

Facile Isolation and Purification of Alkaloids with Strong Cation Exchange Columns on a Flash Chromatography System

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Abstract

The use of a Strong Cation Exchange (SCX) resin is demonstrated to isolate a variety of alkaloids using a “catch-and-release” mechanism. Automatic solvent switching is used to generate multiple gradients with cations of increasing strength that elute the alkaloids using a combination of ionic strength and pH control. The multiple gradients are used within the same purification run to isolate various alkaloids from the same crude extract; green tea extract is used as a model for other plant extracts. Linear gradients allow purification of related compounds from each other. The use of multiple gradients permits purification of unknown alkaloids without prior knowledge of their pKa.

Background

Ion exchange columns represent a powerful method to quickly purify desired natural products from the rest of the extract. One common method to isolate alkaloids include extracting them into an acidic aqueous solution, making the aqueous solution basic, followed by back extraction into an organic solvent. However, extraction does not allow families of alkaloids to be purified from each other. 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 basic compounds. Both methods were combined to isolate alkaloids from green tea extract. Teledyne Isco RediSep 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. RediSep Rf SCX columns can be used with solvents such as dichloromethane.

Experimental and Results

Sample Dissolution

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 were dissolved in acidic solutions of methanol. The presence of acid forces the alkaloids to have a positive charge, allowing interaction with the column. Using methanol caused better binding than dissolving the sample in water.

A 15 g RediSep Rf SCX column (PN 69-2203-391, Teledyne Isco, Lincoln NE) and CombiFlash Rf-200 system (PN 68-5230-006, Teledyne Isco) was used for all experiments. The same column was used for all experiments. All pure compounds were purchased from Sigma Aldrich (St. Louis, MO).

Xanthine Alkaloids

Separate solutions of caffeine and theophylline were prepared by dissolving 200 mg alkaloid in 20 mL methanol containing 5% glacial acetic acid. The mixture run on the column was 2.0 mL of each solution, mixed, and injected onto the column. Solvent A was methanol; Solvent B was water containing 5% glacial acetic acid. The gradient is shown in the chromatogram in *Figure 1*.

Caffeine and theophylline were both displaced by protons from the acetic acid. The two peaks are partially resolved showing that, in addition to being captured and released, the compounds can be partially resolved. Binding of the alkaloids to the column is demonstrated by their eluting only after the gradient is run, starting 10 column volumes (CV) into the run. Substituting water for methanol for Solvent A caused the alkaloids to elute early during the initial 10 CV column wash.

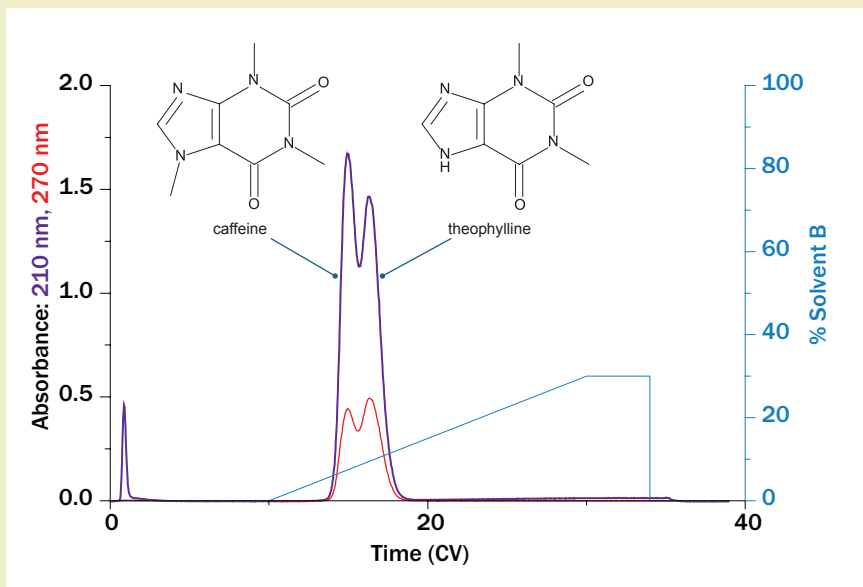


Figure 1

Capture and release of caffeine and theophylline using a SCX column

Nicotine

Nicotine (100 mg) was dissolved in 1 mL methanol containing 5% glacial acetic acid and injected onto the column. Solvent A1 was methanol; Solvent B1 was water containing 5% glacial acetic acid; Solvent B2 was water containing 5% ammonia. The gradient is shown in the chromatogram in *Figure 2*. Acetic acid and ammonia were chosen because they are volatile and easily removed from the purified product compared to other buffers. The third gradient is a wash with 5% acetic acid to recondition the column. Detection was 260 nm. The solvents were automatically switched on the Rf-200 to create the sequential gradients.

Nicotine eluted only during the ammonia gradient.

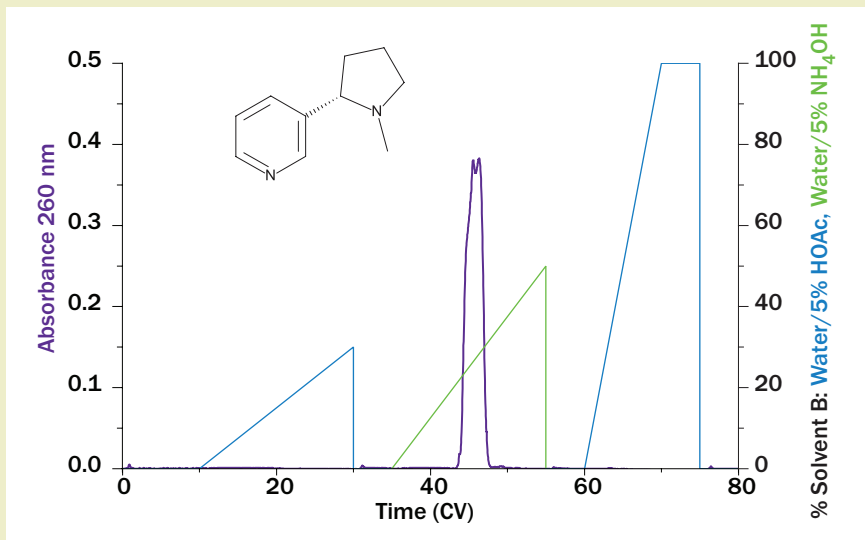


Figure 2

Capture and release of nicotine using a SCX column

Harmine and Harmaline

The alkaloid (50 mg) was dissolved in 1 mL methanol containing 5% glacial acetic acid and injected onto the column. The solvent system and gradients were the same as for nicotine. Detection was at 245 nm for harmine and 360 nm for harmaline; both compounds were also detected with All-Wavelength Collection (220 – 360 nm, 2 minute width).

Both harmine and harmaline were captured by the RediSep Rf SCX column (*Figure 3*). Both compounds eluted at the same time during the second ammonia gradient.

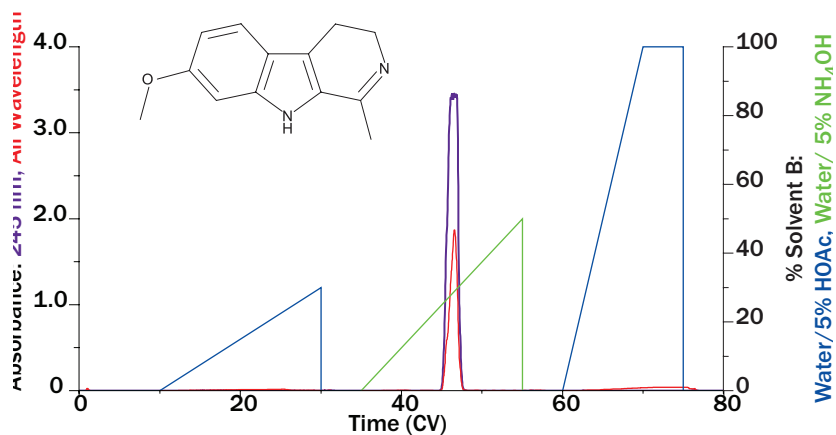
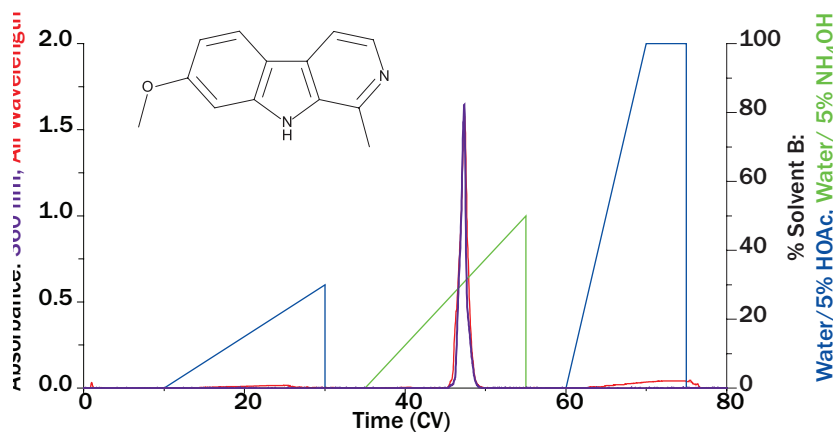


Figure 3

Capture and release of harmine (top) and harmaline (bottom)

Green Tea Extract

Green tea extract is used as a model for other purifications because, in addition to the alkaloids, it also contains other materials in the plant extract that could possibly interfere with alkaloid binding. Green tea was purchased at a grocery store in Lincoln, NE. A mass of 150 g was extracted with methanol. The extract was evaporated under vacuum; 0.5 g was dissolved in 5 mL methanol containing 5% glacial acetic acid and run using the same gradients and solvents as used for nicotine, harmine, and harmaline described previously. Detection was 210 and 270 nm.

Figure 4 shows that acidic and neutral compounds eluted from the column with methanol. Xanthine alkaloids eluted with the acetic acid gradient while an unknown compound eluted during the ammonia gradient demonstrating that the technique is useful for purifying families of alkaloids.

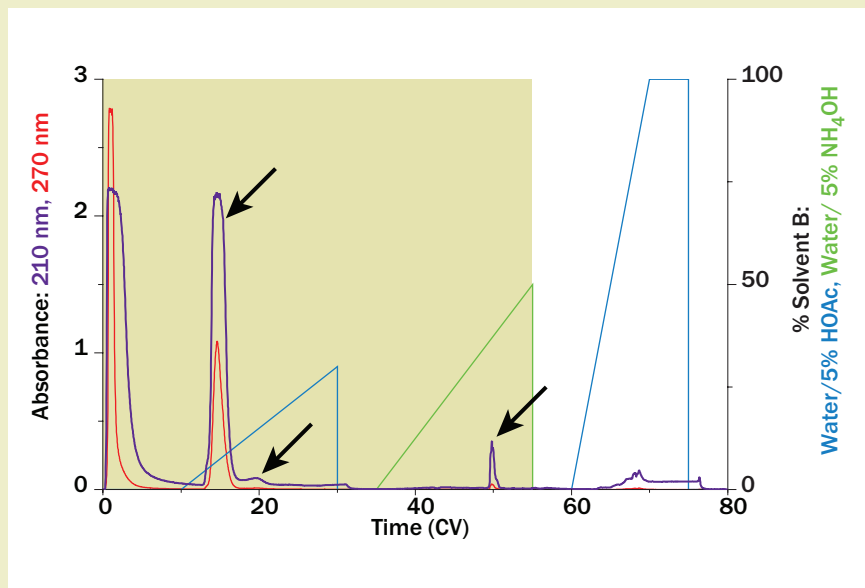


Figure 4

Purification of alkaloids in green tea extract. The shaded area denotes a time window used to collect alkaloids and exclude wash gradient.

Conclusions:

- A multiple-gradient system utilizing sequential acetic acid and ammonia gradients was demonstrated to purify alkaloids without knowledge of their pKa.
- This gradient works with a variety of basic compounds and therefore represents a general procedure.
- Dissolving the alkaloids in acidic methanol allowed the compounds to bind to the SCX column. Only the minimum volume of water required to dissolve the sample should be added to the methanol.
- RediSep Rf SCX columns provide an efficient means of purifying alkaloids.
- Flash chromatography provides a viable technique for these and related ion exchange separations.

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