Advanced Topics for Use of RediSep® Specialty Media Columns in Flash Chromatography

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Abstract

Redi*Sep*® specialty media columns offer synthetic organic chemists practical tools for obtaining optimal purification by flash chromatography. Redi*Sep* Cyano columns are featured as an efficient alternative to normal phase silica gel for high pK_a compound separations. In addition, a practical approach for using specialty media such as Redi*Sep* Cyano and Redi*Sep* Amine columns under reversed phase conditions is discussed.

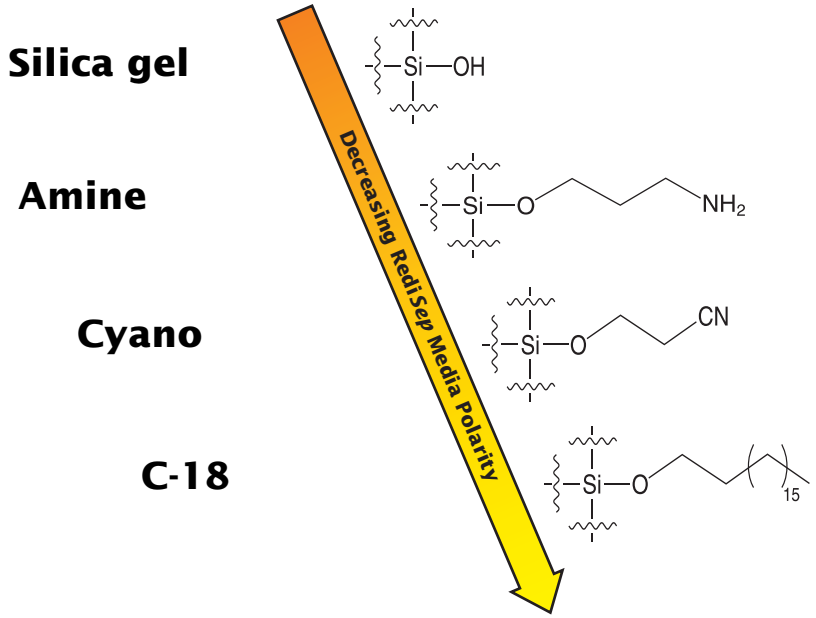
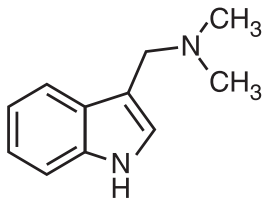


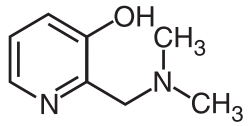
Figure 1.
Solvents demonstrate different strengths with varying media polarity

Separation of High pK_a Compounds on Redi*Sep* Cyano

Silica gel is a widely used stationary phase for normal phase purification of compounds with low to medium polarity. Yet, inefficiencies of silica gel are often encountered by the synthetic chemist when purifying basic or medium-to-high polarity compounds. High pK_a compounds such as 3-(dimethylaminomethyl) indole **(1)** and 2-(dimethylaminomethyl)-3-hydroxypyridine **(2)**, are often fully retained on silica gel due to the presence of acidic silanol groups, requiring the use of basic modifiers, such as ammonia, ammonium hydroxide, or triethylamine in the mobile phase. In addition, such relatively polar compounds have a strong affinity to the polar silanol groups and require elution through silica gel with a mobile phase of higher solvent strength than ethyl acetate in hexane, such as 10% methanol in dichloromethane (Figure 3, top).



(1)



(2)

Redi*Sep* Cyano functionalized silica is a neutral media with a short alkyl chain end capped with a weakly polar nitrile group. High pK_a compounds such as 3-(dimethylaminomethyl) indole **(1)** and 2-(dimethylaminomethyl)-3-hydroxypyridine **(2)** are not retained as strongly (Figure 3, bottom), thereby eliminating the need for basic modifiers and the use of chlorinated solvent systems.

Alternatively, Redi*Sep* Cyano columns also can be used as an efficient alternative to C-18 silica for the separation of compounds of medium to high polarity. The short alkyl chain does not retain moderately polar compounds as strongly in reversed phase mode like C-18 silica with its long alkyl chain. Strong retention of less polar compounds on C-18 can result in broader peak shape and longer elution time. These effects are seen with the broader peak shape of the hydroxypyridine derivative on C-18 and the indole derivative not eluting within the designated time frame (Figure 4, bottom). Separation of moderately polar compounds is enhanced on Redi*Sep* Cyano due to the decrease of retentive properties while maintaining good selectivity.

Table 1 Experimental

Instrument:	Teledyne Isco's Combi*Flash*® Rf
Columns:	Redi*Sep* Flash 4g (Silica gel), Redi*Sep* Cyano 5g, Redi*Sep* C-18 Reversed Phase 4.3g
Sample mass:	40mg
Flow rate:	16ml/min
Detection wavelength:	280nm

Figure 2.
Teledyne Isco's Combi*Flash* Rf automated Flash chromatography system

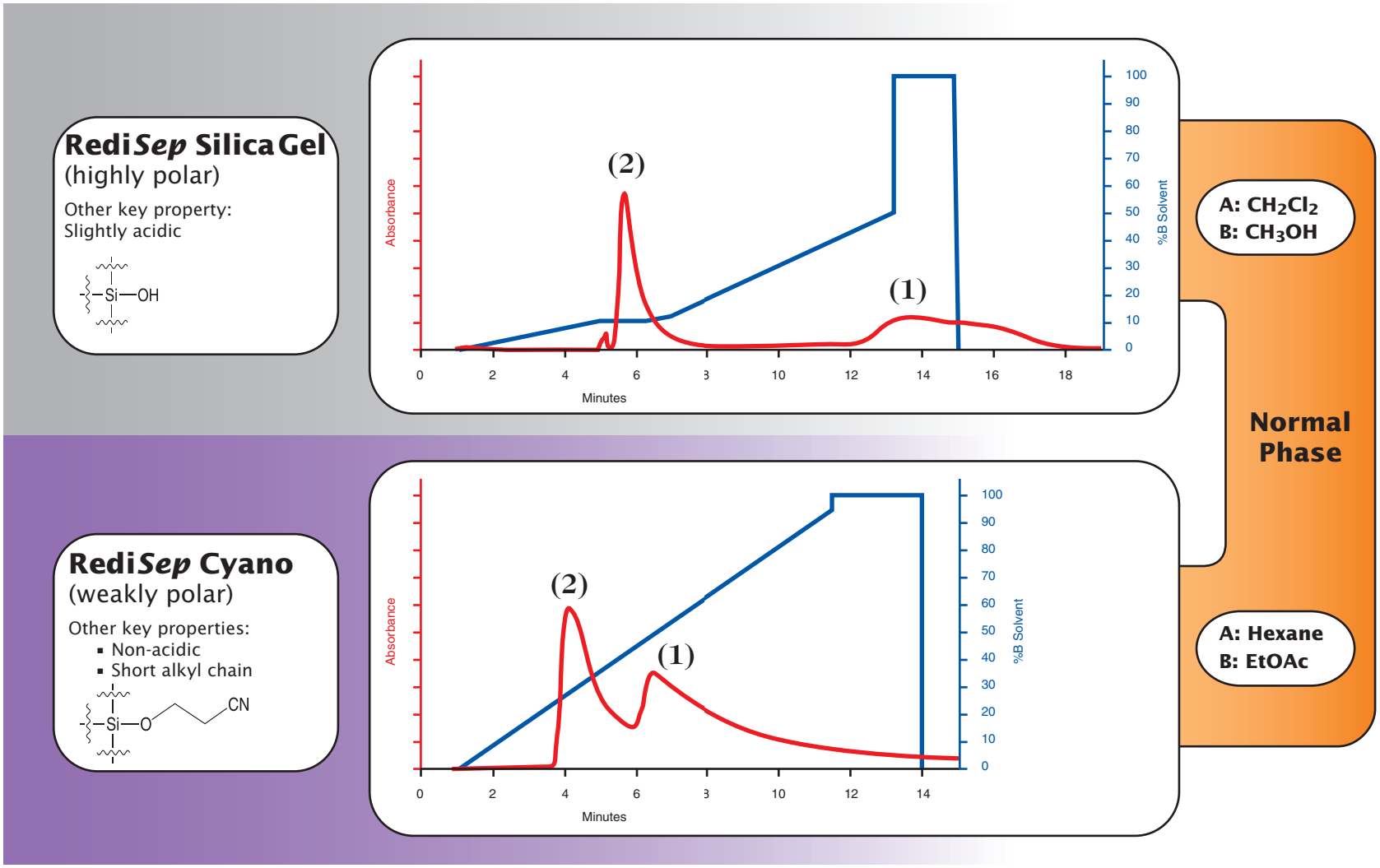


Figure 3.
Normal phase separation of high pK_a compounds

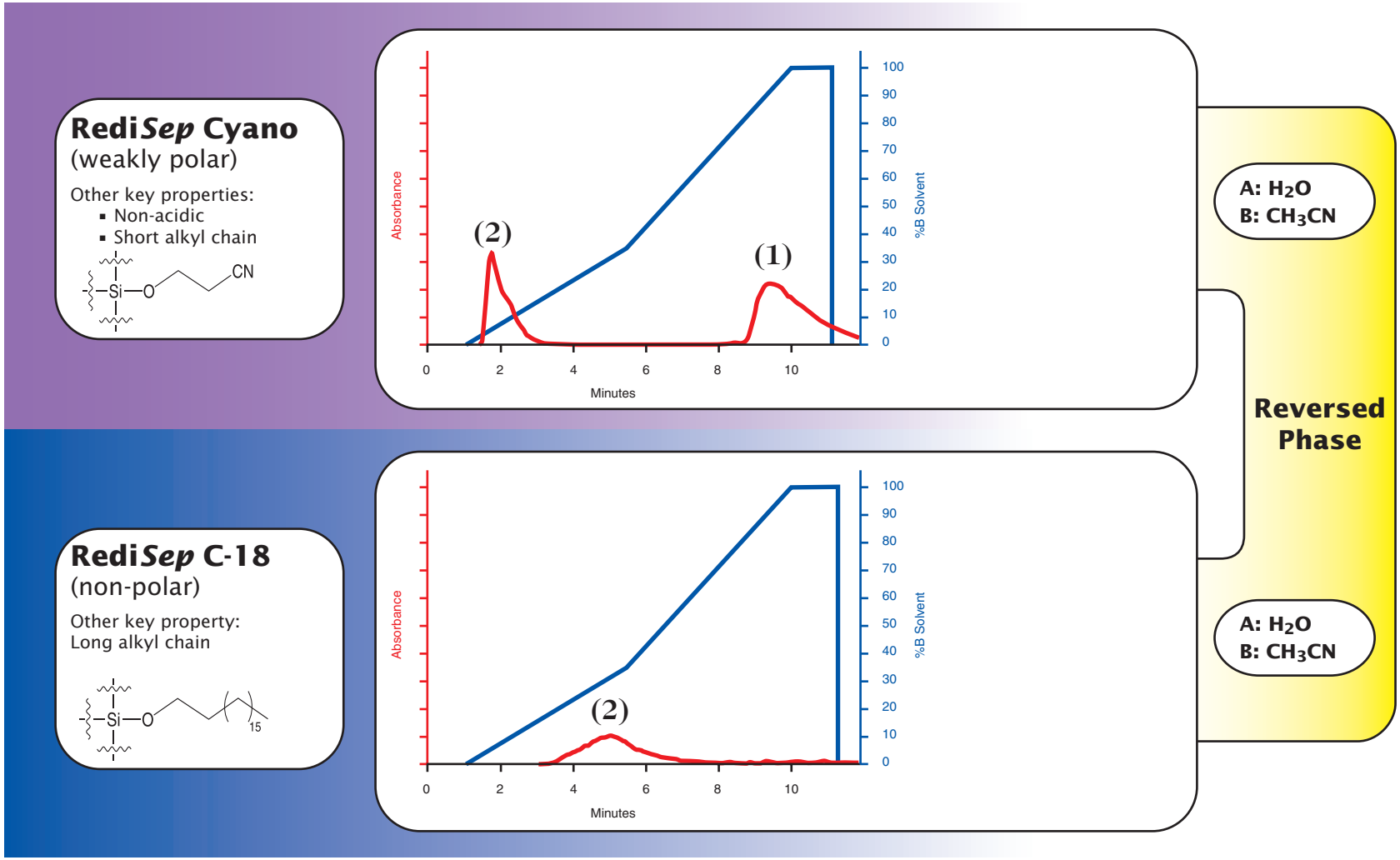


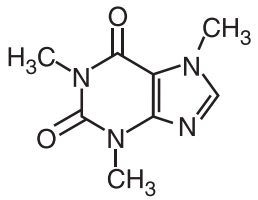
Figure 4.
Reversed phase separation of high pK_a compounds

Separation of Xanthine Derivatives on Redi*Sep* Amine

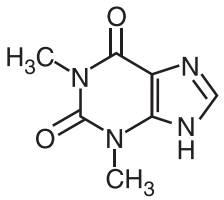
Synthetic organic chemists must develop unique separation methods for compounds that are difficult to separate. Developing a practical separation method requires the appropriate stationary phase and mobile phase combination to create the selectivity needed for an effective separation of such a sample. It is general practice that in reversed phase separation, the non-polar stationary phase is used with the mobile phase increasing in strength with decreasing solvent polarity (*e.g.*, water to acetonitrile), thus eluting the most polar compounds first. As illustrated with the separation of xanthine derivatives on C-18 silica (Figure 5, top), the solvent strength increased from water to acetonitrile and resulted in a partial separation with the relatively more polar compound, theophylline **(4)**, eluting first. Conversely, for the separation of the xanthine derivatives using the Redi*Sep* Amine stationary phase, it can be seen that the compounds have low retention when the same gradient profile is used, eluting the relatively less polar compound, caffeine **(3)**, first (Figure 5, bottom). Such a drastic change in result would suggest a review of the selectivity of the mobile phase with the amino stationary phase for the separation of xanthine derivatives.

The Redi*Sep* Amine stationary phase is functionalized silica with a short alkyl chain end capped with a polar amino group. In an attempt to use the Redi*Sep* Amine column in a reverse-phase separation mode (*i.e.*, water as the weak solvent), a fast elution of the xanthine derivatives resulted with an inversion of compound elution order. It is general practice that in normal phase separation, solvent strength increases with increasing solvent polarity, typically eluting the less polar compounds first. Using these reversed phase solvents on the

Redi*Sep* Amine stationary phase resulted in complete separation of the xanthine derivatives when using the appropriate solvent, acetonitrile, as the weak solvent; eluting the relatively less polar compound, caffeine **(3)**, first as would be expected for a normal phase mode of separation. It can be seen that retention of the xanthine derivatives was increased when beginning the gradient profile with the appropriate "weak" solvent, acetonitrile.



(3)



(4)

Table 2 Experimental

Instrument:	Teledyne Isco's Combi*Flash*® Rf
Columns:	Redi*Sep* C-18 Reversed Phase 4.3g, Redi*Sep* Amine 4.7g
Sample mass:	40mg
Flow rate:	16ml/min
Detection wavelength:	280nm

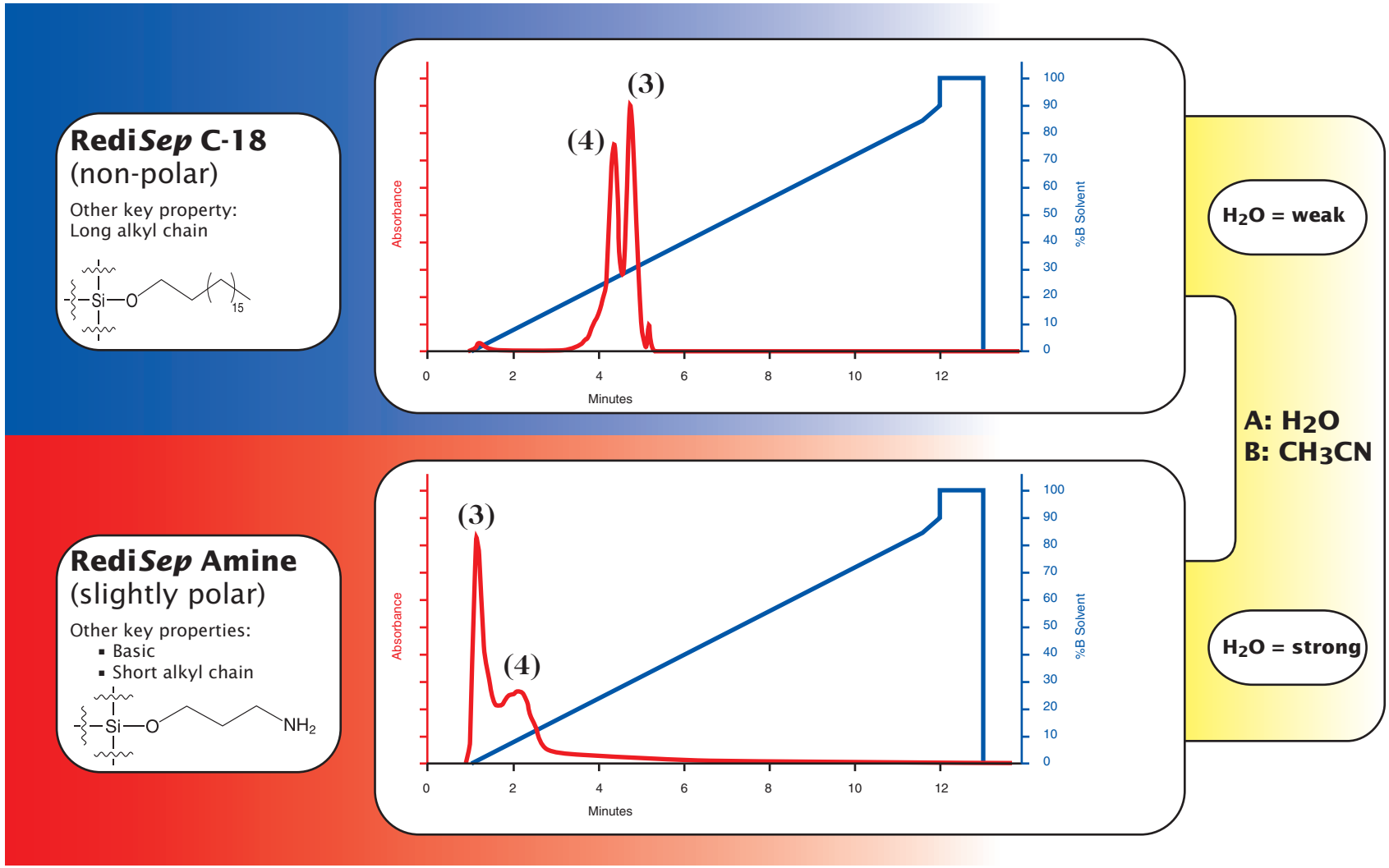


Figure 5.
Comparison of separations on Redi*Sep* C-18 and Amine columns with H_2O and CH_3CN mobile phase

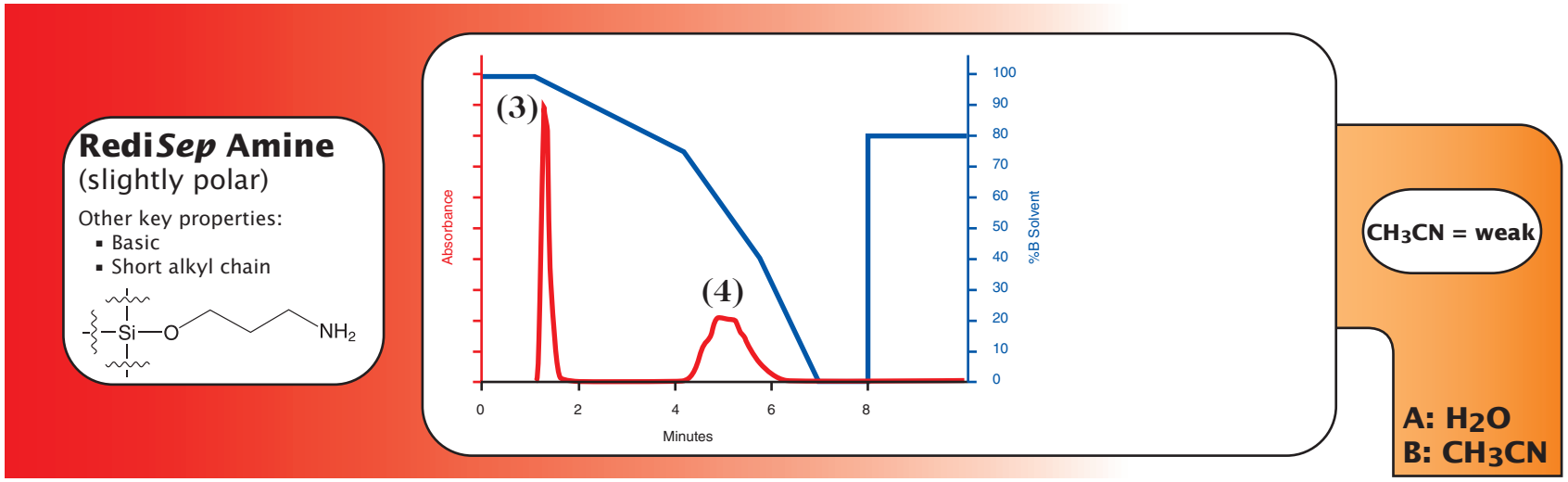


Figure 6.
Normal phase separation with H_2O and CH_3CN on Redi*Sep* Amine column. Gradient profile initiated with the weak solvent CH_3CN.

Figure 7.
Teledyne Isco's Redi*Sep* C-18 reversed phase chromatography columns

Summary

It is demonstrated that the Redi*Sep* Cyano stationary phase is an effective alternative to silica for the purification of basic compounds due to the media being far less acidic than normal phase silica. In addition, Redi*Sep* Cyano can be used as an efficient alternative to C-18 silica for the separation of compounds of medium polarity by decreasing elution time and enhancing peak shape.

It has been illustrated that Redi*Sep* Amine stationary phase is favorable for performing separations in a normal phase mode employing a wide variety of solvents—including those typically used in reversed phase separations.

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