

Flash chromatography purification of high pKa organic compounds with Teledyne Isco's specialty *RediSep*[®] columns

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Abstract

Normal phase silica gel is not always the best choice for a stationary phase for purification by flash chromatography for complex and difficult separations.

Specialty media can provide the synthetic organic chemist a useful alternative. A new efficient and practical approach to the purification by flash chromatography of high pKa organic compounds featuring the Teledyne Isco specialty RediSep columns will be described.

Background

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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 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.



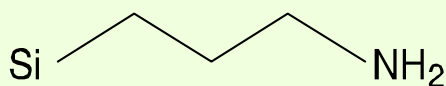
Removing this modifier involves additional extraction or washing with a suitable solvent, or concentrating the mixture down to an oil and placing the oil on a high vacuum overnight.

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 solvents switch after the run may also be needed.

Teledyne Isco offers several specialty RediSep columns suitable for convenient and efficient flash chromatography purification of high pKa organic compounds without the usual inconveniences:

- RediSep Amine,
- RediSep Basic Alumina,
- RediSep SCX (Strong Cation Exchange),
- RediSep Florisil columns.

RediSep amine column

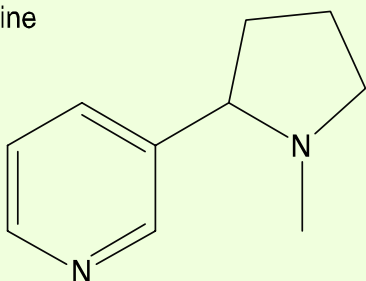


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The amine functionalized silica is a carbon tether end-capped with a primary amine functionality and can be used under normal- or reversed-phase conditions.

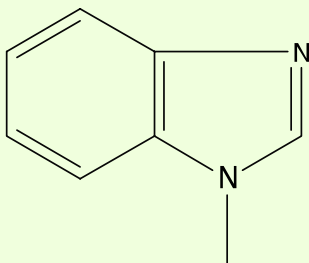
To illustrate the benefits offered when considering this media for a flash chromatography purification, the separation of a mixture of nicotine and 1-methylbenzimidazole was investigated.

Nicotine



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

1-methylbenzimidazole



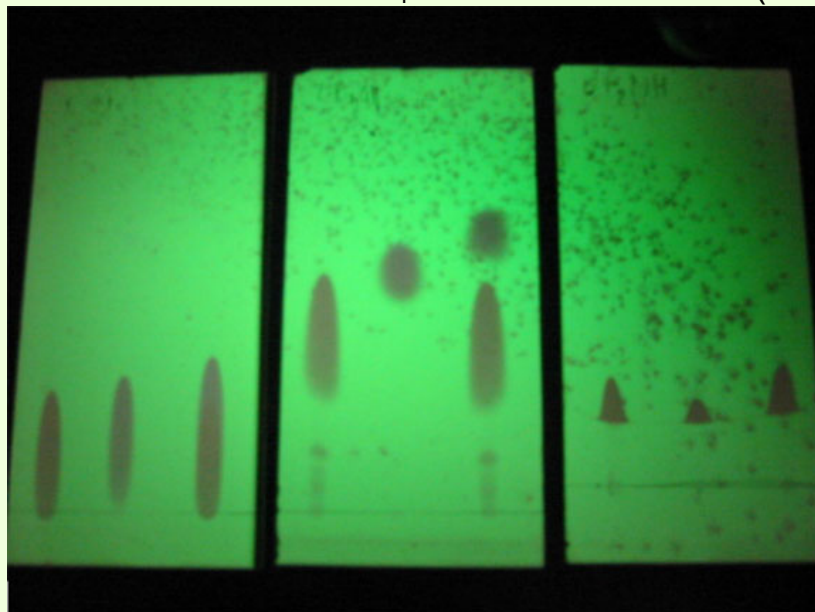
TELEDYNE ISCO
A Teledyne Technologies Company

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.

CH₂Cl₂/MeOH 98:2
No modifier With NH₄OH(1%) With TEA (1%)



TLC runs on RediSep 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).

Figure 2: RediSep amine functionalized phase silica TLC plates of heterocycles mixture

- Left spot: Nicotine
- Middle spot: Mixture of nicotine and 1-methylbenzimidazole
- Right spot: 1-methylbenzimidazole.

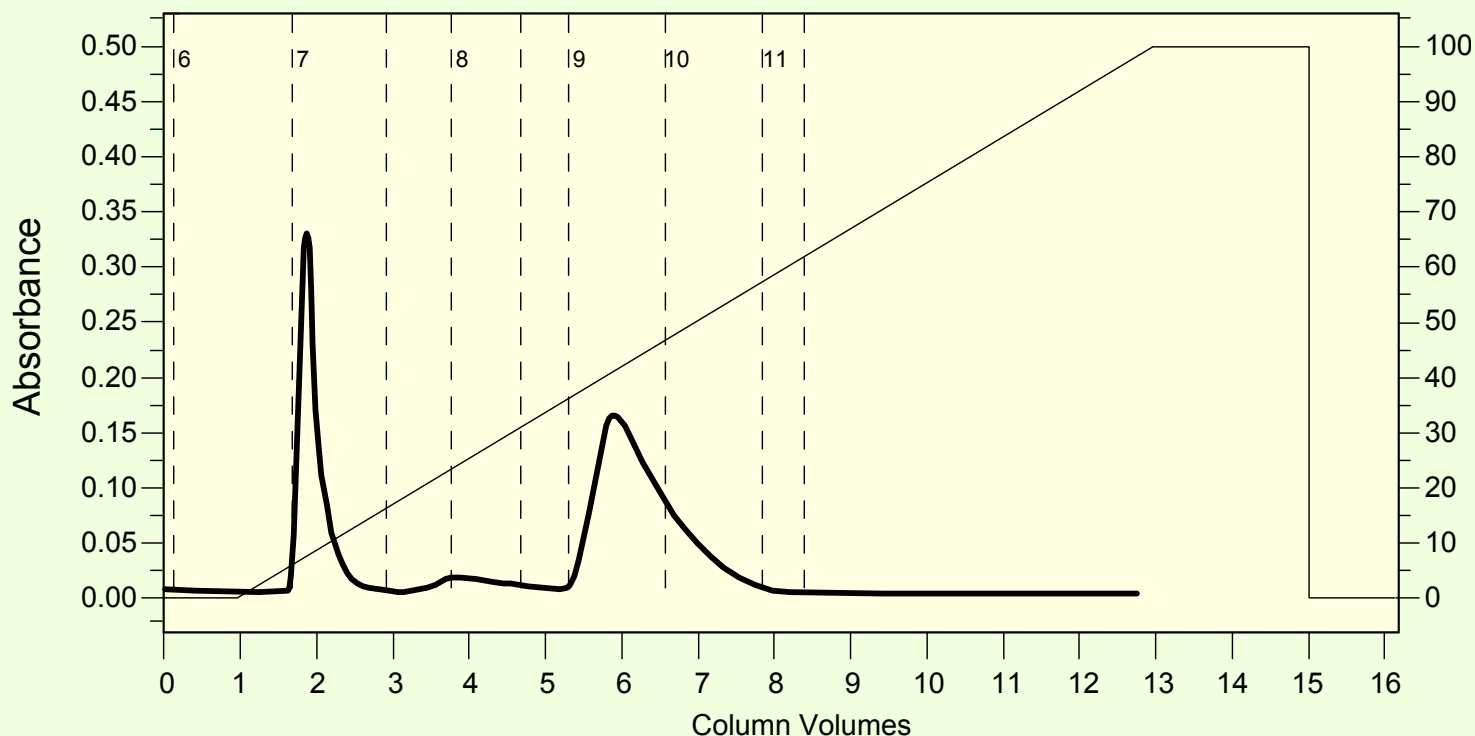
Ethyl acetate



Indeed, flash chromatography of the heterocycles mixture on an amine functionalized RediSep column fully separated the products with hexane/ethyl acetate as the mobile phase (figure 3).

Figure 3: Chromatogram of amine functionalized column with hexane/ethyl acetate

- Heterocycles mixture successfully separated

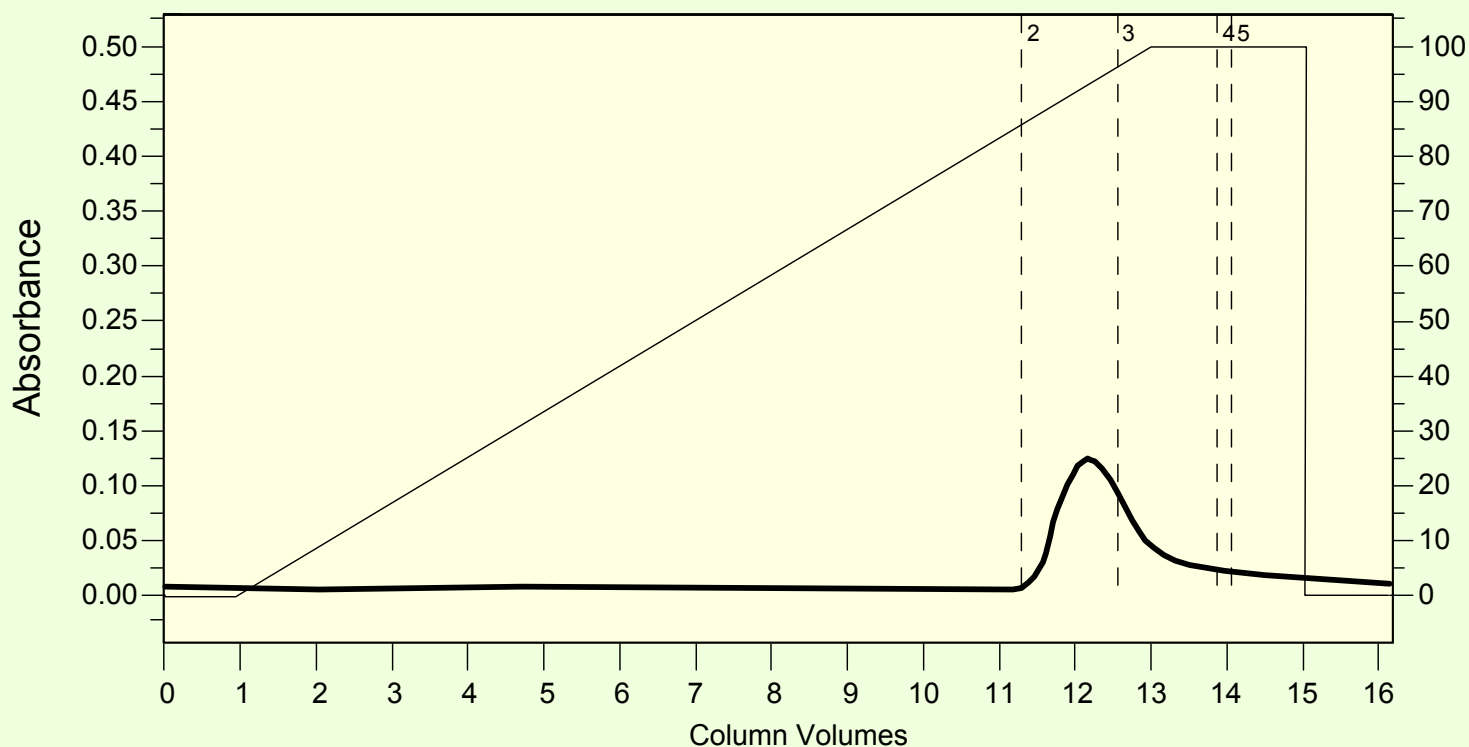


The normal phase column failed to separate them (figure 4).

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

Figure 4: Chromatogram of normal phase column elution with hexane/ethyl acetate

- Heterocycles mixture failed to separate.



Experimental

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Table 1: Method Parameters & Results

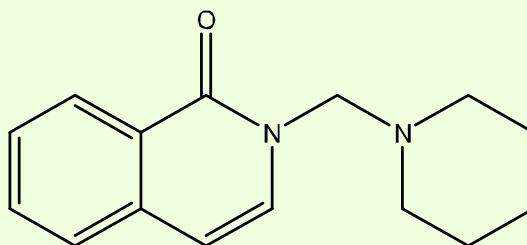
Instrumentation:	Isco CombiFlash [®] Companion [®] 4x	
Columns	4g Normal Phase RediSep 4.7g Amine Functionalized RediSep	
Sample Loading Method	66 mg pre-loaded on silica gel	
Wavelength	254 nm	
Mobile phase:	Solvent A: Hexane Solvent B: Ethyl Acetate	
Flow Rate:	18 mL/minute	
Equilibration Volume:	7 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: 95%	1-methylbenzimidazole: 99%

RediSep Basic Alumina column

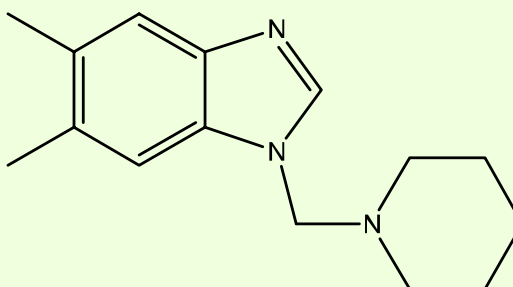
Basic alumina is a mixture of different aluminum oxides partially dehydrated.

In order to illustrate the benefits offered when considering this media for a flash chromatography purification, the separation of a mixture of quinazolinone and benzimidazole derivatives was investigated.

3-(1-Piperidinylmethyl)-4(3H)-Quinazolinone



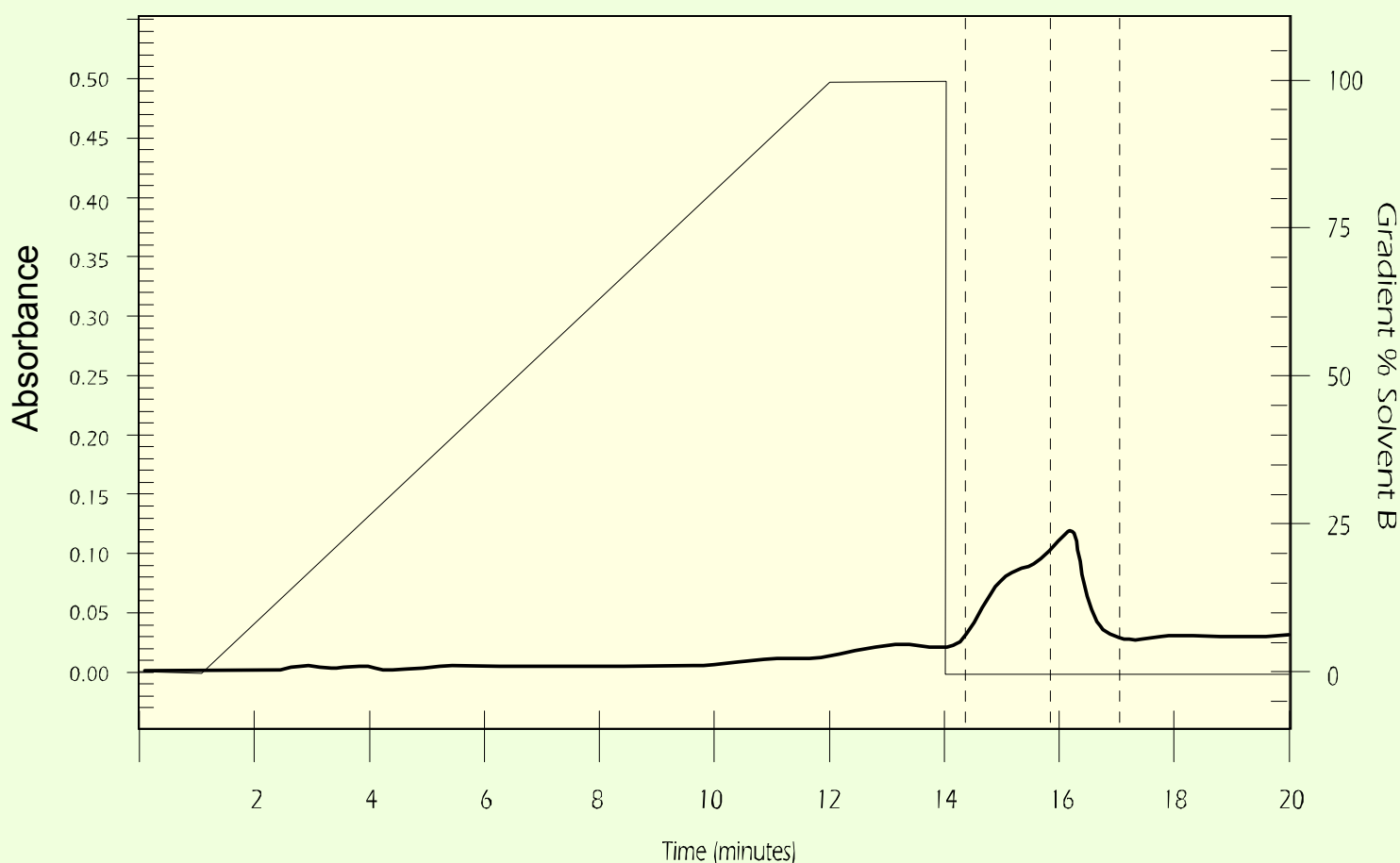
5,6-Dimethyl-1-(piperidinomethyl)benzimidazole



Flash chromatography of the heterocycles mixture on a normal phase RediSep column failed to separate the two products (figure 5).

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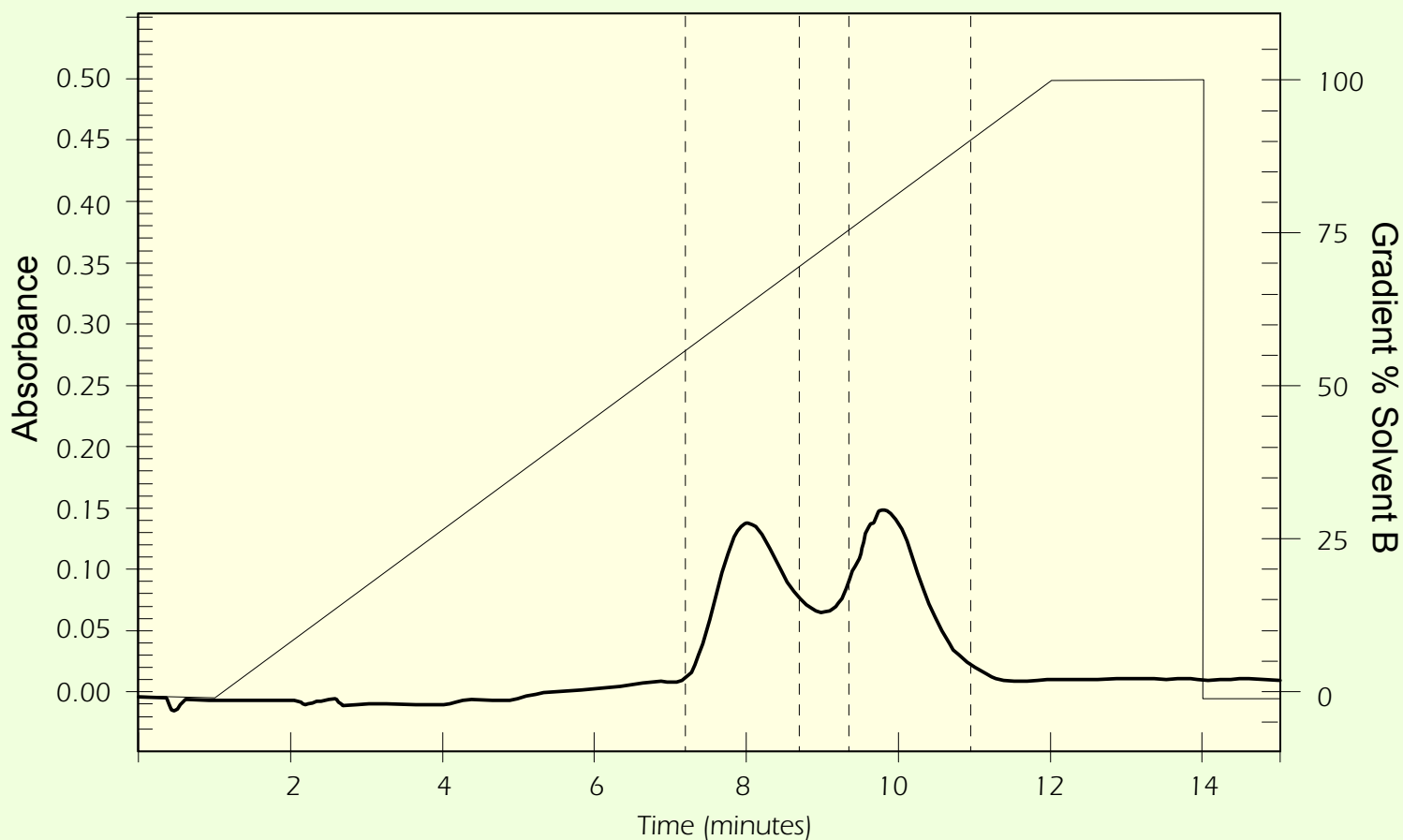
Figure 5: Chromatogram of normal phase column, elution with hexane/ethyl acetate



However, the use of basic alumina RediSep column successfully separated the two nitrogen-containing heterocycles (figure 6).

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Figure 6: Chromatogram of basic alumina column, elution with hexane/ethyl acetate



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

RediSep basic alumina columns are single use columns.

Experimental

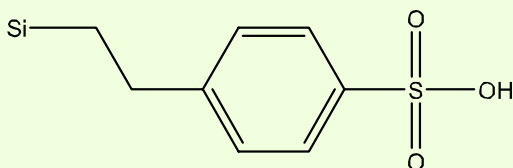
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Table 2: Method Parameters & Results

Instrumentation:	Isco CombiFlash Companion 4x	
Columns:	4g Normal Phase RediSep 8g Basic Alumina RediSep	
Sample Loading Method:	34 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:	Quinazolinone product: 95%	Benzimidazole product: 96%



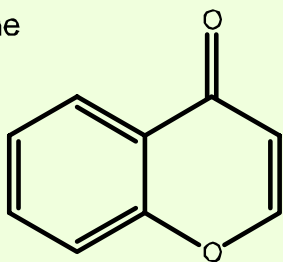
RediSep SCX column



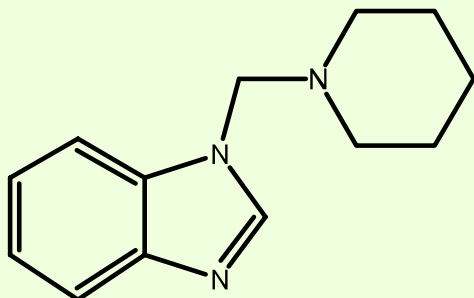
The SCX media is a silica-bound tosic acid.

The strong acidity of this media induces the full retention of any compounds with basic properties subjected through a RediSep SCX column. This intrinsic media property can be exploited several ways.

Chromone



1-(1-Piperidinylmethyl)-1H-Benzimidazole

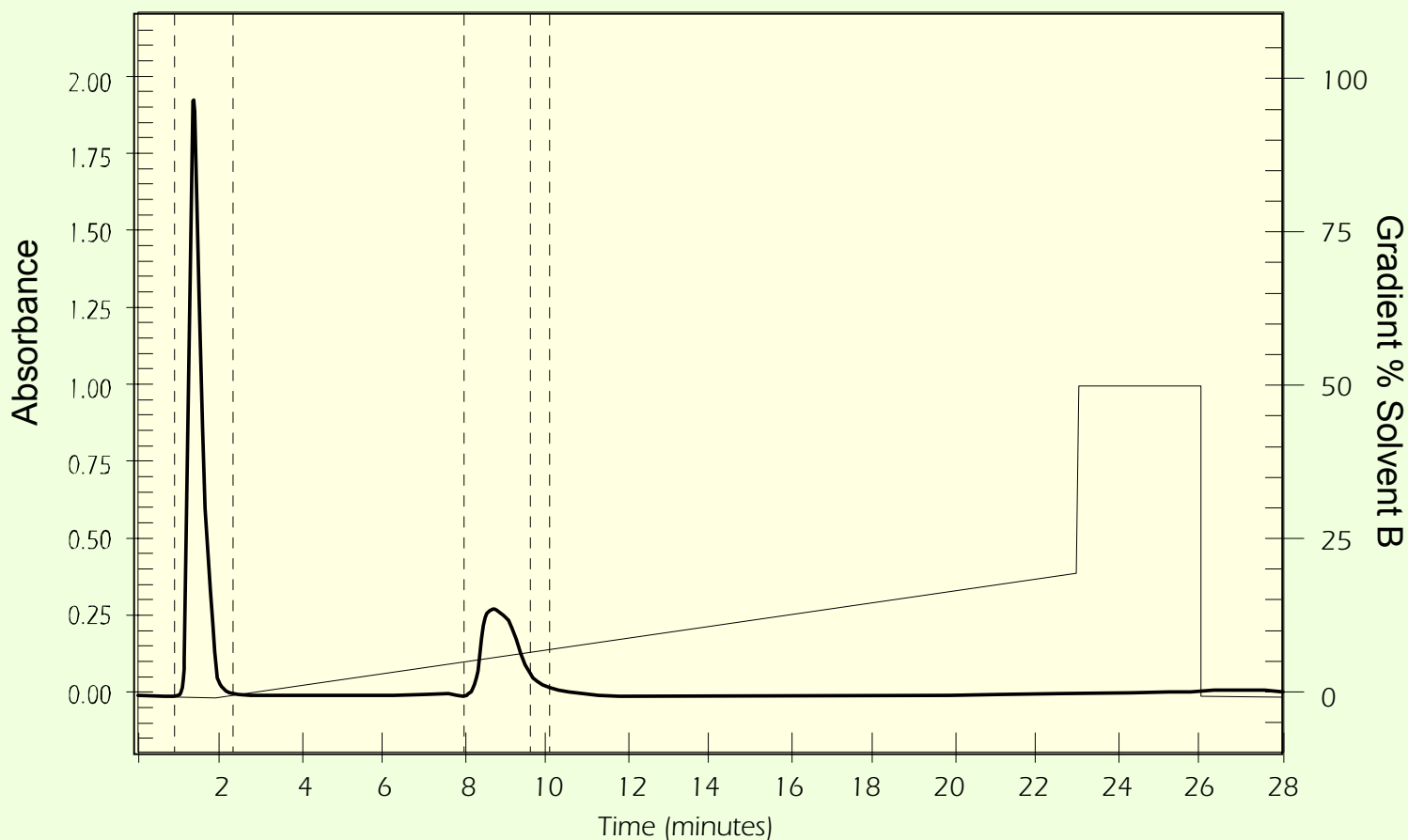


To illustrate the benefits offered when considering this media for a flash chromatography purification, separation of a mixture of chromone and a benzimidazole derivative was investigated.

Flash chromatography of the mixture on a normal phase RediSep column showed release of the two products (figure 7).

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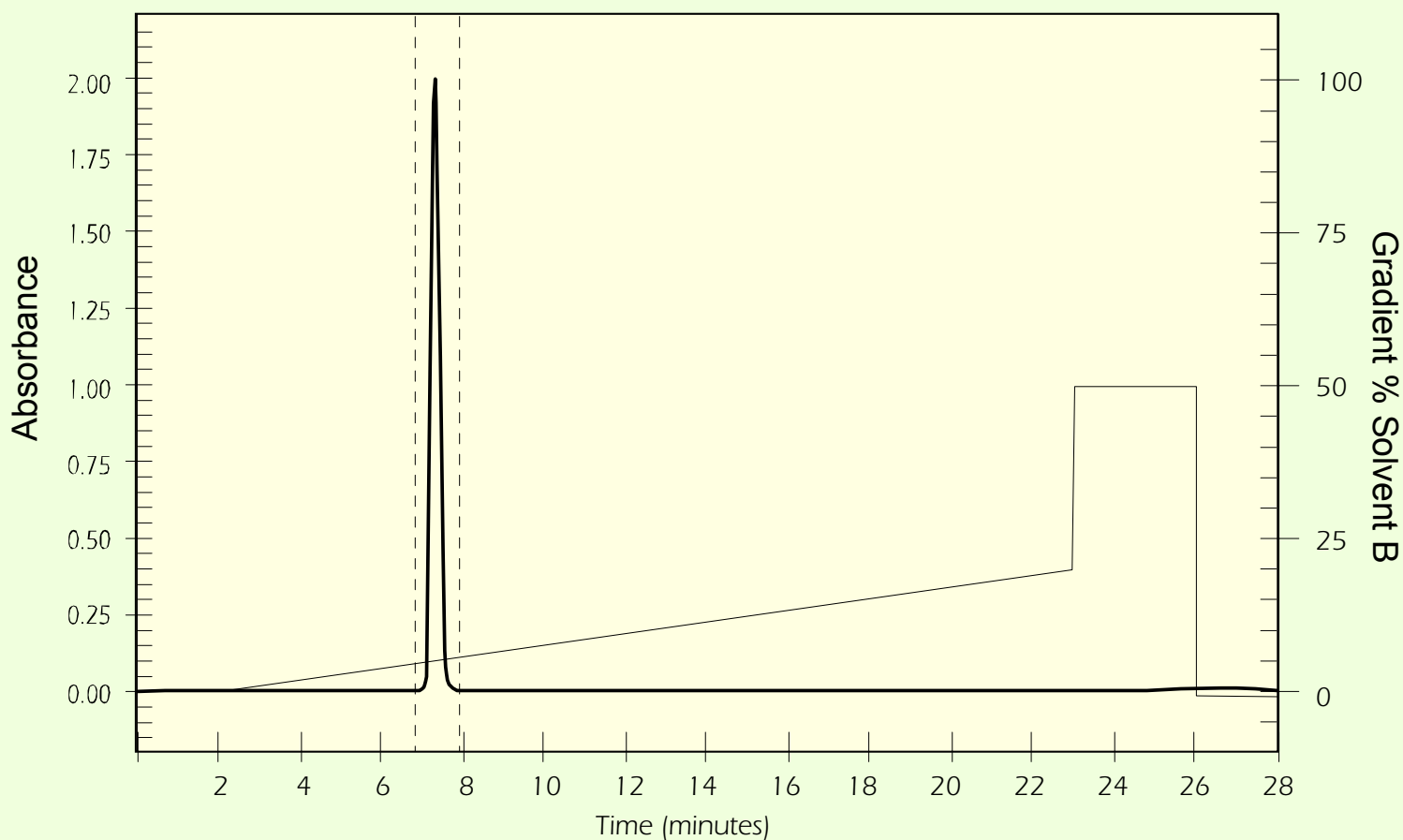
Figure 7: Chromatogram of normal phase column, elution with dichloromethane/methanol



The use of a RediSep SCX column showed total retention of the benzimidazole derivative onto the column and release of the chromone (figure 8).

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Figure 8: Chromatogram of SCX column, elution with dichloromethane/methanol

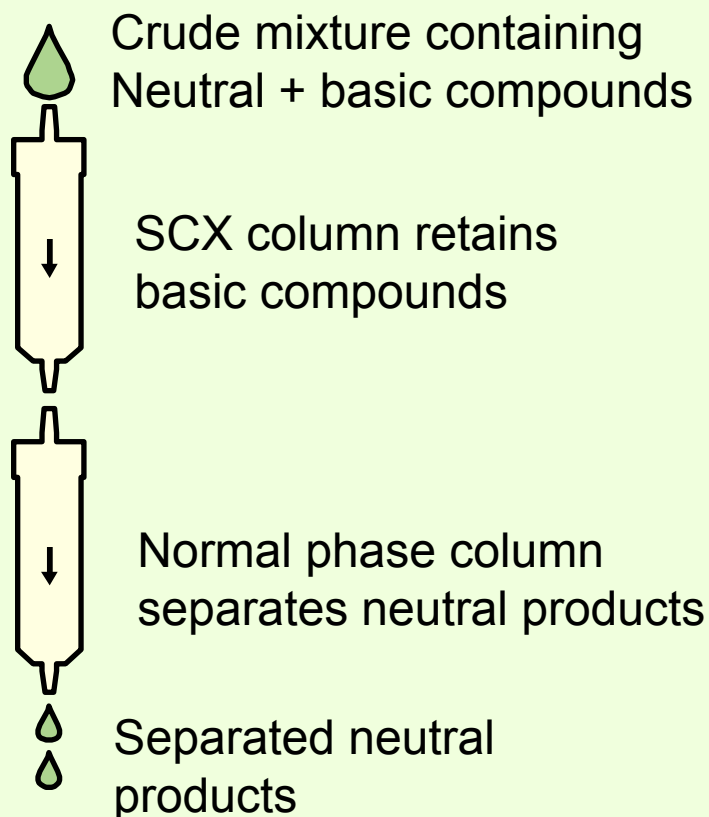


This isolation of the basic compound, while allowing one or more organic compounds holding neutral properties to migrate freely through the column, demonstrates that the column can be effective as a clean up tool.

If the elute from the SCX column contains multiple compounds holding neutral properties that require further purification, column stacking can be an option. Stacking an SCX column on top of a normal phase column (figure 9) would first strip the compounds holding basic properties, and then separate the remaining neutral compounds. A solvent system should be selected to provide satisfactory resolution on normal phase silica.



Figure 9: Stacked columns of different media as a purification tool



Conversely, the SCX column also represents a practical tool for the isolation of desired compounds holding basic properties. In this case, the contaminants would be the neutral compounds which would be immediately released then discarded by the SCX column run.

The compounds holding basic properties retained by the SCX column are liberated by injecting 8 column volumes of a 5% ammonia in methanol solution. This solution can be directly injected through the column with a syringe flush. In this case, the SCX column works as a catch and release process.

The RediSep column not only scavenges compounds holding basic properties such as amine or nitrogen-containing heterocycles, it can also retain borohydrides, nickel, and silver species.

The RediSep SCX column media will not degrade when using common protic or aprotic organic solvents and it is reusable if regenerated after each use with 5 column volumes of a solution of 1M HCl in acetonitrile.



Experimental

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Table 3: Method Parameters & Results

Instrumentation:	Isco CombiFlash [®] Companion [®] 4x	
Columns:	4g Normal Phase RediSep 5g SCX RediSep	
Sample Loading Method:	45 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:	Chromone product: 99%	Benzimidazole product: 99%

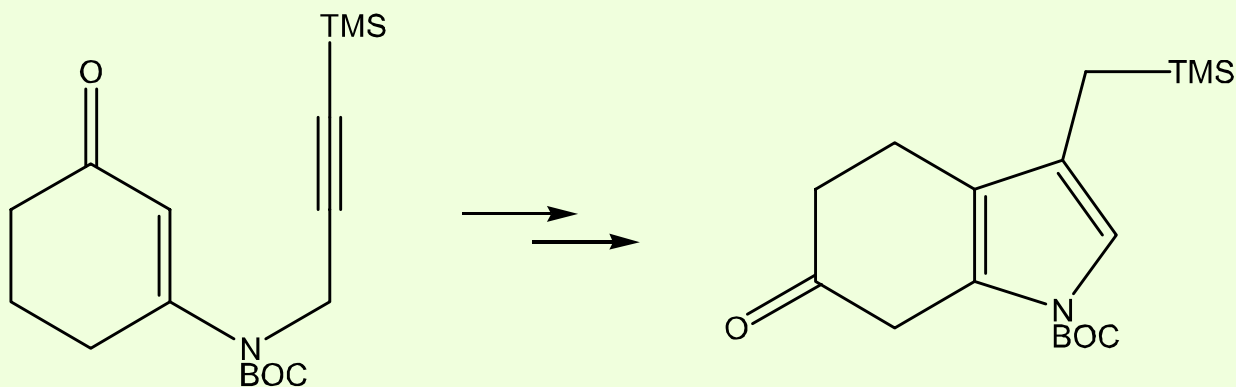
RediSep Florisil® column

Florisil media is a magnesium silicate containing 15% magnesium oxide and 85% normal phase silica gel.

The magnesium oxide confers Florisil a slight basicity which can be exploited for different applications.

The synthesis via radical cyclisation and purification of some unstable pyrrolo derivatives have been reported in the literature¹.





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These 3-methylsilane pyrrolo products show acid sensitivity and degrade when subjected onto normal phase silica gel flash chromatography purification. However, successful isolation has been achieved when using Florisil media as stationary phase.

Thus, Florisil media can be considered for weakly basic organic or acid sensitive compounds flash chromatography purification.

RediSep Florisil columns are single use columns.

Method development

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 RediSep columns on a CombiFlash 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 RediSep 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 subjected using the appropriate column size with respect to the crude sample quantity and the resolution observed during method development.

Conclusion

Flash chromatography purifications of compounds holding basic properties with specialty RediSep columns avoiding the need for a basic mobile phase modifier in the solvent system have been described.

These specialty media provide chemists a panoply of tools to select from when purification of this class of compound is considered. Since each sample has a unique basicity and affinity with each specialty media, method development is recommended in order to select the appropriate stationary phase.

Amine, Basic Alumina, SCX, and Florisil RediSep columns offer chemists highly practical and efficient tools for optimal high pKa organic compounds separation.



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- 1 Chin-Kang Sha, Wei-Hong Tseng, Kuan-Tsau Huang, Kuan-Miao Liu, Heng-Yih Lin and San-Yan Chu, J. *Chem. Soc., Chem. Commun.*, **1997**, 239.

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