Redi*Sep*® C-18 reversed phase column

Purification of halogenated heterocyclic compounds

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Chromatography Application Note AN50

Overview

Reversed phase chromatography offers both analytical and preparative applications in the area of small organic compounds separation and purification. Molecules that possess wide range of hydrophobic properties can be separated by reversed phase chromatography with excellent recovery and resolution. This application describes the purification of a mixture of halogenated heterocycles using Teledyne Isco's Redi*Sep* C-18 reversed phase column as the stationary phase.

Background

The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the molecules in the mobile phase and the stationary phase. The actual nature of the hydrophobic binding interaction itself is believed to be the result of a favorable entropy effect. The initial mobile phase binding conditions used in reversed phase chromatography are primarily aqueous, which indicates a high degree of organized water structure surrounding both the sample and the stationary phase. As molecules bind to the hydrophobic stationary phase, the hydrophobic area exposed to the solvent is minimized. Therefore, the degree of organized water structure is diminished with a corresponding favorable increase in system entropy. In this way, it is advantageous from an energy point of view for the hydrophobic moieties, *i.e.* sample and stationary phase, to associate.

Reversed phase chromatography is an adsorptive process by experimental design, which relies on a partitioning mechanism to effect separation. The sample partitions (*i.e.* an equilibrium is established) between the mobile phase and the stationary phase. The distribution of the sample between the two phases depends on the binding properties of the medium, the hydrophobicity of the sample and the composition of the mobile phase. The degree of molecules binding to the reversed phase medium can be controlled by manipulating the hydrophobic properties of the initial mobile phase. Subsequently, the mobile phase composition is modified to favor desorption of the molecules from the stationary phase back into the mobile phase.

In summary, separation in reversed phase chromatography depend on the reversible adsorption/desorption of molecules with varying degrees of hydrophobicity to a hydrophobic stationary phase.

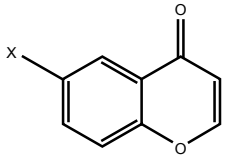
The selectivity of the reversed phase medium is predominantly a function of the type of ligand grafted to the surface of the medium. Generally speaking, linear hydrocarbon chains (n-alkyl groups) are the most popular ligands used in reversed phase applications.

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 practical drawback associated with reversed phase chromatography is the subsequent removal of water. Nowadays, water can be effectively removed by using a lyophilizer or a low-vacuum concentrator.

Results and Discussion

The separation of a mixture of halogenated chromones was investigated.



X= F, Br, Cl

Fluoro- (A),
Bromo- (B), and
Chlorochromones (C)

Flash chromatography of the heterocycles mixture on a normal phase Redi*Sep* column failed to separate the products (Figure 1). However, the use of C-18 reversed phase Redi*Sep* column successfully separated one of the three halogenated heterocycles (Figure 2 and Table 1).

C-18 reversed phase media can represent a useful alternative to normal phase silica gel media as illustrated in this paper. Beyond just representing an alternative, C-18 reversed phase silica also potentially is a media for systematic small organic compounds purification.

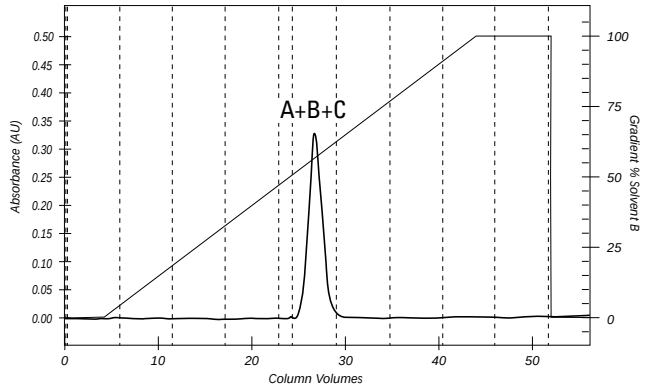


Figure 1: Chromatogram of normal phase column
Elution with hexane/ethyl acetate

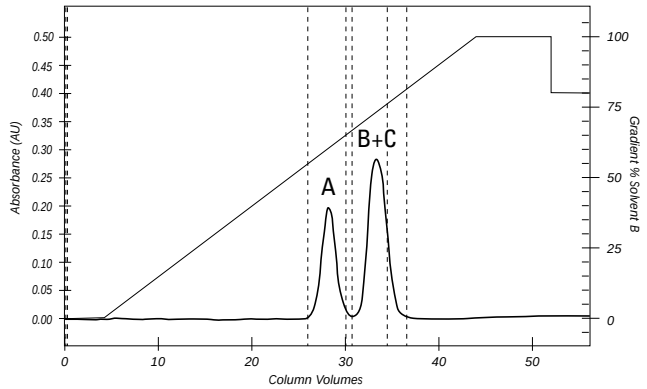


Figure 2: Chromatogram of C-18 reversed phase column Elution with water/methanol

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

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 Combi*Flash*® 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 Redi*Sep* columns on a Combi*Flash* automated flash chromatography instrument. Sample sizes are kept small, *e.g.* 30–40 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 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.

Redi*Sep* C-18 reversed phase columns are reusable if never dried after a run and stored on a mixture of 80% organic solvent (usually acetonitrile or methanol) and 20% water.

Experimental

Table 1: Method Parameters and Results

Instrumentation:	Teledyne Isco Combi*Flash*® Companion™	
Column	4.3g C-18 Reversed Phase Redi*Sep*	
Sample Loading Method	40 mg pre-loaded on celite (J.T. Baker celite 503)	
Wavelength	254 nm	
Mobile phase:	Solvent A: Water	Solvent B: Methanol
Flow Rate:	12 mL/minute	
Equilibration Volume:	7 column volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	4.0
	100	40.0
	100	8.0
	80	0.0
	80	4.0
Recovery yields:	fluorochromone product (A): 99%	unseparated chloro- and bromochromone product (B+C): 90%

Conclusion

The selective isolation of a fluoro heterocycle from a mixture of multihalogenated heterocycles was successfully achieved by using C-18 reversed phase media as the stationary phase.

This application illustrated the use of C-18 reversed phase Redi*Sep* column as a highly practical and efficient tool for the purification of medium to low polarity heterocycles.

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