Alkaloid Purification Strategies Using Flash Chromatography

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Abstract

Alkaloids are common compounds derived from natural products. Many alkaloids are also synthesized for their medicinal properties. They are challenging to purify on silica due to their polarity and basicity. The use of appropriate solvents and columns allow facile purification of this class of compounds. Examples are provided for silica, C18, ion exchange, and alternative media such as diol and amine columns.

Background

Alkaloids include a diverse class of nitrogen containing compounds that often exhibit pharmacological effects. These compounds are often found in plants, although they are also commonly synthesized compounds. As these compounds are ubiquitous in medicinal chemistry, strategies to purify this class of compounds is of great interest.

Experimental and Results

All experiments were run on a Combi*Flash*® Rf 200 Flash chromatography system (PN 68-5230-006). Pure chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Other details are described in each section below. All columns were Redi*Sep* Rf Gold® packed with spherical 20–40 micron media.

Silica Strategies

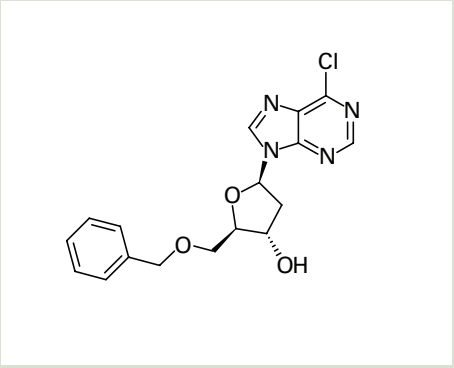
Silica is the most common means to purify alkaloids because the media is relatively inexpensive and silica can be used to purify a wide variety of compounds. Choosing appropriate methods to purify alkaloids can be difficult due to the polarity range exhibited by these compounds. Some useful guidelines include:

- Less polar alkaloids require less polar solvents such as hexane/ethyl acetate. These alkaloids have nonpolar substituents or few basic centers (nitrogen).
- Alkaloids of intermediate polarity are purified using dichloromethane and methanol.
- Very polar alkaloids can be purified with mobile phases containing a large percentage of methanol or water with Redi*Sep* Rf Gold silica.

An example of a relatively nonpolar alkaloid is shown in **Figure 1**. The non-polar substituents on the purine ring allow purification with gradients from 50 to 75% ethyl acetate[1]. As the number of substituents decrease, or as they become more polar, dichloromethane and methanol are required to elute the compound.

Figure 1

The polarity of 9-(5-O-benzoyl-b-D-2-deoxyribofuranosyl)-6-chloropurine is dominated by the non-polar side chain which allows purification with a hexane/ethyl acetate gradient.

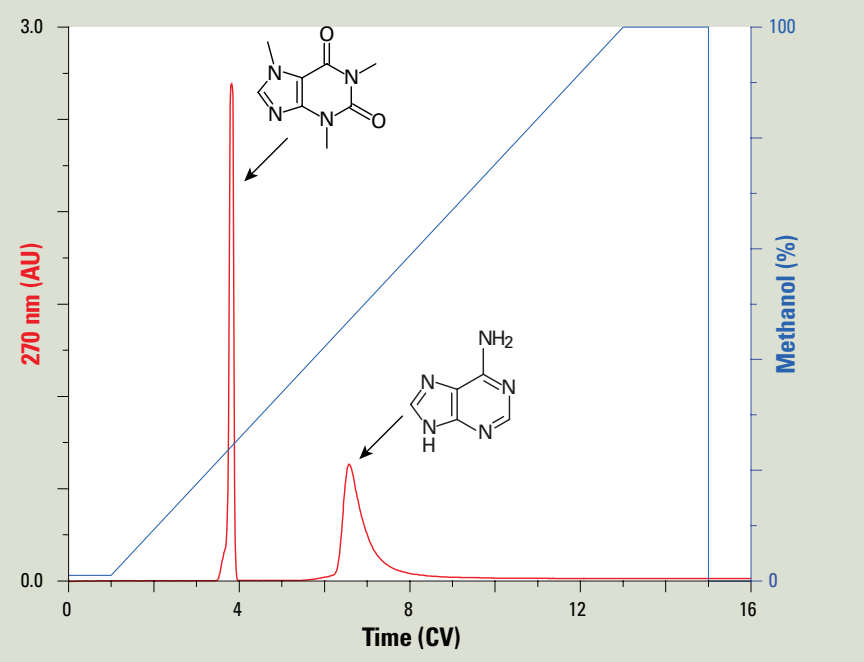


Redi*Sep* Rf Gold spherical silica is compatible with high concentrations of methanol making these columns useful for purifying very polar alkaloids. The columns can be run to 100% methanol.

Adenine (145 mg) and caffeine (200 mg) were adsorbed on 1.3 g silica and dried. A 24 g Redi*Sep* Rf Gold silica column was used (PN 69-2203-346). The gradient started at 1% methanol and ran to 100% methanol (**Figure 2**). The column was equilibrated at 1% methanol prior to sample introduction using a solid load cartridge.

Figure 2

Purification of adenine from caffeine using a dichloromethane/methanol gradient on Redi*Sep* silica columns.

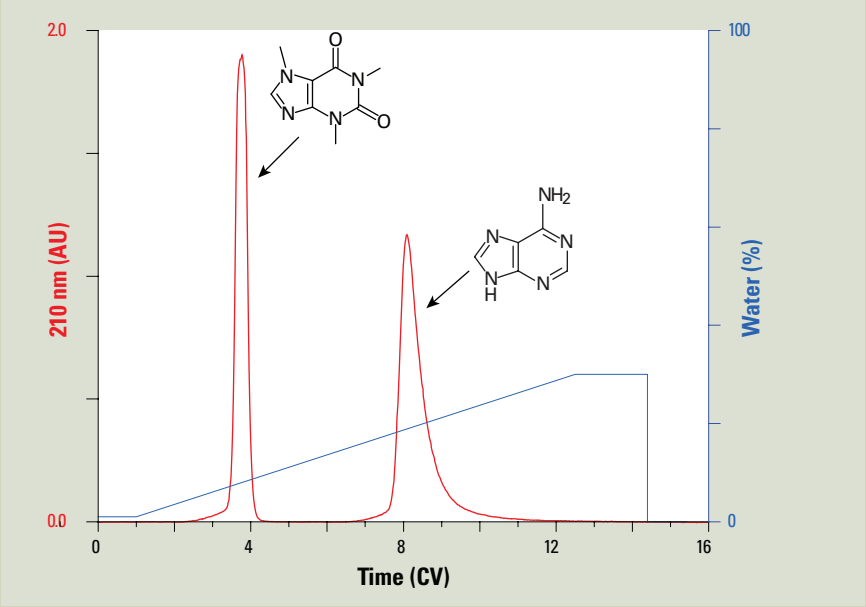


Using *Aqueous Normal Phase* is another way of purifying the alkaloids (**Figure 3**). Water can be used as a "greener" alternative to chlorinated solvents with Redi*Sep* columns. No degradation of the Redi*Sep* Rf Gold silica was noted running either in 100% methanol or in water.

Adenine and caffeine (145 and 200 mg, respectively) were adsorbed on 1.3 g silica and dried. A 24 g Redi*Sep* Rf Gold silica column was used (PN 69-2203-346). The column was equilibrated and gradient started at 1% water and ran to 30% water.

Figure 3

Purification of adenine from caffeine using a methanol/water gradient.



Amine Column Strategies

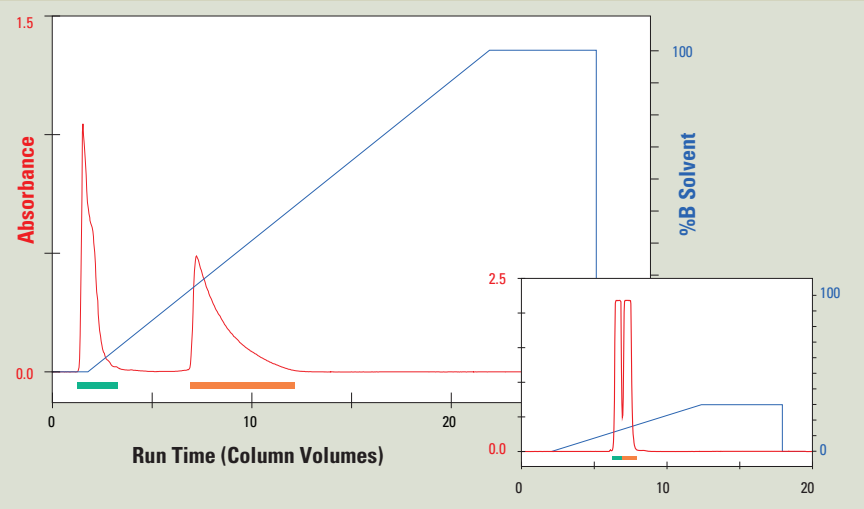
An alternative to silica is the amine column.

Both columns were loaded with 0.15 g total alkaloids. The amine column was a 15.5 g Redi*Sep* Rf Gold Amine column (PN 69-2203-505) using an acetonitrile/water gradient (water = solvent B); a 12 g Redi*Sep* Rf Gold Silica (PN 69-2203-345) column was eluted with a dichloromethane/methanol gradient (inset).

The amine column under HILIC (**H**ydrophilic **I**nteraction **LI**quid **C**hromatography) conditions exhibited greater resolution between the alkaloids compared to silica (**Figure 4**). In addition, the purification was achieved without the use of chlorinated solvents. Both compounds eluted with less than 50% water; greater resolution could be achieved by reducing the maximum gradient to 50% water. The amine column can also be used with organic solvents as a normal phase column[2].

Figure 4

Caffeine and theophylline are easily resolved on an amine column compared to standard silica media (inset).



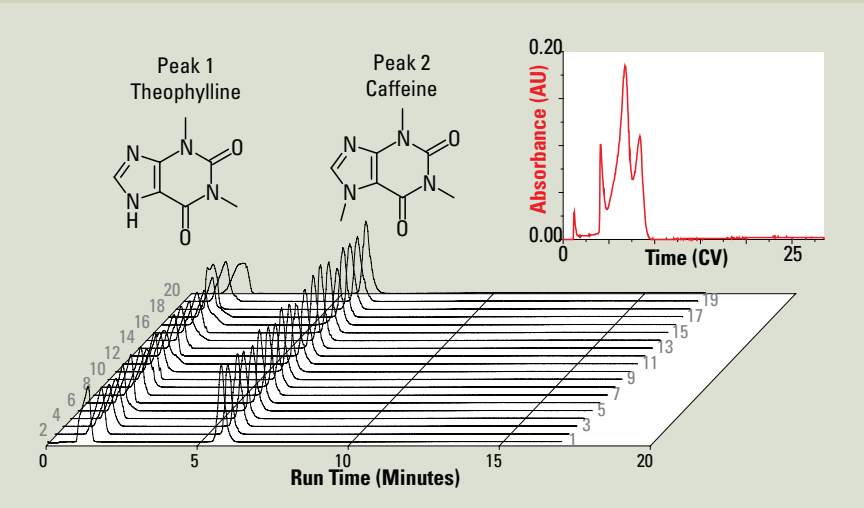
C18 Strategies

Caffeine and theophylline were dissolved in water and repeatedly injected onto a Redi*Sep* Rf Gold C18 15.5 g column. A standard C18 method was run on a Combi*Flash* Rf 200 system with a gradient from 5 to 100% B. Solvent A was deionized water adjusted to a pH of 10.0 with ammonium hydroxide; solvent B was methanol. The compound mixture was injected 20 times. Prior to each run, the column was equilibrated with 5 column volumes of 5:95 methanol:pH 10 water.

An end-capped C18 column, such as the Redi*Sep* Rf Gold, can be run many times at a high pH without degradation. This is useful when the alkaloids fail to resolve at low pH, such as the commonly used 0.1% TFA (**Figure 5**, inset). Running at high pH is useful for acid labile compounds. Like TFA, ammonia is volatile and can be removed during lyophilization.

Figure 5

Caffeine and theophylline are easily and reproducibly resolved on Redi*Sep* Rf Gold C18 at high pH compared to low pH with TFA (inset).



Diol Strategies

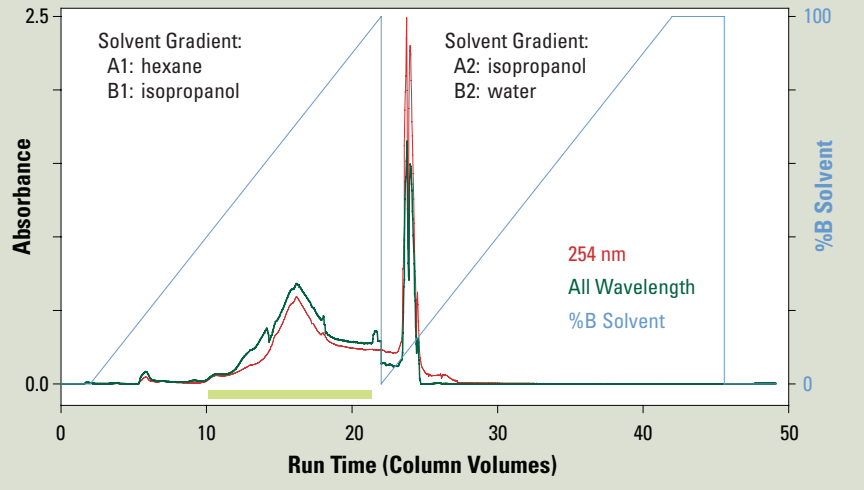
Like the amine column, diol can be used in normal phase separations with organic solvents[3] and with water. Being relatively non-polar, diol can elute a wide variety of compounds including those that might bind to silica. Diol is useful for "Wide-Polarity Range" chromatography where the elution begins in a nonpolar solvent and ends with water. This is a useful technique for natural products where the desired product is an unknown compound.

Green tea methanolic extract (1 g) was dissolved in methanol and adsorbed onto Celite 545 (Acros Organics) in a Redi*Sep* solid load sample cartridge (PN 69-3873-235). The compound was eluted with a hexane/isopropanol gradient (**Figure 6**) followed by an isopropanol/water gradient on a Redi*Sep* Rf Gold Diol column (PN 69-2203-371). The alkaloids and catechin compounds eluted together while the tannins eluted early in the water gradient. Fractions were collected using the All-wavelength Collection feature on the Combi*Flash* Rf 200 system.

A single gradient run captured nearly all the compounds ranging from very nonpolar to water soluble. The diol column is functionalized and can be reused many times.

Figure 6

Alkaloids are purified between 10 and 20 CV using a diol column.

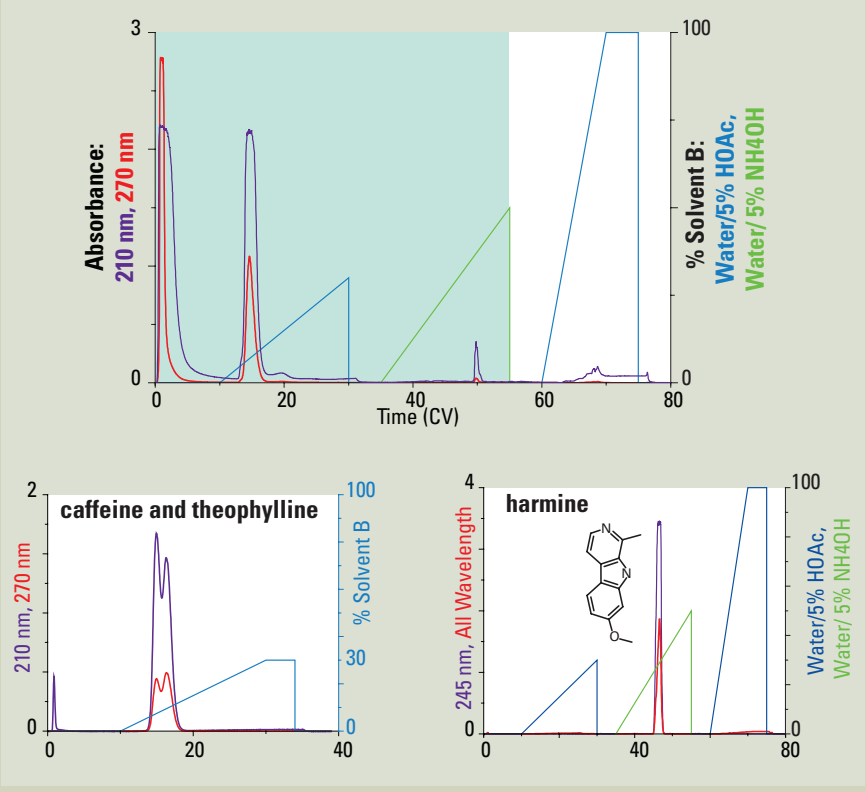


Ion Exchange Strategies

Alkaloids were dissolved in methanol containing 5% glacial acetic acid and injected onto a 15 g Redi*Sep* Rf SCX column (PN 69-2203-391). Xanthine alkaloids (caffeine and theophylline) eluted with a water gradient containing 5% acetic acid (**Figure 7**, upper inset). Harmine failed to elute with acetic acid, but required a gradient using 5% ammonium hydroxide in water to elute (**Figure 7**, lower inset). The main image in **Figure 7** used both gradients to purify alkaloids found in green tea; most alkaloids eluted during the acetic acid gradient but another alkaloid eluted during the ammonium hydroxide gradient.

Figure 7

Capture and release of alkaloids with an SCX column. Lower-left inset shows caffeine and theophylline; lower-right inset shows harmine; main illustration shows purification of green tea alkaloids. Shaded area denotes time window used to only collect compounds of interest.



Conclusions

- Silica is useful to purify many alkaloids. It can be used with methanol and water for very polar alkaloids without degradation.
- Amine is a useful alternative to silica with a different selectivity. It can be run as a normal phase column, including aqueous normal phase.
- Redi*Sep* Rf Gold C18 can be used under basic conditions without degradation to purify compounds that are difficult to resolve using TFA.
- Diol columns are useful for purifying natural products when the desired compound is unknown because they can be used over a wide polarity range.
- Ion exchange columns work well in a "catch-and-release" mode for purifying alkaloids from acidic and neutral compounds. The double-gradient is a powerful and flexible technique for purifying families of compounds.

References

[1] Ikejiri, M.; Ohshima, T.; Kato, K.; Toyama, M. Murata, T.; Shimotohno, K.; Maruyama, T. *Bioorg. Med. Chem.* **2007**, 15, 6882–6892

[2] Silver, J.E.; Bellinghausen, P.; Fowler, N. Pipes, R. *Method development strategies for amine bonded phase columns for medium pressure liquid chromatography*. Presented at the 239th meeting of the American Chemical Society, March 2010, poster MEDI 79

[3] Silver, J.E.; Bellinghausen, P.; Fowler, N. Pipes, R. *Diol columns– pretend they're normal phase.* Presented at the 239th meeting of the American Chemical Society, March 2010, poster MEDI 78

Part Numbers (PN) are Teledyne Isco catalog numbers, unless otherwise stated.

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