RediSep® basic alumina column

Purification of high pKa organic compounds
Case Study 1

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Chromatography Application Note AN35

Overview

Purifying organic compounds holding inherent basic properties (*e.g.* amines or nitrogen-containing heterocycles) present technical challenges when using normal phase silica flash chromatography.

This application note describes a practical and efficient purification of a mixture of basic heterocycles using Teledyne Isco's Redi*Sep* basic alumina column instead of normal phase silica to overcome these challenges.

Background

Acidic and basic organic compounds interact with residual surface silanol groups on normal phase chromatographic support. This interaction causes peak streaking and tailing, which will ultimately cause multiple or overlapping fractions during chromatographic purification.

To improve resolution in a separation of basic or acidic compounds, chemists typically add a mobile phase modifier to reduce peak tailing and sharpen peaks. For components containing a 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 modifier 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. An additional purge and solvent switch after the run may also be needed.

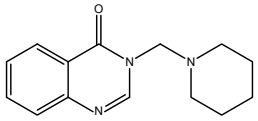
A Redi*Sep* basic alumina column offers an efficient and user-friendly alternative to normal phase silica, eliminating the need for a mobile phase modifier.

Basic alumina is a mixture of different aluminum oxides that are partially dehydrated. Redi*Sep* basic alumina columns are single use columns.

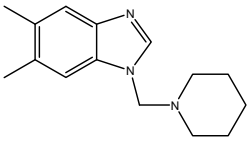
Results and Discussion

The separation of a mixture of quinazolinone and benzimidazole derivatives was investigated.

3-(1-piperdinylmethyl)-4(3H)-quinazolinone (A)



5,6-dimethyl-1-(piperdinomethyl)benzimidazole (B)



Flash chromatography of the heterocycles mixture on a normal phase Redi*Sep* column did not separate the two products successfully (Figure 1).

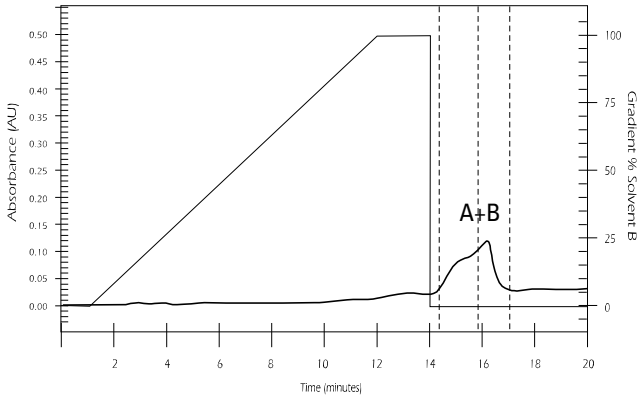


Figure 1: Chromatogram of normal phase column
Elution with hexane/ethyl acetate

However, the use of basic alumina Redi*Sep* column successfully separated the two nitrogen-containing heterocycles (Figure 2).

Although the two product peaks on the chromatogram show incomplete baseline resolution, analytical examination of resulting fractions has shown limited cross-contamination.

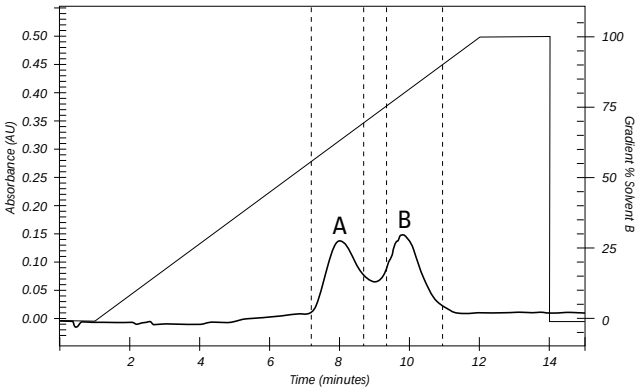


Figure 2: Chromatogram of basic alumina column
Elution with hexane/ethyl acetate

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

When Thin-layer Chromatography (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 Redi*Sep* 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 have been identified, the full sample batch can then be purified using the appropriate column size with respect to the crude sample quantity and the resolution observed during method development.

Experimental

Table 1: Method Parameters and Results

Instrumentation:	Isco Combi*Flash*® Companion™ 4x	
Columns	4g Normal Phase Redi*Sep* 8g Basic Alumina Redi*Sep*	
Sample Loading Method	34 mg pre-loaded on celite (J.T. Baker celite 503)	
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:	quinazolinone (A):	benzimidazole products (B):
	95%	96%

Conclusion

The preparative separation of a mixture of two heterocycles holding basic properties was successfully achieved without the presence of a mobile phase modifier by using basic alumina media as the stationary phase.

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

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