Purification of Alkaloids

with Redi*Sep*® Rf SCX columns

TELEDYNE ISCO
Everywhereyoulook™

Chromatography Application Note AN40

Abstract

The purification of alkaloids using Redi*Sep* Strong Cation Exchange (SCX) columns is described. Several alkaloids are used as models for those extracted from plant species. The alkaloids are separated from neutral and acidic compounds, and are purified from each other. Solvent switching is used to purify increasingly basic compounds.

Background

Why use SCX?

Ion exchange media separate molecules by net charge. Because ions have differing affinities for the ion exchange media, it is possible to selectively remove ions from solutions and release them later. SCX columns can be used in a "catch-and-release" mechanism where the basic compounds are removed from the crude mixture and released after the impurities are washed away. Alternatively, the ionic strength and pH of the solvent system can be altered to purify a collection of bases. Both methods were combined to isolate alkaloids from green tea extract.

Teledyne Isco Redi*Sep* Rf SCX columns consist of a sulfonic acid moiety bound to silica. Because the ion exchanger is chemically bound to silica, the media will not swell when organic solvents are used; Redi*Sep* Rf SCX columns can be used with solvents such as dichloromethane.

General Methods

Many small molecules of interest are weak bases and are difficult to adsorb onto SCX columns. Salts of these compounds are often very soluble in water, reducing interaction with the ion-exchange column when the sample is loaded. To overcome these issues, the samples can be dissolved in acidic solutions of organic solvent such as methanol. The presence of acid forces the alkaloids to have a positive charge, allowing interaction with the column.

Load capacity and determination of sample size

The load limit for ion exchange columns is determined by the number of ion exchange sites on the column, generally expressed in millimoles. To determine the sample load, use the following equation:

$$\text{Sample load (g)} = \frac{\text{mmol} \times \text{Compound Molecular Wt.}}{1000}$$

where *mmol* is read from Table 1.

Table 1: Load capacity for Redi*Sep* Rf SCX columns

SCX Column			
Part Number	Column Size (grams)	Maximum Sample Load (mmol)	Sample weight (MW=200 AMU, grams)
69-2203-390	5	1.8	0.35
69-2203-391	15	5.3	1.05
69-2203-392	30	10.5	2.1
69-2203-393	50	17.5	3.5
69-2203-396	100	35.0	7.0
69-2203-394	150	52.5	10.5
69-2203-398	275	96.3	19.25
69-2203-395	410	143.5	28.7

Table 1 lists various Redi*Sep* Rf SCX columns and their load capacities. The capacity, in milligrams, of a typical alkaloid is also listed. The small columns are useful for extracting small quantities of compound for screening and also for method development.

Column Storage

Redi*Sep* Rf ion exchange columns can be reused. After the run is complete, regenerate the column with ten column volumes of 0.1 N strong mineral acid (sulfuric or phosphoric acid), followed by washing with ten column volumes of water. Store the column in 80% methanol in water or 100% isopropyl alcohol.

Experimental and Results

Xanthine Alkaloids

Separate solutions of caffeine and theophylline were prepared by dissolving 200 mg of alkaloid in 20 mL methanol containing 5% glacial acetic acid. The mixture run on the column was made by mixing 2.0 mL of each solution. This mixture (40 mg alkaloids) was injected onto a 15 g Redi*Sep* Rf SCX column (PN 69-2203-391). Detection was at 210 and 270 nm. Solvent A was methanol; Solvent B was water containing 1% glacial acetic acid. The column was washed with 10 column volumes (CV) of methanol before initiating a linear gradient to 30% Solvent B over 20 CV.

The resulting chromatogram is illustrated in Figure 1.

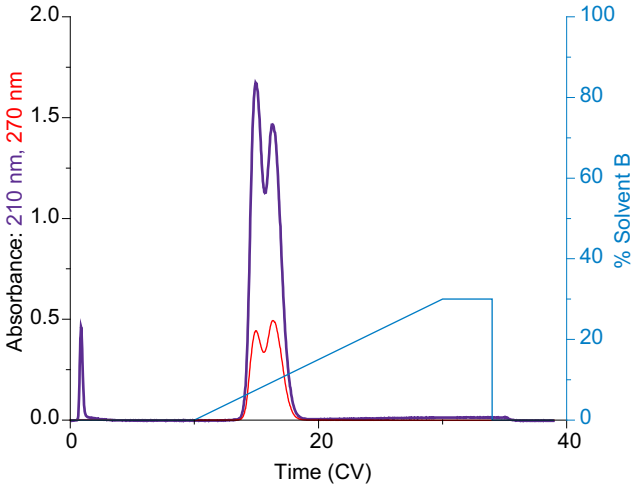

Figure 1: Catch and release of xanthine alkaloids
Caffeine is the first peak eluted.

Nicotine

Nicotine was run in a similar fashion as the xanthine alkaloids. Nicotine (100 mg) was dissolved in 1.0 mL methanol containing 5% acetic acid and injected onto a 15 g Redi*Sep* Rf SCX column. A gradient of 5% acetic acid was followed by a gradient of 5% ammonium hydroxide. The column was regenerated with 5% acetic acid. Detection was 260 nm.

The resulting chromatogram is illustrated in Figure 2

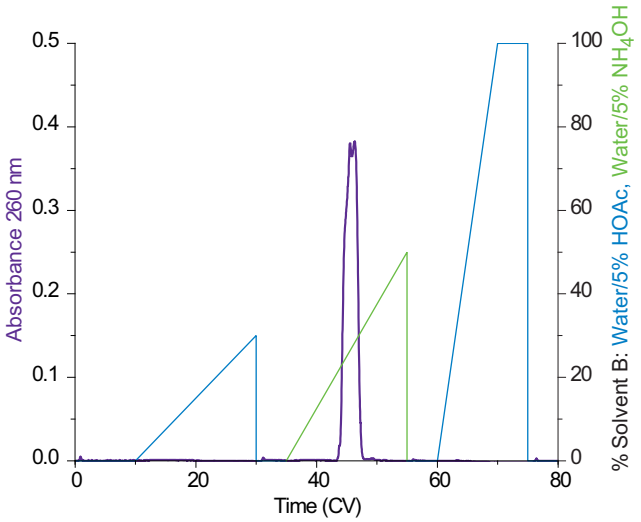

Figure 2: Catch and release of nicotine
on a Redi*Sep* Rf SCX column

Unlike the xanthine alkaloids, nicotine is not displaced by hydrogen ions but requires the "stronger" ammonium ions for elution.

Harmine and Harmaline

These compounds were run similar to nicotine. The alkaloid (50 mg) was dissolved in 1 mL methanol containing 5% acetic acid followed by injection onto a 15 g Redi*Sep* Rf SCX column. A gradient of 5% acetic acid was followed by a gradient of 5% ammonium hydroxide. The column was regenerated with 5% acetic acid. Detection was 245 nm (harmine) or 360 nm (harmaline). Both compounds were also detected with All-Wavelength Detection (range 220–360 nm).

The resulting chromatograms are illustrated in Figures 3 and 4.

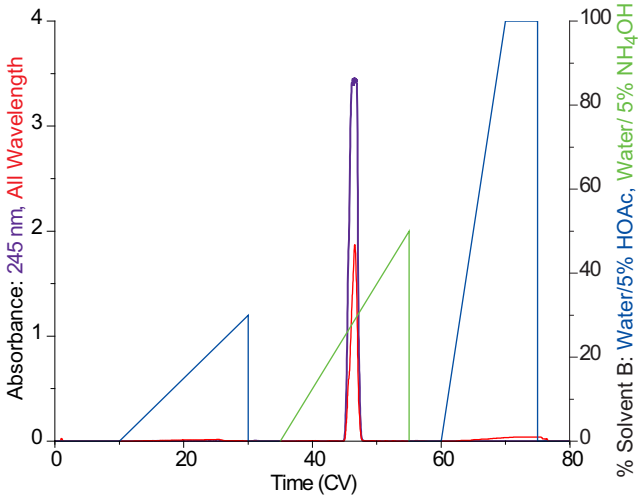

Figure 3: Catch and release of harmine on a Redi*Sep* Rf SCX column

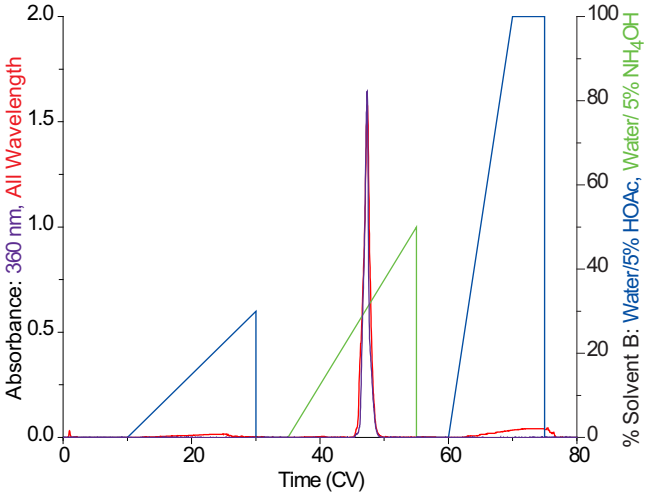

Figure 4: Catch and release of harmaline on a Redi*Sep* Rf SCX column

Like nicotine, harmine and harmaline were not displaced by the hydrogen ion, but required the ammonium ion to displace the alkaloid.

Green Tea Extract

Green tea extract is used as a model to demonstrate the technique using an actual plant extract. Green tea was extracted in methanol and the extract dried. A portion of this extract (0.524 g) was dissolved in 5.0 mL methanol containing 5% glacial acetic acid; the entire sample was injected onto a 15 g Redi*Sep* Rf SCX column (PN 69-2203-391). Detection was at 210 and 270 nm. A gradient using 5% acetic acid in water followed by a gradient containing 5% ammonium hydroxide in water was used to isolate the alkaloids.

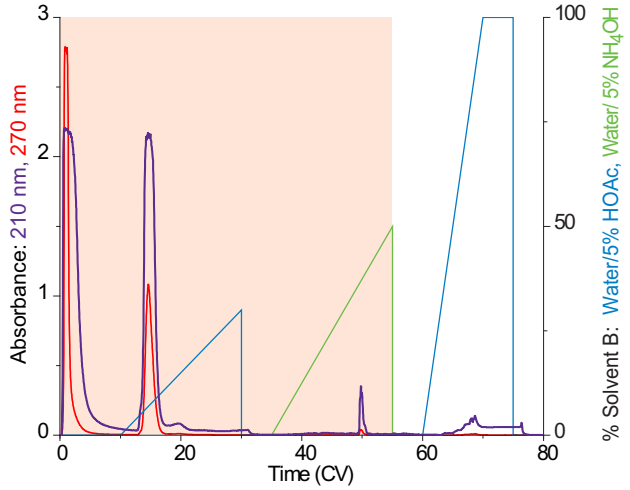

Figure 5: Purification of alkaloids from green tea extract. The time window (shaded region) excludes collection during the column wash/regeneration.

The non-polar and acidic compounds in the plant extract are washed off the column early in Figure 5. The alkaloids elute as the acetic acid gradient is run. Other basic compounds elute during the second gradient utilizing ammonium hydroxide. The column is regenerated in the last gradient for a subsequent purification. A time window was utilized so that fractions were only collected during the first two gradients and not during the column regeneration.

Acetic acid and ammonium hydroxide are used because they are relatively easily removed from the sample after purification compared to other acids or bases. Other useful acids include formic and trifluoroacetic acid.

Conclusion

Teledyne Isco Redi*Sep* Rf SCX columns are useful for isolating basic compounds. The columns can be used for "catch-and-release," or to purify a mixture of basic compounds. Redi*Sep* Rf SCX columns are useful for purifying alkaloids from complex mixtures. Several alkaloids were captured and released suggesting this technique to be general. Dissolving the alkaloids in an acid solution causes them to be cationic for capture. In the case of the xanthine alkaloids, dissolving them in methanol without any acid caused them to elute through the column without binding.

Last modified November 8, 2012

Teledyne Isco
P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091
E-mail: IscoInfo@teledyne.com