

RediSep[®] C-18 reversed phase column

Purification of low-solubility polar heterocycles

Mikael Mahler, Stephen Swartz, Veronica Thomason
Teledyne Isco, Inc.



Chromatography Application Note AN55

Overview

Purification of highly polar compounds by flash chromatography is generally difficult and lengthy when using normal phase silica gel as the stationary phase.

C-18 reversed-phase media is widely used in HPLC instruments for analytical separations of most classes of organic compounds including polar ones. C-18 reversed-phase flash chromatography pre-packed columns have recently become available to synthetic organic chemists for convenient preparative separations with automated flash chromatography instrumentation.

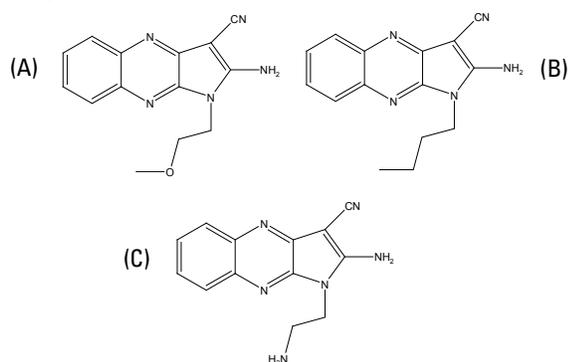
This application describes the purification of a mixture of low-solubility polar heterocycles using Teledyne Isco's RediSep C-18 reversed phase column as the stationary phase.

Background

For background information, please refer to Chromatography Application Note AN50.

Results and Discussion

The separation of a mixture of quinoxalines was investigated:



Flash chromatography of the quinoxalines mixture on C-18 reversed phase 4.3g RediSep column fully separated the products with water + 0.1% TFA/acetonitrile+0.1% TFA as the mobile phase (Figure 1).

The sample was loaded using the solid sample technique by dissolving the sample (7 mg) in a necessarily high amount of methanol (15 ml). 100 mg of C-18 reversed-phase silica was then added and the solvent was removed in vacuo. The resulting pre-coated sample was placed in an empty RediSep solid load cartridge.

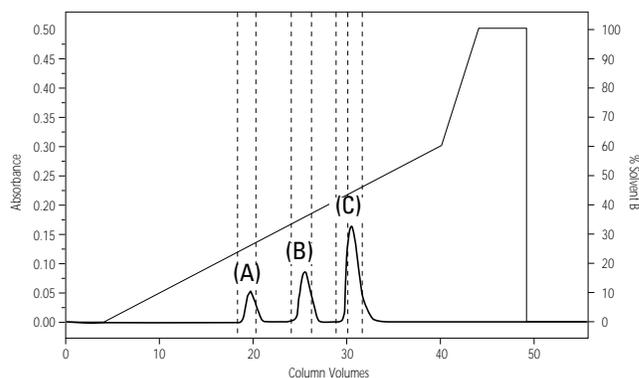


Figure 1: Quinoxaline mixture chromatogram on C-18 reversed phase RediSep column with water + 0.1% TFA/acetonitrile+ 0.1% TFA

In addition to C-18 reversed phase RediSep columns, amine or basic alumina RediSep columns are also options to consider for the purification of low-solubility polar heterocycles. Method development would indicate which column and solvents to select.

LC-MS is an analytical technique routinely used for sample analysis which can also serve as a method development technique prior to preparative scale separation.

Provided that adequate separation is observed, LC-MS conditions of crude reaction mixture can easily be transposed to a CombiFlash[®] automated flash chromatography instrument for full batch separation. If LC-MS isn't routinely used, other method development techniques are available.

When TLC plates are available for a stationary phase under consideration, method development would begin by investigating whether an exploitable optimal selectivity 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. 10–20 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 separation 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.

About Teledyne Isco C-18 Reversed-phase RediSep columns, cartridges and TLC plates

Teledyne Isco C-18 Reversed-phase RediSep columns are available in 4.3g, 13g, 43g, 130g and 360g sizes with a recommended loading capacity between 0.1% and 0.5%. The recommended column equilibration is 7 column volumes. 0.1% TFA is routinely added to the solvents used with C-18 RediSep columns.

C-18 Reversed-phase RediSep columns are reusable up to 25–30 times if used with fully worked-up crude reaction mixture (filtration of solids, neutralized and extracted) and if stored using one of the following solvents after use: ethanol, methanol, or 80% acetonitrile + 20% water.

C-18 Reversed-phase RediSep pre-packed cartridges are convenient to use when injecting via solid sample loading technique especially in situations when sample solubility is limited.

C-18 Reversed-phase RediSep TLC plates are available for method development purposes.

Experimental

Table 1: Method Parameters and Results

Instrumentation:	Teledyne Isco CombiFlash [®] Companion [®] Touchscreen	
Column	4.3g C-18 Reversed Phase RediSep	
Sample Loading Method	7 mg pre-loaded on C-18 Reversed-phase powder	
Wavelength	254 nm	
Mobile phase:	Solvent A: Water + 0.1% TFA	Solvent B: Acetonitrile+ 0.1% TFA
Flow Rate:	12 mL/minute	
Equilibration Volume:	7 column volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	4.0
	60	36.0
	100	4.0
	100	5.0
	0	4.0

Conclusion

The preparative separation of a quinoxaline mixture was successfully achieved by using C-18 reversed phase media as the stationary phase.

This application illustrated the use of C-18 reversed phase RediSep column as a highly practical and efficient tool for the purification of low-solubility polar heterocycles.

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Teledyne ISCO

P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091
www.teledyneisco.com

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