Redi*Sep*® amine functionalized column

Purification of high pKa organic compounds Case Study 1

Mikael Mahler
Teledyne Isco, Inc.

TELEDYNE ISCO
Everywhereyoulook™

Chromatography Application Note AN31

Overview

Organic compounds holding inherent basic properties (*e.g.* amines or nitrogen containing heterocycles) present technical challenges at the purification stage via normal phase silica flash chromatography.

This application describes a practical and efficient purification of a mixture of basic heterocycles using Isco Redi*Sep* amine functionalized column.

Background

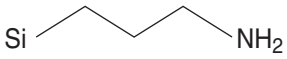
Acidic and basic organic compounds interact with residual surface silanol groups on normal phase chromatographic support, resulting in peak streaking and tailing. Streaking and tailing will ultimately cause multiple fractions and overlapping fractions during chromatographic purification.

Typically, chemists add a mobile phase modifier to reduce peak tailing and sharpen peaks, thereby generating maximum resolution for a separation of basic or acidic compounds. For components containing basic moiety, triethylamine or ammonium hydroxide are common modifiers added to the mobile phase.

Two time-consuming issues are usually encountered after adding a mobile phase modifier. First, the mobile phase modifier (TEA or NH_4OH) remains after evaporation of the volatile solvents, usually dichloromethane and methanol. Removing this involves additional extraction or washing with a suitable solvent, or concentrating the mixture down to an oil and placing the oil on a high vacuum overnight.

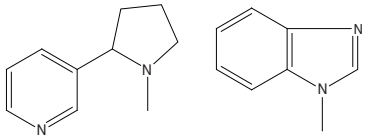
Second, the solvent system needs to be swapped and primed on an automated flash chromatography device, usually from a hexane/ethyl acetate to a dichloromethane/methanol solvent system. A purge and solvents switch after the run may also be needed.

The use of an amine functionalized Redi*Sep* column offers an efficient and user-friendly alternative to normal phase silica associated with the need of a mobile phase modifier. Amine functionalized silica is a carbon tether end-capped with a primary amine functionality and can be used under normal or reverse phase conditions.



Results and Discussion

The separation of a mixture of nicotine and 1-methylbenzimidazole was investigated.



nicotine (A) 1-methylbenzimidazole (B)

Analytical and preparative separations of the mixture on normal phase and amine functionalized silica were examined.

TLC runs on normal phase silica plates show the effect of the mobile phase modifier on the mixture spots form. The sharpness of the peak is enhanced by adding ammonium hydroxide or triethylamine providing a slightly improved separation resolution (Figure 1).

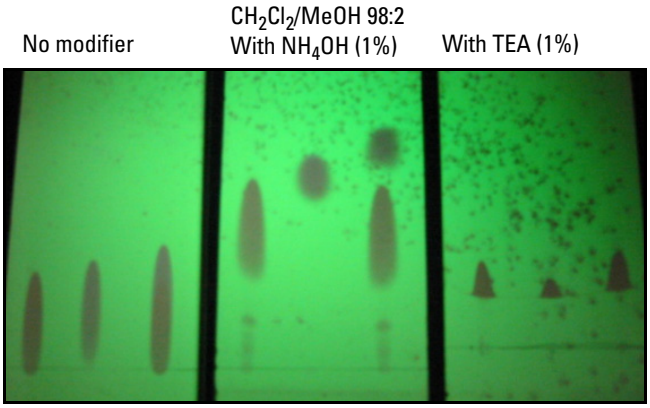


Figure 1: Normal phase silica TLC plates of heterocycles mixture Left spot: Nicotine; Middle spot: 1-methylbenzimidazole; Right spot: Mixture of nicotine and 1-methylbenzimidazole.

TLC runs on amine functionalized silica plates show a mixture of heterocycles resolved in one slightly streaking spot and one plain spot, predisposing a successful preparative separation of the two heterocyclic compounds using an amine functionalized silica column (Figure 2).

Indeed, flash chromatography of the heterocycles mixture on an amine functionalized Redi*Sep* column fully separated the products with hexane/ethyl acetate as the mobile phase (Figures 3 and 4). The normal phase column did not separate them correctly.

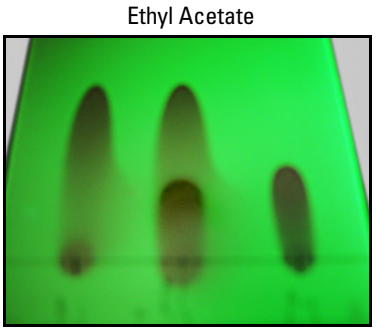


Figure 2: Amine functionalized phase silica TLC plates of heterocycles mixture Left spot: Nicotine; Middle spot: Mixture of nicotine and 1-methylbenzimidazole; Right spot: 1-methylbenzimidazole.

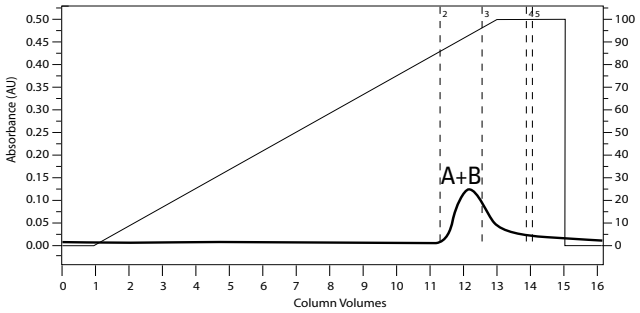


Figure 3: Chromatogram of normal phase column elution with hexane/ethyl acetate Heterocycles mixture did not separate.

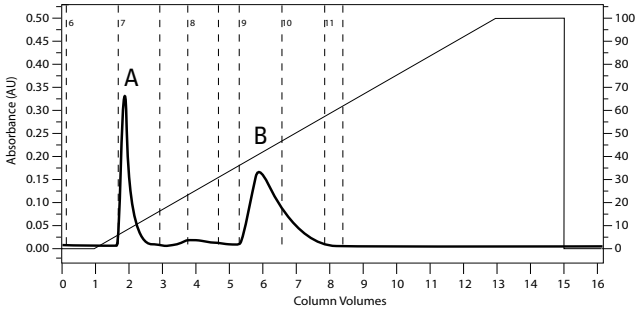


Figure 4: Chromatogram of amine functionalized column with hexane/ethyl acetate Heterocycles mixture successfully separated.

To decide which stationary phase to use for a flash chromatography purification, method development is helpful.

When TLC plates are available for stationary phases under consideration, method development begins by investigating whether an exploitable optimal selectivity with limited streaking can be obtained. TLC trials are run on the corresponding TLC plates using various solvent systems.

Without TLC plates, method development consists of running small-scale purifications using RediSep columns on a Combi*Flash*® automated flash chromatography instrument. Sample sizes are kept small, *e.g.* 30 to 40 mg, to avoid committing the full sample batch. The smallest-size specialty media Redi*Sep* column being considered is examined with various solvent systems.

Once mobile and stationary phases for optimal sample purification are identified, the full sample batch can then be subjected using the appropriate column size with respect to the crude sample quantity and the resolution observed during method development.

Redi*Sep* amine functionalized columns may also be used as a scavenger for acid chlorides. Redi*Sep* amine columns are reusable if stored under isopropanol immediately after each use.

Experimental

Table 1: Method Parameters and Results

Instrumentation:	Teledyne Isco Combi*Flash*® Companion™ 4x	
Columns	4g Normal Phase Redi*Sep* 4.7g Amine Functionalized Redi*Sep*	
Sample Loading Method	66 mg pre-loaded on celite	
Wavelength	254 nm	
Mobile phase:	Solvent A: Hexane	Solvent B: Ethyl Acetate
Flow Rate:	18 mL/minute	
Equilibration Volume:	3 column volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	1.0
	100	12.0
	100	2.0
	0	0.0
	0	1.0
Recovery yields:	nicotine (A): 95%	1-methylbenzimidazole (B): 99%

Conclusion

The analytical and preparative separation of a mixture of two heterocycles holding basic properties was successfully achieved without the presence of a mobile phase modifier by using amine functionalized silica as the stationary phase.

Amine functionalized Redi*Sep* columns offer chemists a highly practical and efficient tool for high pKa organic compounds separation.

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