

Flash chromatography purification of polar organic compounds with Teledyne Isco's C-18 Reversed-phase RediSep[®] column

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Abstract

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.

Practical flash chromatography purification of high polarity organic compounds featuring the Teledyne Isco C-18 Reversed-phase RediSep column will be described.

Background

Separation in reversed-phase chromatography depends on the reversible adsorption/desorption of molecules with varying degrees of hydrophobicity to a hydrophobic stationary phase.

The selectivity of the reversed-phase medium is predominantly a function of the type of ligand grafted to the surface of the medium. Generally speaking, linear hydrocarbon chains (n-alkyl groups) are the most popular ligands used in reversed-phase applications.

Although a large variety of organic solvents can be used in reversed-phase chromatography, in practice only a few are routinely employed. The two most widely used organic solvents are acetonitrile and methanol, although acetonitrile is the more popular choice. All solvents, including water, are essentially UV transparent. This is a crucial property for reversed-phase chromatography since column elution is typically monitored using UV detectors. In addition, the use of ion pairing modifiers in the mobile phase allows reversed-phase chromatography of charged molecules.

A common drawback associated with reversed-phase chromatography is the subsequent removal of water. With current technology, water can be effectively removed by using a lyophilizer or a low-vacuum concentrator.

About Teledyne Isco C-18 Reversed-phase RediSep columns

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.

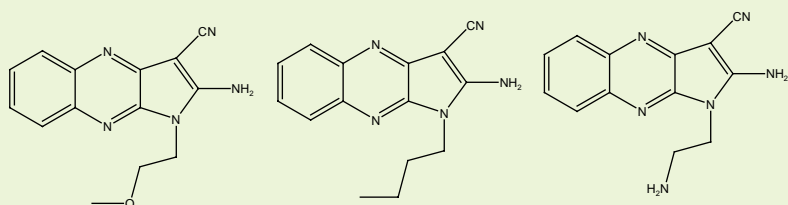
C-18 Reversed-phase RediSep columns are reusable up to 25-30 times if used with fully worked-up crude reaction mixture (filtration of solids, neutralized and extracted) and if stored using one of the following solvents after use: ethanol, methanol, or 80% acetonitrile + 20% water.

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.



Low-solubility polar heterocycles

Preparative separation of low-solubility polar heterocycles using C-18 Reversed-phase RediSep column was illustrated with the purification of a mixture of three quinoxaline derivatives (below).



These quinoxalines showed little solubility in usual organic solvents and were soluble only in highly diluted methanol.

Flash chromatography of the mixture of heterocycles on a 4.3g C-18 Reversed-phase 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 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. Heterocycles mixture chromatogram on C-18 Reversed-phase RediSep column with water + 0.1% TFA/acetonitrile + 0.1% TFA

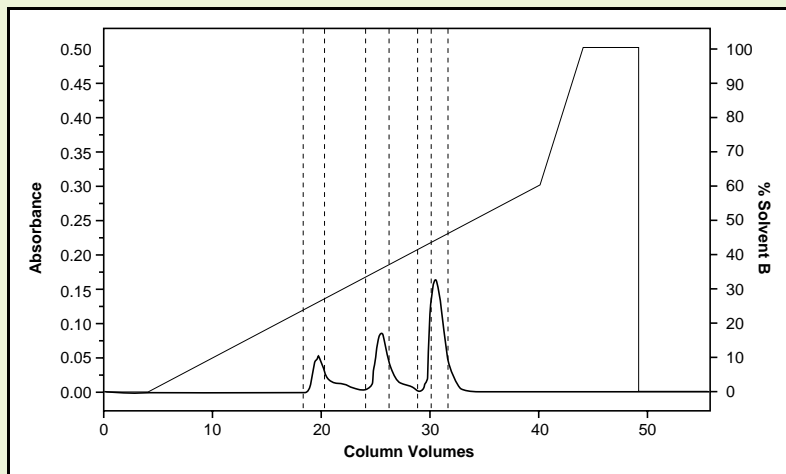


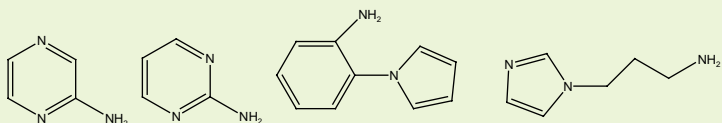
Table 1 Method Parameters

Instrumentation:	Teledyne Isco CombiFlash Companion Touchscreen	
Column:	4.3g C-18 Reversed-phase RediSep Column	
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	0.0
	0	6.0

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Primary amines

Preparative separation of amines using a C-18 Reversed-phase RediSep column is illustrated with the purification of a mixture of 4 primary amines (below).



Flash chromatography of the mixture of primary amines on a 4.3g C-18 Reversed-phase RediSep column separated the products with water/acetonitrile as the mobile phase (Figure 2).

Figure 2. Primary amines mixture chromatogram on C-18 Reversed-phase RediSep column with water/acetonitrile

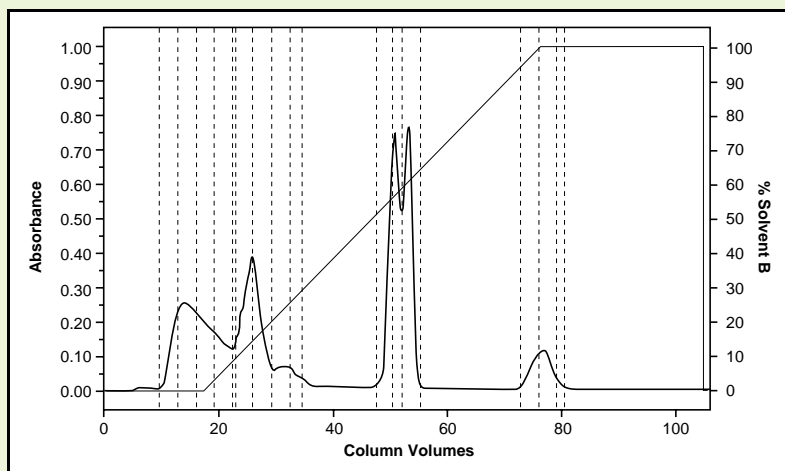


Table 2 Method Parameters

Instrumentation:	Teledyne Isco CombiFlash Companion 4x	
Column:	4.3g C-18 Reversed-phase RediSep Column	
Sample Loading Method:	30 mg pre-loaded on C-18 5g pre-packed cartridge	
Wavelength:	220 nm	
Mobile phase:	Solvent A: water Solvent B: acetonitrile	
Flow Rate:	12 mL/minute	
Equilibration Volume:	7 Column Volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	18.0
	100	58.0
	100	30.0
	0	0.0
	0	2.0

Peptides

Preparative separation of peptides using a C-18 Reversed-phase RediSep column is illustrated with the purification of a mixture of two peptides:

Gly-Pro-Ala

Val-Tyr-Val

Flash chromatography of the mixture of peptides on a 13g C-18 Reversed-phase RediSep column fully separated the products with water + 0.1% TFA/acetonitrile + 0.1% TFA as the mobile phase (Figure 3).

Figure 3. Peptide mixture chromatogram on C-18 Reversed-phase RediSep column with water + 0.1% TFA/acetonitrile + 0.1% TFA

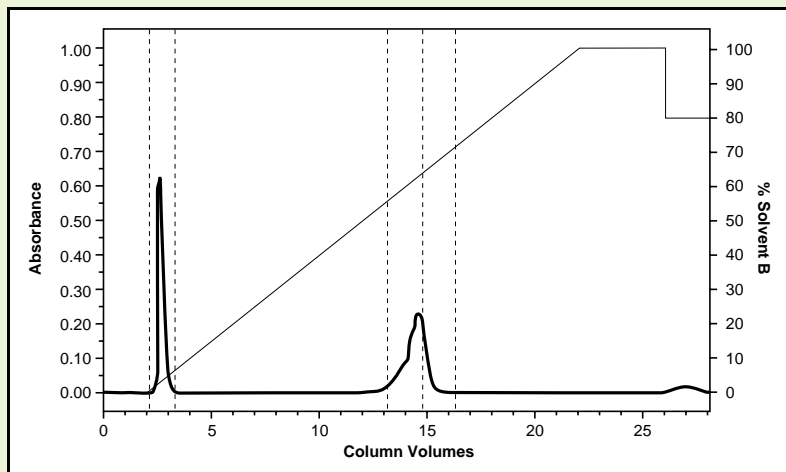
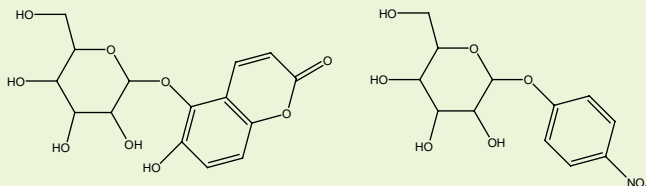


Table 3 Method Parameters

Instrumentation:	Teledyne Isco CombiFlash Companion 4x	
Column:	13g C-18 Reversed-phase RediSep Column	
Sample Loading Method:	67 mg pre-loaded on C-18 5g pre-packed cartridge	
Wavelength:	214 nm	
Mobile phase:	Solvent A: water + 0.1% TFA Solvent B: acetonitrile + 0.1% TFA	
Flow Rate:	20 mL/minute	
Equilibration Volume:	7 Column Volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	2.0
	100	20.0
	100	4.0
	80	0.0
	80	2.0

Carbohydrates

Preparative separation of carbohydrates using a C-18 Reversed-phase RediSep column is illustrated using a purification of a mixture of two carbohydrates (below).



Flash chromatography of the mixture of carbohydrates on a 4.3g C-18 Reversed-phase RediSep column fully separated the products with water/acetonitrile as the mobile phase (Figure 4).

Figure 4. Carbohydrates mixture chromatogram on C-18 Reversed-phase RediSep column with water/acetonitrile

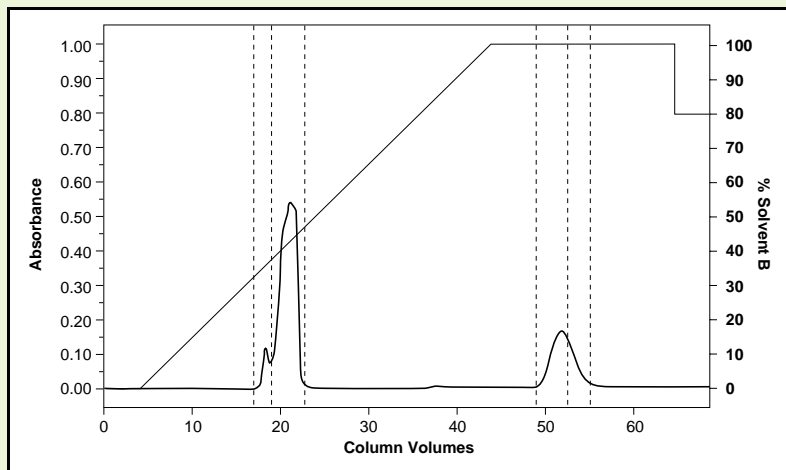


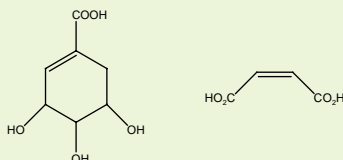
Table 4 Method Parameters

Instrumentation:	Teledyne Isco CombiFlash Companion 4x	
Column:	4.3g C-18 Reversed-phase RediSep Column	
Sample Loading Method:	22 mg pre-loaded on C-18 5g pre-packed cartridge ^a	
Wavelength:	214 nm	
Mobile phase:	Solvent A: water Solvent B: acetonitrile	
Flow Rate:	12 mL/minute	
Equilibration Volume:	7 Column Volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	4.0
	100	40.0
	100	20.0
	80	0.0
	80	4.0

a.

Carboxylic acids

Preparative separation of carboxylic acids using a C-18 Reversed-phase RediSep column was illustrated with the purification of a mixture of two acids (below).



Flash chromatography of the carboxylic acid mixture on a 13g C-18 Reversed-phase RediSep column fully separated the products with water + 0.1% TFA/acetonitrile + 0.1% TFA as the mobile phase (Figure 5).

Figure 5. Carboxylic acids mixture chromatogram on C-18 Reversed-phase RediSep column with water + 0.1% TFA/acetonitrile + 0.1% TFA

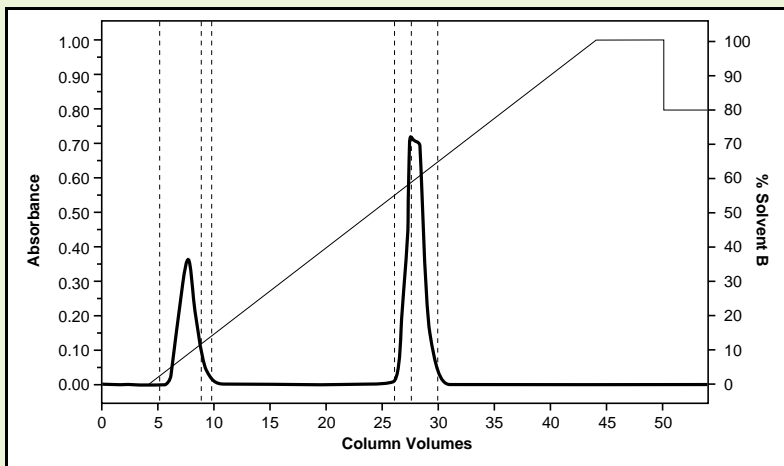


Table 5 Method Parameters

Instrumentation:	Teledyne Isco CombiFlash Companion 4x	
Column:	13g C-18 Reversed-phase RediSep Column	
Sample Loading Method:	15 mg pre-loaded on C-18 5g pre-packed cartridge	
Wavelength:	214 nm	
Mobile phase:	Solvent A: water + 0.1% TFA Solvent B: acetonitrile + 0.1% TFA	
Flow Rate:	15 mL/minute	
Equilibration Volume:	7 Column Volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	4.0
	100	40.0
	100	6.0
	80	0.0
	80	5.0

Conclusion

Flash chromatography purifications of various high-polarity compounds with C-18 Reversed-phase RediSep columns have been described.

Preparative separation of common polar organic compounds such as low solubility polar heterocycles, primary amines, carbohydrates, peptides, and carboxylic acids can be effectively and easily obtained using automated flash chromatography instrumentation such as the CombiFlash[®] systems.

C-18 Reversed-phase RediSep columns offer chemists a convenient pre-packed stationary phase for high polarity organic compounds separation.

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