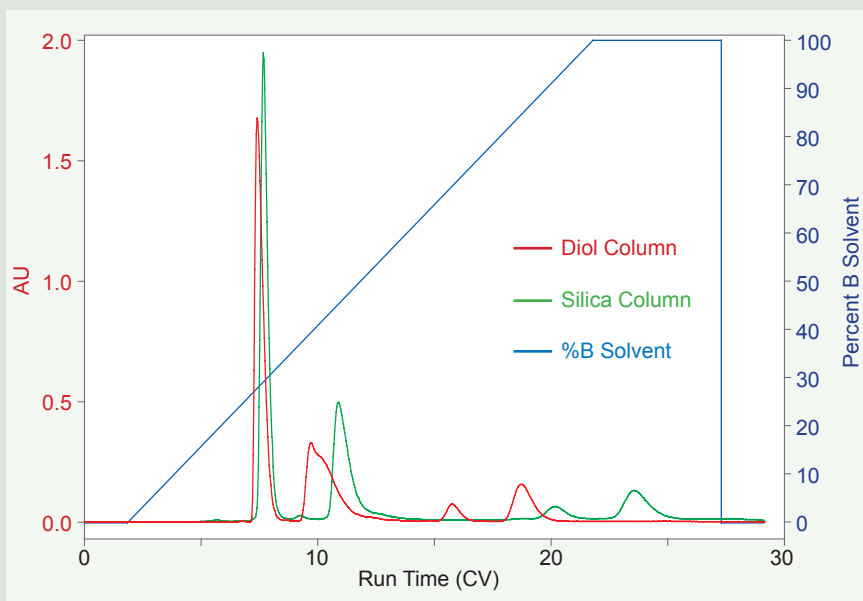
Figure 1:  
Initial purification of green tea extract using a diol column with multiple solvents



## Purification of 3-(2-nitrophenyl amino) propionitrile

This experiment demonstrates the utility of a diol column in normal phase with a compound readily purified on silica gel. Figure 2 shows the diol generates a similar chromatogram as silica gel. Compounds generally elute slightly earlier due to the reduced polarity of diol compared to silica gel.

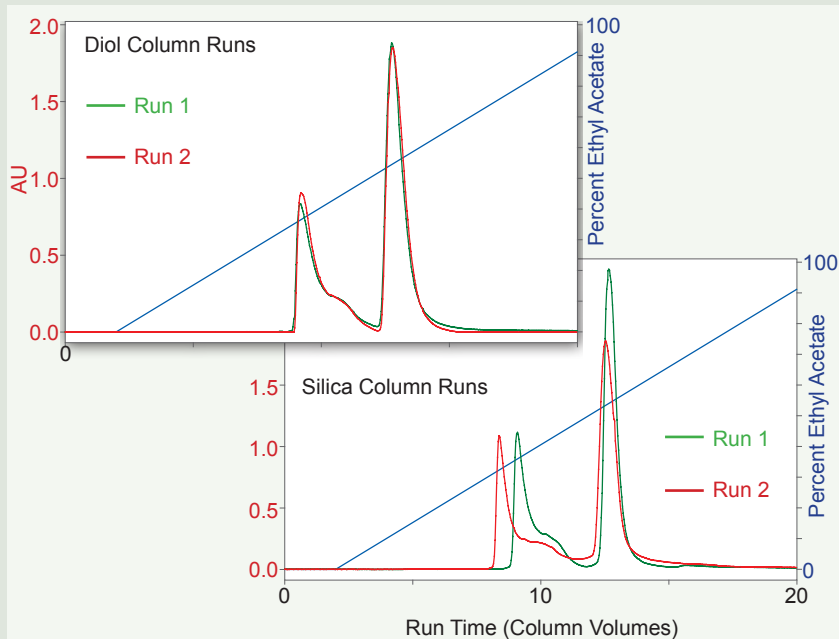
Figure 2:  
Purification of 3-(2-nitrophenyl amino) propionitrile on RediSep Gold diol and RediSep Gold silica columns using a hexane/ethyl acetate gradient



## Column Reuse Experiments

The diol column exhibited identical retention time and peak shape of the test compounds upon reuse. The silica column showed changes in retention time. In addition, the first peak of the silica column was broader. This continued behavior would lead to unresolved peaks on subsequent runs. Similar chromatographic degradation could be expected on multiple runs of more complex mixtures.

**Figure 3:**  
Diol columns can be reused without changes in peak shape or retention time



## Conclusion

RediSep Gold diol columns are a useful alternative to silica columns. Method development is simple; they can be simply considered to be normal phase columns. TLC plates can be used in the same fashion as silica gel to determine the best solvent system to purify compounds.

The wide solvent polarity range allows diol columns to purify compounds that are difficult with silica gel. Diol columns are compatible with strong solvents such as methanol and water.

Diol columns work well as a reusable replacement for bare silica for common purifications although the selectivity may be slightly different. Diol columns may be repeatedly reused because the diol protects the silica layer. The protected silica also offers a broader range of solvents than bare silica, from hexane through water.

<sup>1</sup><http://www.justchromatography.com/wiki/diol-phase>, 18 Feb 2009

<sup>2</sup>Effective Organic Compound Purification— Guidelines and Tactics for Flash Chromatography, 4th Ed, Teledyne Isco, 2010, p 9

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