Hints for Strong Ion Exchange Resins

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Chromatography Application Note
AN98

Abstract

Ion exchange columns are a powerful means of isolating and purifying compounds, but their use is limited due to lack of a general procedure for using these columns. This application note provides hints that allow purification of compounds without prior knowledge of their pKa or ionic strength. Additionally, this application note covers the use of Redi*Sep*® Strong Anion ion eXchange (SAX) and Strong Cation eXchange (SCX) columns.

Background

The term "strong" ion exchange refers to the functional group on the silica, which may be strongly acidic or basic. The Redi*Sep*® Rf SAX column possesses a strongly basic tertiary methyl amine group. The Redi*Sep*® Rf SCX column uses a strongly acidic sulfonic acid group. The SAX column exchanges anions while the SCX column exchanges cations.

General Hints

Column conditioning

The column should be conditioned so that the weakest ion possible is on the column. This makes it easier for the desired compound to displace the ion from the column.

For Redi*Sep*® Rf SCX columns, the ionic strength is:
$\mathrm{Li}<\mathrm{H}<\mathrm{NH_4}<\mathrm{Mg}<\mathrm{Ca}<\mathrm{Fe}<\mathrm{Cu}<\mathrm{Pb}<\mathrm{Ag}<\mathrm{Ba}$

For most small molecules, putting the SCX column into the hydrogen form is sufficient to allow binding. Washing the SCX column with 10 column volumes (CV) of 0.1 N acid (sulfuric or phosphoric preferred) followed by 10 column volumes of water will convert the column to the hydrogen form.

For Redi*Sep*® Rf SAX columns, ionic strength follows the order:
$\mathrm{F}<\mathrm{OH}<\mathrm{actate}<\mathrm{fomate}<\mathrm{Cl}<\mathrm{Br}<\mathrm{CO_3}<\mathrm{PO_4}<\mathrm{citrate}<\mathrm{tosylate}$

For general usage, the column should be put into the acetate form prior to use by washing with 20 column volumes of 5% acetic acid.

☑ **Note**

The Redi*Sep*® Rf SAX column is shipped with the chloride counter-ion, and so requires conversion to the acetate ion. Running the column in the chloride form may cause the desired compound to bind poorly (Figure 1).

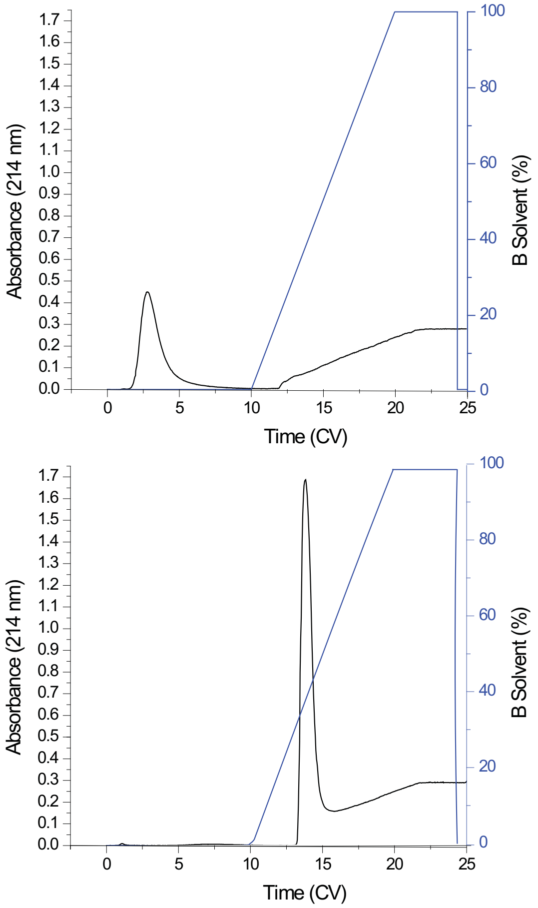

Figure 1: Chromatogram of catechin elution. The top column was run as delivered with chloride ion, while the bottom column was conditioned with acetate ion, demonstrating that columns should be conditioned with "weak" ions prior to use.

Sample Preparation

Samples need to be ionized prior to injection; for basic compounds, dissolving them in 5% solution acetic acid works well while 5% ammonium hydroxide ionizes acidic compounds. The sample should be dissolved in a minimum volume of water.

Column Storage

The columns should be conditioned as described under the "column conditioning" section for future experiments. After conditioning, the columns should be washed and stored in 2-propanol. This solvent is miscible with most other solvents, and prevents the column body from swelling.

SCX Examples

Suggested gradient

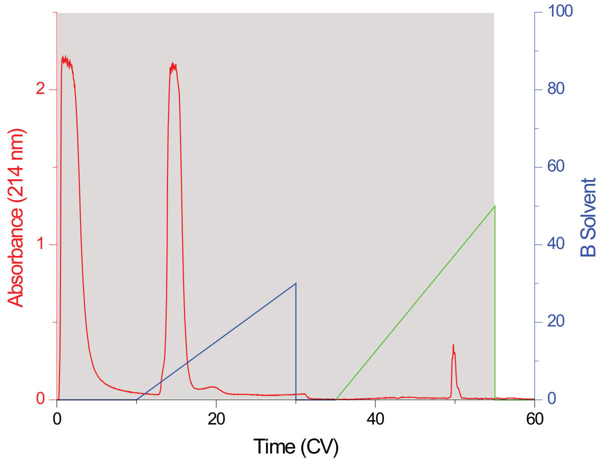

Figure 2: Alkaloids captured and released with multiple gradient; shaded area denotes peak collection window

For the run in Figure 2, 0.5 g green tea methanolic extract was dissolved in methanol containing 5% acetic acid and loaded onto a 15 g Redi*Sep*® Rf SCX column (PN 69-2203-391). The column was washed with methanol and the compounds eluted with a gradient (5% acetic acid in water) followed by a basic gradient (5% ammonium hydroxide in water). Weak bases, such as xanthine alkaloids, are displaced by hydrogen ions. Stronger bases are eluted with the ammonium ion. The basic ammonium gradient also tends to reduce the charge on eluted compounds reducing their affinity for the column. The peaks at 0 to 5 CV are acidic or neutral compounds. Alkaloids eluted between 12 and 21 CV, and at 50 CV. Acetic acid and ammonia are both volatile and can be removed by rotary evaporation or lyophilization. Formic acid also works well in this application.

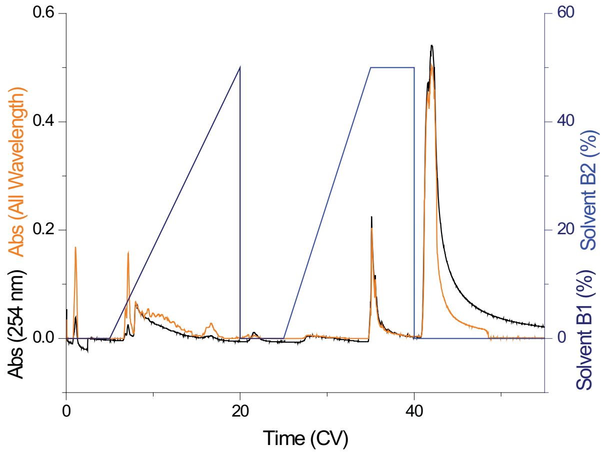

Figure 3: Cationic compounds eluted from a tar sample

Figure 3 demonstrates the double gradient used to purify basic compounds from a tar sample. The tar was dissolved in toluene containing 5% glacial acetic acid. After the sample was loaded, the column was manually washed with toluene to elute un-retained material followed by 2-propanol to remove the toluene. A variety of compounds were eluted. The large peak beginning at ~42 CV probably was insoluble in the aqueous solution as a free base and eluted in the 2-propanol wash.

Table 1: Suggested gradient for SCX column

Segment Length (CV)	%B	B Solvent
Start	0	5% acid in water (B1)
10	0	5% acid in water (B1)- wash off un-retained material
20	50	5% acid in water (B1)- elute weak bases
0	0	5% acid in water (B1)
5	0	5% acid in water (B1)- wash off acid
15	50	5% NH_4OH in water (B2)- elute stronger bases
5	50	5% NH_4OH in water (B2)
0	0	5% acid in water (B1)
15	0	5% acid in water (B1)- wash off slow eluting, neutral compounds

☑ ***Note***

The acid could be acetic or formic acid. The alcohol could be methanol, ethanol, or propanol.

Dissolution in a Non-Aqueous Solvent

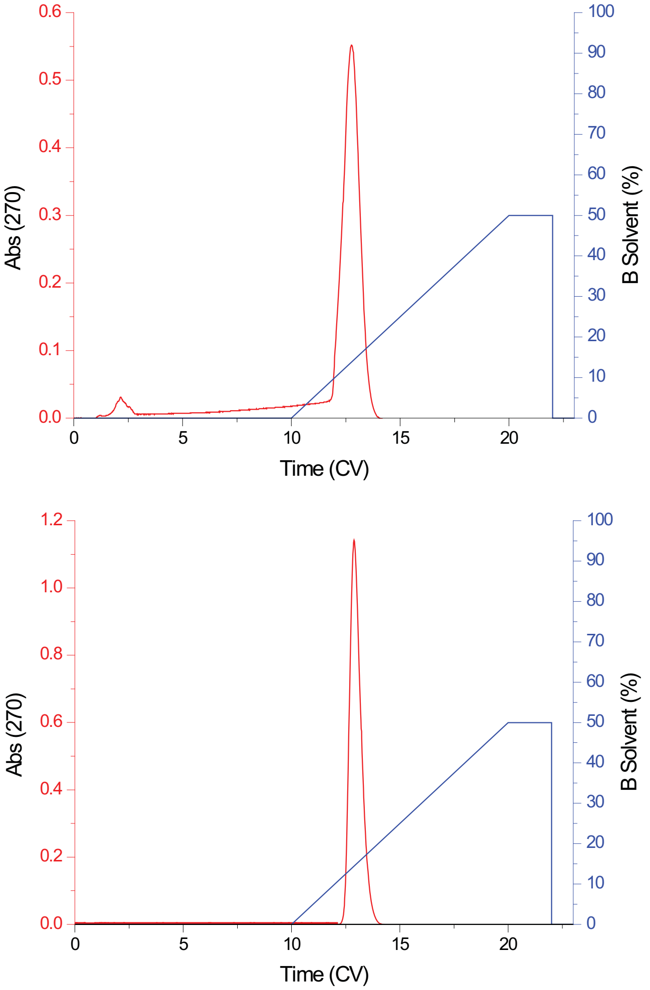

Figure 4: Caffeine dissolved in acidic water (top) exhibits bleed-through compared to the same compound dissolved in acidic methanol (bottom)

The compounds need to be ionized in order to interact with the column, so the sample is dissolved in acid. Dissolving the sample in minimum water improves the peak shape and reduces early elution from the column. Figure 4 shows an equivalent sample of caffeine eluted using the same method. The sample on the top of Figure 4 was dissolved in acidic water and bleeds off the column during the wash. The second sample dissolved in acidic methanol remains entirely retained until the gradient is run. For both runs, the eluting solvent is 5% acetic acid in water.

Using Water as the “A” Solvent

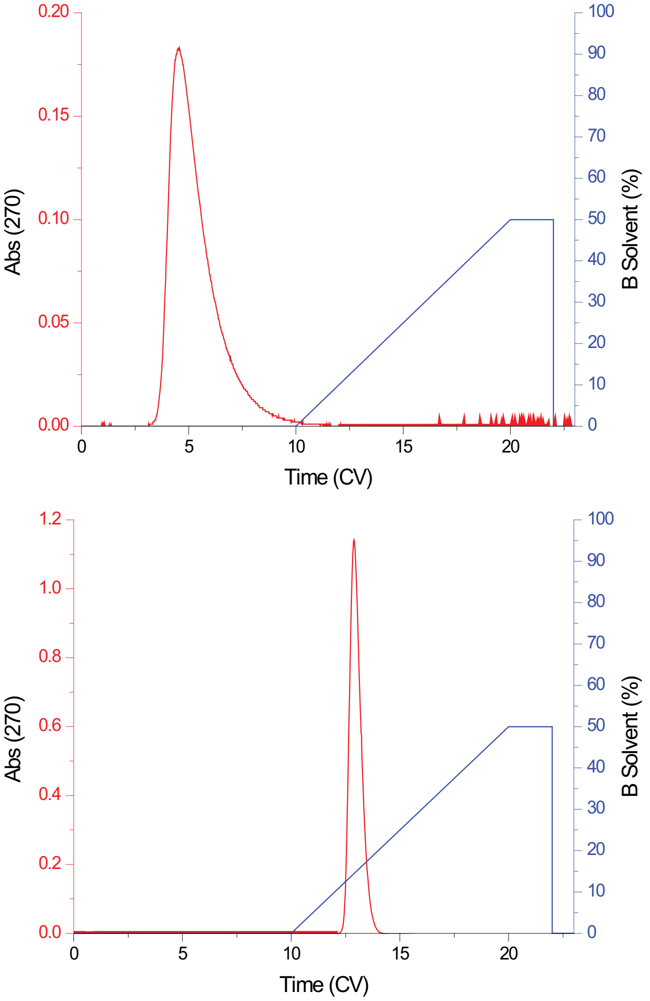

Figure 5: Washing the column with water (top) elutes the compound early compared to washing with methanol (bottom)

For the experiments in Figure 5, caffeine was run in the same fashion except that solvent A was freshly drawn and degassed 17.4 MΩ water. Water was able to displace caffeine from the column, causing it to elute (Figure 5, top) while using methanol as the A solvent caused the compound to be retained. The eluting solvent was 5% acetic acid in water in both experiments. The wash solvent could be a water-miscible solvent such as alcohols, acetonitrile, or acetone. The experiment in Figure 3 was washed with toluene, followed by 2-propanol.

SAX Examples

Running an SAX column uses a single gradient as the sample is displaced by acetate or formate. Concurrently, the pH is being lowered to generate the free acid.

Green Tea Extract

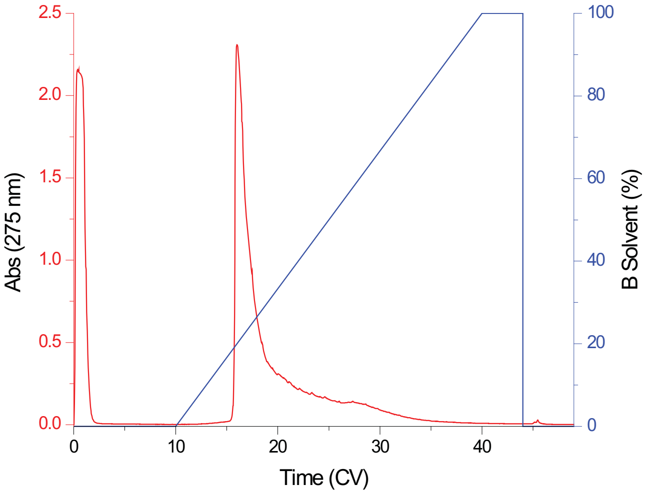

Figure 6: Green tea extract on a Redi*Sep* SAX column

An example of SAX purification is shown in Figure 6. A methanolic extract of green tea (0.75 g) was dissolved in methanol containing 5% ammonium hydroxide and the mixture was loaded onto a 17 g Redi*Sep*® Rf SAX column (PN 69-2203-392). The column was washed with 10 CV methanol to elute basic and neutral compounds (peak prior to 5 CV) followed by a gradient with 5% acetic acid in water. The anti-oxidants eluted after 15 CV.

Tar

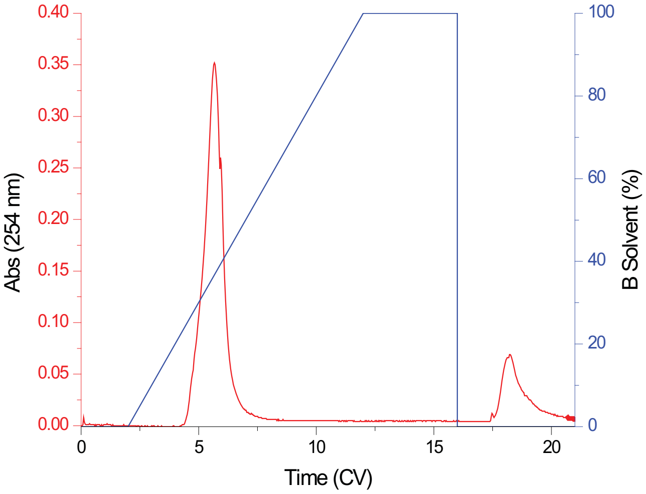

Figure 7: SAX purification of anionic components from tar

The tar sample (11.7 g) was dissolved in 25 mL toluene; 3 mL concentrated ammonium hydroxide was added. To this mixture was added 2-propanol until the mixture was homogeneous (5 mL). This was loaded onto a 17 g Redi*Sep*® Rf SAX column (69-2203-392) previously conditioned and then washed with toluene. The column was then manually washed with toluene to elute non-binding material followed by 10 CV 2-propanol. The acidic compounds were eluted with 5% acetic acid in water. For this run, some material eluted after the gradient was complete, likely an acidic compound that is insoluble in the aqueous eluting solvent.

A general solvent method for the SAX column would be:

Segment Length (CV)	%B	B Solvent (Acetic or Formic Acid)
Start	0	5% acid in water (B1)
10	100	5% acid in water (B1)
5	100	5% acid in water (B1)
0	0	5% acid in water (B1)
5	0	5% acid in water (B1)

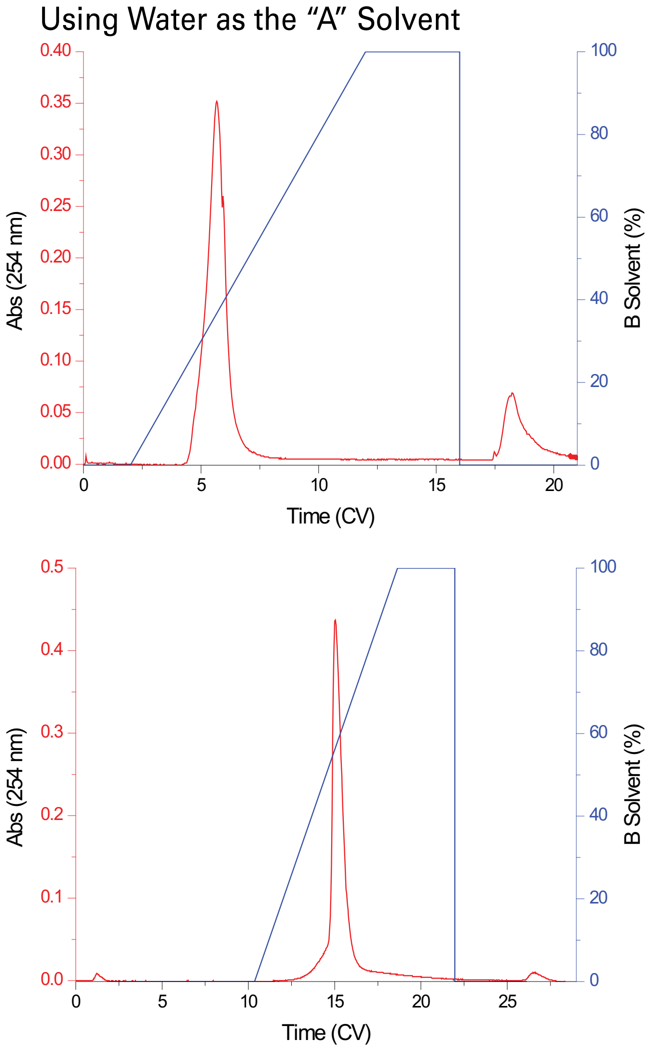

Figure 8: Column washed with water (top) and methanol (bottom); sample was retained better with methanol

In Figure 8, both experiments were run with 50 mg catechin. The column in the top example was washed with water while the column in the bottom example was washed with methanol. Similar to the SCX column, washing the column with water caused the sample to elute early. It is recommend, like in the SCX experiments, to use a minimal amount of water when working with compounds.

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