Method Development Strategies for Amine Bonded Phase Columns for Medium Pressure Liquid Chromatography

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Abstract

Amine is a useful alternative to silica gel because it is less polar and has a basic character. Compounds that require basic mobile phase modifiers such as triethyl amine or ammonia on silica can be purified without these additives on amine columns, simplifying the purification. An amine column can be run as a normal phase or reverse phase column, which can cause confusion for method development.

Treating the amine column as if it were normal phase for all solvents simplifies method development for these columns. Amine columns can be changed between solvent systems and are reusable, facilitating method development. Examples of method development using amine TLC and columns are provided.

Background

Amine bonded phase columns are a useful substitute to bare silica columns. They can be reused many times while producing reproducible chromatograms. They are less polar than silica which allows them to be used for a wide range of compounds. They also are compatible with a wide range of solvents from hexane through water.

Users are often confused about how to develop methods for amine columns. This confusion stems from comments indicating that amine columns are "useful in normal or reverse phase[1]". Although amine columns can be run in either fashion, a user is confused about which mode and solvents to use.

Treating the amine column entirely as normal phase greatly simplifies method development for most compounds. The column can be run in normal phase with solvents considered reversed phase, such as isopropanol and water, although this isn't commonly required. This allows methods to be rapidly developed on amine thin layer chromatography (TLC) plates.

Experimental

TLC Method Development

Redi*Sep*® amine TLC plates (P/N 69-2203-573, Teledyne Isco, Lincoln, NE, 68512, USA) were used to develop methods. Solvents were ACS grade from BDH (VWR, Brandywine, PA, USA) unless otherwise specified. Purifications were run on a Combi*Flash*® Rf 200 (P/N 68-5230-006, Teledyne Isco). Test compounds were from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Redi*Sep* Rf Gold® silica columns were used for comparison because they have spherical particles in the same size range as the Redi*Sep* Rf Gold amine columns.

Purification of 3-(2-nitrophenyl amino) propionitrile

Crude 3-(2-nitrophenyl amino) propionitrile from another experiment was used. Methods were developed with Redi*Sep* silica TLC plates (P/N 69-2203-400, Teledyne Isco) and Redi*Sep* amine TLC plates. The Rf-to-Gradient program in the Peak*Trak*™ software in the Combi*Flash* Rf 200 system was used to develop a purification gradient. The compound was purified with a 12 g Redi*Sep* Rf Gold silica column (P/N 69-2203-345, Teledyne Isco) or 15.5 g Redi*Sep* Rf Gold amine column (P/N 69-2203-505, Teledyne Isco). Detection was at 254 nm.

Purification of Xanthine Alkaloids

Equal amounts (4.0 g each) of caffeine (J.T. Baker, Phillipsburg, NJ) and theophylline were dissolved in dichloromethane and methanol. This mixture was added to 72.0 g Celite 545 (Acros Organics, NJ) to make a slurry. The solvents were evaporated until dry. TLC plates were run for method development. In addition, a 5.5 g Redi*Sep* Rf Gold amine column (P/N 69-2203-504) was used for method development. The mixture was scaled to run on a 15.5 g Redi*Sep* Rf Gold amine column using the Peak*Trak* method scale-up feature; this column was run at a 1% and 10% total sample load (1.5 or 15.5 g sample/Celite mixture; 0.15 or 1.5 g total alkaloids). A 12 g Redi*Sep* Rf Gold silica column was run at 1% sample load with the same method. Compounds were detected at 270 nm with a Combi*Flash* Rf 200 system.

Results and Discussion

TLC Method Development

One premise to method development is that TLC plates are able to predict when the compound would elute from a column. A series of TLC experiments were run to confirm that the amine TLC plates both acted in a manner consistent with normal phase chromatography and were able to predict how compounds would be able to elute from columns.

With a minimal amount of compound, TLC plates can be used to rapidly scout solvent systems and predict chromatography. The relationship between the TLC retention factor (Rf) and the elution time for a compound is Rf=1/CV where CV= the number of column volumes[2].

Table 1 demonstrates that amine TLC plates provide a reasonable prediction of how a variety of compounds will behave in a column. As the polarity of the solvent is increased, the compounds show higher retention factors demonstrating that the amine is running as normal phase. This predictability is useful because the Rf-to-Gradient feature in the Peak*Trak* software may be used to optimize the method for improved purification. Amine columns and TLC plates can be run with a variety of solvents.

Table 1
TLC of compounds on amine plates with comparisons of estimated and actual retention times using various solvents.

Compound	Hexane %	Ethyl Acetate %	Rf	Predicted Retention (CV)	Actual Retention (CV)
caffeine	50	50	0.23	4.4	4.5
nicotine	50	50	0.66	1.5	1.5

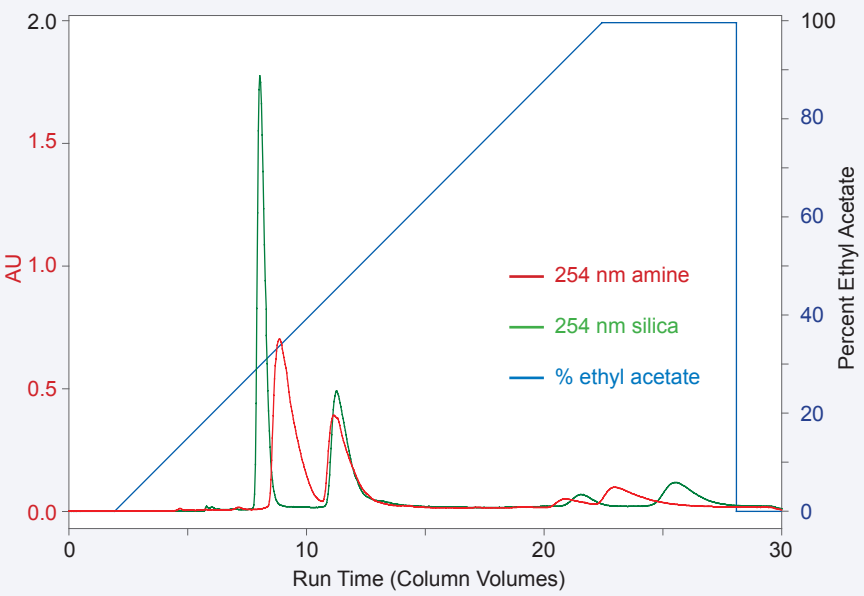
Compound	Hexane %	Isopropanol %	Rf	Predicted Retention (CV)	Actual Retention (CV)
phenol	80	20	0.45	2.2	2.4
	60	40	0.65	1.5	1.7
hydroquinine	80	20	0.11	9.1	10.4
	60	40	0.33	3.3	3.6

Compound	Dichloro-methane %	Methanol %	Rf	Predicted Retention (CV)	Actual Retention (CV)
caffeine	100	0	0.3	3.3	1.5
	80	20	0.4	2.6	1
theophylline	80	20	0	No elution	5

Purification of 3-(2-nitrophenyl amino) propionitrile

This experiment demonstrates the utility of a amine column in normal phase with a compound readily purified on silica gel. Figure 1 shows the amine column generates a similar chromatogram as silica gel. Compounds generally elute slightly earlier due to the reduced polarity of amine compared to silica gel. TLC plates indicated that the mixture could be purified on either silica or amine.

Figure 1
Purification of 3-(2-nitrophenyl amino) propionitrile on silica and amine columns using a hexane/ethyl acetate gradient



Purification of Xanthine Alkaloids

A small column was used to develop a method for caffeine and theophylline. The run was completed in ~15 minutes, including column equilibration. The method was scaled up from a 5.5 g to a 15.5 g column using the scale-up feature in the Peak*Trak* software in the Combi*Flash* Rf 200 (Figure 2). The alkaloids are better purified on the amine column as compared to silica. It was noted that caffeine was the first peak eluted on the amine column and second on the silica.

Due to the greater peak spacing, the amine column was able to purify 10 times the sample load as the silica column (Figure 3).

Figure 2
Scale-up and purification of caffeine and theophylline on amine and silica columns with a dichloromethane/methanol gradient

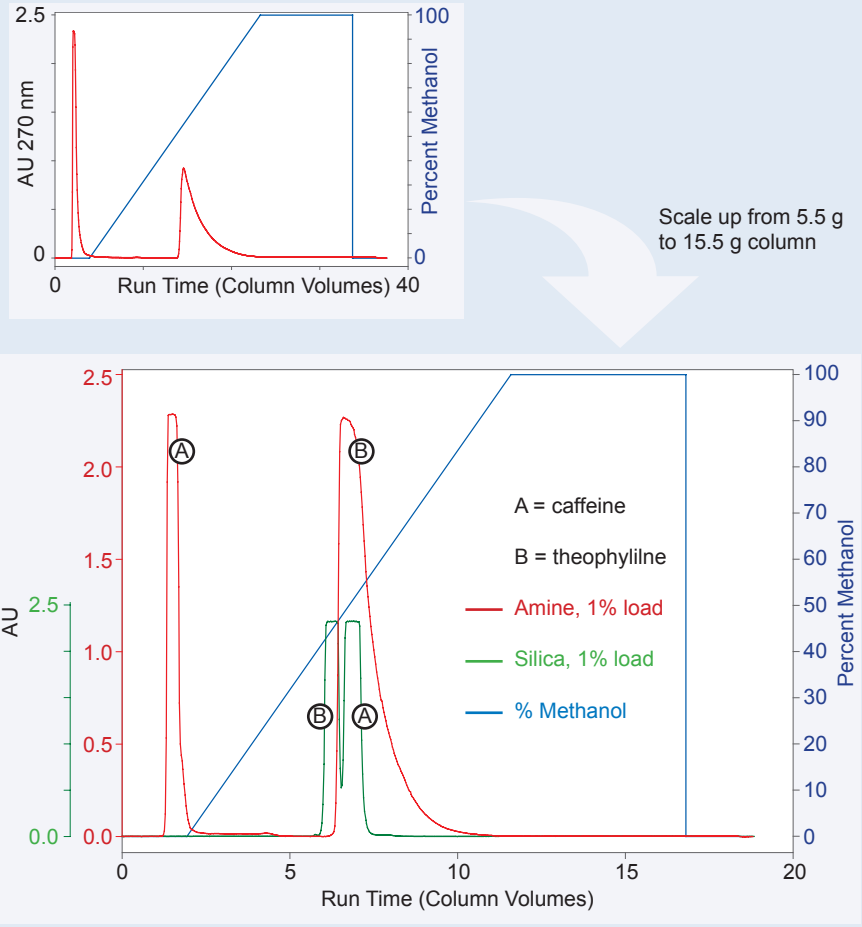
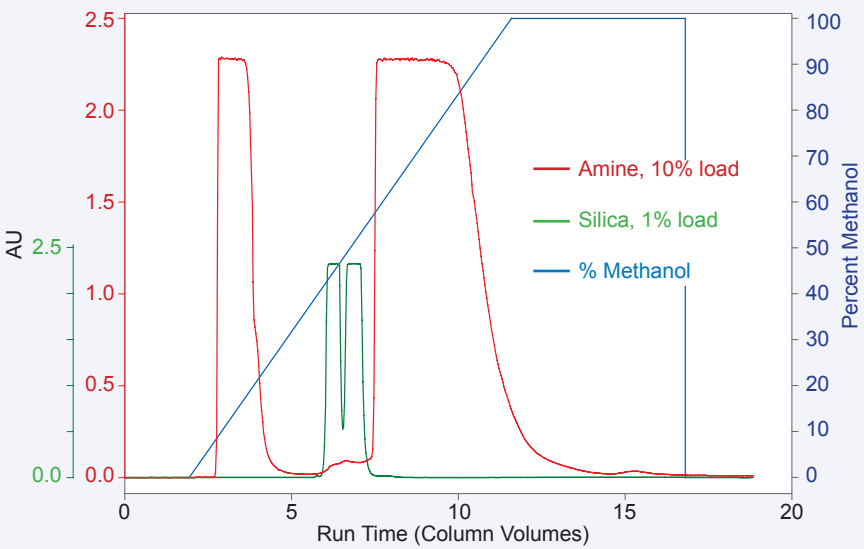


Figure 3
Purification of caffeine and theophylline on an amine column with a heavy (10%) sample load with a dichloromethane/methanol gradient. The silica column was limited to a 1% load.



Conclusion

Redi*Sep* Rf Gold amine columns are a useful alternative to silica columns. Method development is simple; they can be simply considered to be normal phase columns. TLC plates can be used in the same fashion as silica gel to determine the best solvent system to purify compounds. A method can also be developed on a small column and scaled up using the PeakTrak software on the Combi*Flash* instrument.

Amine columns possess different selectivity than silica, which in many cases may allow purifications to be more easily performed. Amine columns also may allow increased loading. Although none of the purifications in this poster required modifiers, the basic character of amine columns often precludes the need for basic modifiers that may be required for silica gel.

[1] http://www.chromatographyshop.com/html/polar_bonded_phase.html, 23 Feb 2009

[2] Effective Organic Compound Purification–Guidelines and Tactics for Flash Chromatography, 4th Ed., Teledyne Isco, 2010, p.9

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