Redi*Sep*® Rf Strong Anion Exchange Column Applications

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Chromatography Application Note AN87

Abstract

Strong Anion Exchange (SAX) columns are used to purify acidic compounds from a mixture. This application note explores the use of SAX columns in a catch-and-release mode on pure compounds, a mixture of anthocyanins, and a green tea extract that serves as a model of natural product purifications. This note also describes a general purpose procedure using Redi*Sep* Rf SAX columns that allows purification of basic compounds from a matrix without *a priori* knowledge of the desired compound's pKa.

Background

The compounds of interest to synthetic and natural product chemists are often weak acids such as phenols or carboxylic acids. Such compounds include anti-oxidants, pesticides, natural colors, and pharmaceuticals. The use of the SAX column allows the acidic compound to be captured while the basic and neutral compounds are washed from the column. The purified acids can be washed from the column with at least partial resolution. The Redi*Sep* Rf SAX column is reusable, therefore the same column was used for all experiments in this application note.

Table 1 lists the loading capacity of Redi*Sep* Rf SAX columns.

Table 1: Loading capacity of Redi*Sep* Rf SAX columns

Part Number	Column Size (grams)	Maximum Sample Load (mmol)	Sample Weight (MW=200 AMU, grams[+])
69-2203-381	5.7	3.8	0.7524
69-2203-382	17	11.2	2.244
69-2203-383	34	22.4	4.488
69-2203-384	57	37.6	7.524
69-2203-387	114	75.2	15.048
69-2203-385	170	112.2	22.44
69-2203-389	313	206.6	41.316
69-2203-386	470	310.2	62.04

[+]Sample load = mmol * compound molecular weight / 1000

Experimental and Results

General Procedure

Column Conditioning — The column was first washed with five column volumes of methanol. Redi*Sep* Rf SAX columns are packed with a chloride counterion. The conjugate bases of the desired compounds fail to displace the chloride ion leading to poor binding. To improve the binding capabilities of the column, it was run twice using the gradient in *Table 1*. Compounds would reliably bind to the column after the second wash, because the column was completely converted to the acetate counterion.

Column Method — A Redi*Sep* Rf SAX column (17 g, PN 69-2203-382) was used for all runs on a *CombiFlash*® Rf 200 system. The samples were run according to the gradient method in *Table 2*. Solvent A was methanol; Solvent B was deionized water containing 5% glacial acetic acid. The column was equilibrated for five column volumes prior to the injection. After the run, the column was stored in methanol. *Table 2* lists the detection wavelength for each compound. The gradient segment lengths are listed as column volumes (CV), as runs in column volumes are easily scaled to larger runs. Segment 1 is kept long at ten CV to both demonstrate binding of the compounds and allow washing of neutral and basic compounds from the column prior to eluting the desired acids.

The samples were dissolved in methanol containing 5% ammonia during column equilibration. The ammonia ensures the acids are in the form of their conjugate bases and are able to interact with the column. Experiments showed that samples dissolved in methanol without the ammonia showed poor binding.

Table 2: Gradient table for purifications except as noted

Segment Number	Segment Length (CV)	%B
0	Start	0
1	10	0
2	10	100
3	4	100
4	0	0
5	0	0

Gallic Acid

Gallic Acid (16 mg, Sigma Aldrich, St. Louis, MO) was dissolved in 0.5 mL methanol containing 5% ammonia and run according to the method described in *Table* 2. The detection wavelength was 270 nm.

The compound was retained on the column even with a 10 CV methanol wash, demonstrating binding to the column. The compound was released ~ 7 CV into the gradient. The system isolated gallic acid from an unknown impurity.

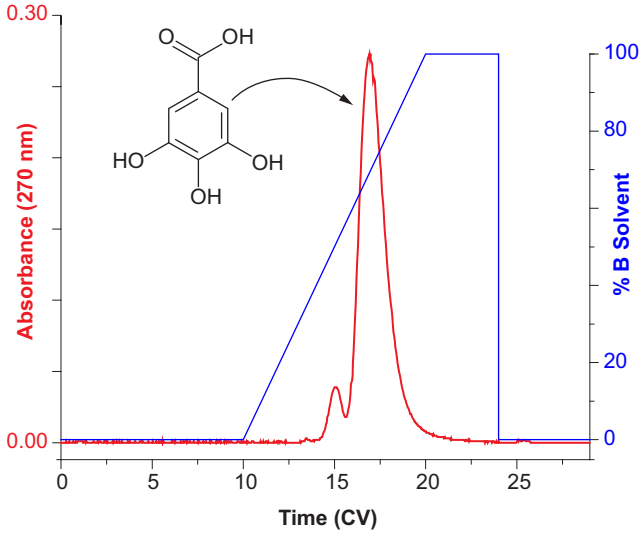

Figure 1: Purification of Gallic Acid on a Redi*Sep* Rf SAX column

Phenolic Flavanoid Compounds

Epicatechin (16 mg, Sigma Aldrich) was dissolved in 0.5 mL methanol containing 5% ammonium hydroxide and run according to the method in *Table* 2. All-Wavelength collection was used with a wavelength range of 200 – 250 nm, and a peak width of 2 minutes. All-Wavelength collection is a detection technique unique to Combi*Flash* Rf 200, Rf 200i, and Torrent systems which allow detection and collection of compounds with unknown absorbance. All-Wavelength collection is also used for baseline suppression. The shoulder in *Figure* 2 is solvent end-adsorption before recognition by All-Wavelength collection.

Anthocyanin

An unknown anthocyanin (25 mg) was mixed with 1 mL methanol containing 5% ammonia. Water was added until the sample was in solution (~0.5 mL). The mixture was injected and run according to the method in *Table* 2.

All-Wavelength collection (200 – 250 nm, 2 min peak width) was used to detect the anthocyanin compound. The shoulder at 16 CV was determined to be another anthocyanin compound. There is also a weak peak appearing at ~22 CV.

Green Tea Extract

Green Tea was extracted in methanol, and dried of which 0.75 g was dissolved in 15 mL methanol containing 5% ammonia. The solvent was limited to 15 mL to prevent sample loss as this volume is similar to the void volume of the column. The neutral and basic compounds do not bind to the column and eluted during loading and column wash. The sample was run similar to the others except that the gradient was changed from 10 to 30 CV (*Table 2,* segment 2). The fractions were collected at 275 nm. Even at this high loading, there was no "breakthrough" of the acidic compounds.

HPLC separation against reference compounds showed the peak eluted during the gradient contained catechin and other unidentified compounds. The gradient was lengthened compared to the other runs to allow these minor components to be better purified from catechin.

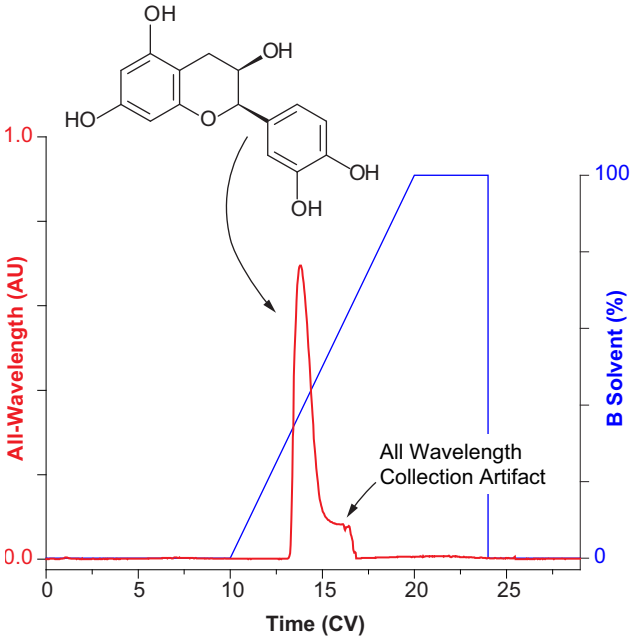

Figure 2: Epicatechin on a Redi*Sep* Rf SAX column

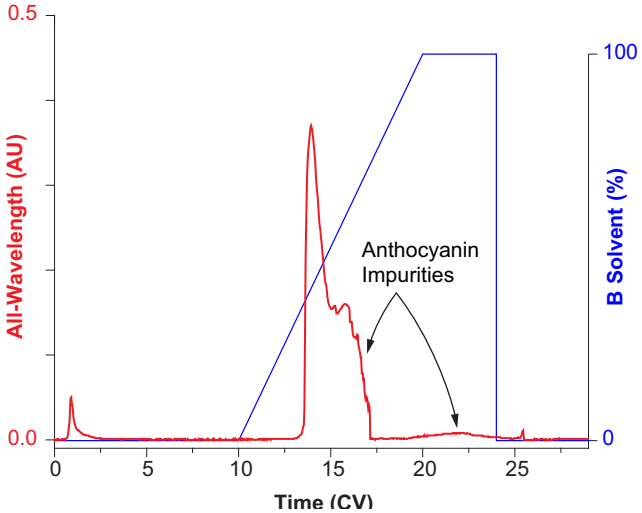

Figure 3: Anthocyanin purified on a Redi*Sep* Rf SAX column

Conclusion

The Redi*Sep* Rf SAX columns are efficient in purifying weakly acidic compounds from a mixture of neutral and basic compounds. A general gradient method was found to be useful for a variety of weak acids without prior knowledge of their pKa. Dissolving the compounds in a basic solution with minimum water provides good interaction with the SAX column. The use of a gradient allows acids to be purified from each other. The SAX column has a high capacity and is reusable.

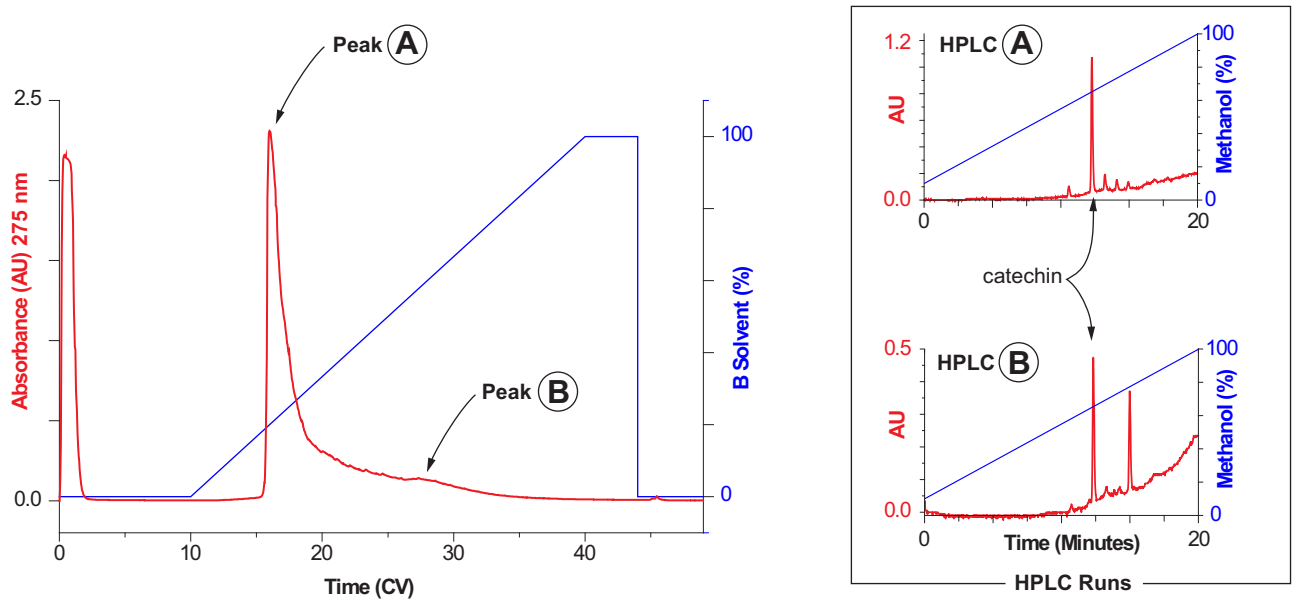

Figure 4: Green Tea Extract purified on a Redi*Sep* Rf SAX column.
HPLC A is from the fraction containing the major peak (A) eluting at 17 CV; HPLC B is the fraction containing the shoulder peak (B) eluting at 27 CV.

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