

RediSep[®] SAX column

Purification of acidic compounds with a multipurpose scavenger column.

Case Study 1

Mikael Mahler, Veronica Thomason

Teledyne Isco, Inc.



Chromatography Application Note
AN44

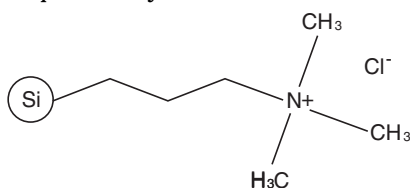
Overview

Organic compounds holding inherent acidic properties (*e.g.* carboxylic acids) present technical challenges at the purification stage via normal phase silica flash chromatography.

This application describes how Teledyne Isco's RediSep SAX column can be used as a practical and efficient tool for the selective isolation of either acidic or non-acidic compounds from a crude reaction mixture containing both. Use of the RediSep SAX column as a crude reaction mixture clean-up tool among other possible applications is also discussed.

Background

The SAX (Strong Anion Exchange) media is a silica-bound quaternary amine:

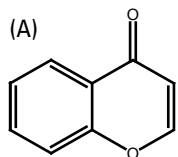


Any compounds with acidic properties including weakly acidic compounds subjected through a RediSep SAX column will be fully retained.

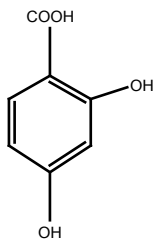
Results and Discussion

The separation of a mixture of chromone and 2,4-dihydroxybenzoic acid was investigated.

Chromone (A)



2,4-Dihydroxybenzoic acid (B)



Flash chromatography of the mixture on a normal phase RediSep column showed release of the two products (Figure 1).

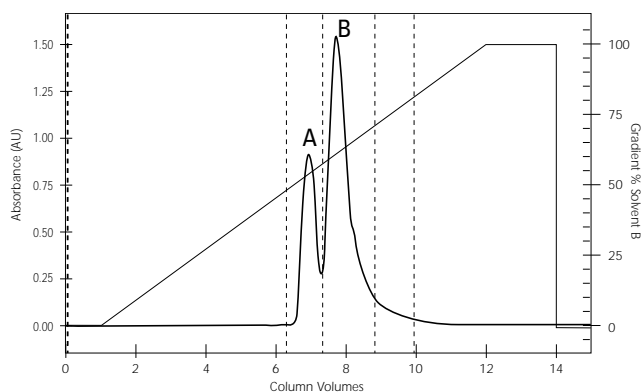


Figure 1: Chromatogram of normal phase column
Elution with hexane/ethyl acetate

The use of a RediSep SAX column showed total retention of the aromatic carboxylic acid compound onto the column and release of the chromone (Figure 2).

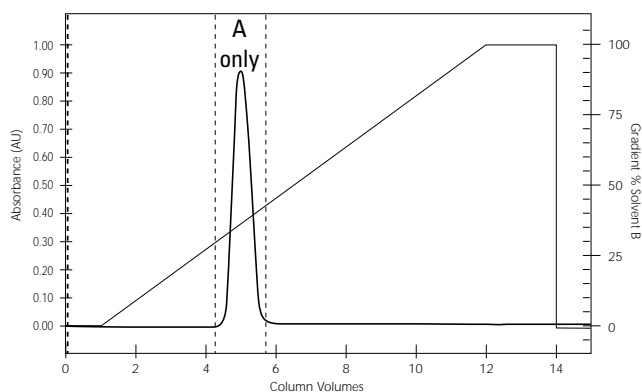


Figure 2: Chromatogram of SAX column
Elution with hexane/ethyl acetate

This isolation of the acidic 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 SAX column contains multiple compounds holding neutral properties that require further purification, column stacking can be an option. Stacking a SAX column on top of a normal phase column (Figure 3) would first strip the compounds holding acidic properties, and then separate the remaining neutral compounds. A solvent system should be selected to provide satisfactory resolution on normal phase silica.

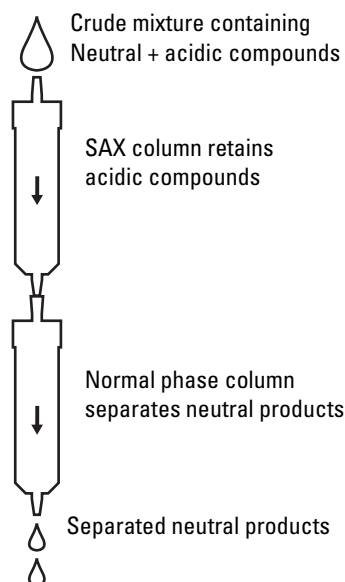


Figure 3: Stacked columns of different media as a purification tactic

RediSep columns' Luer-lok[®] fittings and Teledyne Isco's Column Stacker offer a convenient solution for column stacking.

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

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

The RediSep SAX column media will not degrade when using common protic or aprotic organic solvents and it is reusable immediately if an acetic acid in methanol column flush was used for product release. The remaining acetate counteranions are less retained than the initial chloride ones and should facilitate the next separation.

Experimental

Table 1: Method Parameters and Results

| | | |
|-----------------------|--|--|
| Instrumentation: | Isco CombiFlash [®] Companion [™] 4x | |
| Columns | 5.7g SAX RediSep | |
| Sample Loading Method | 48 mg pre-loaded on celite (J.T. Baker celite 503) | |
| 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 | 11.0 |
| | 100 | 2.0 |
| | 0 | 0.0 |
| | 0 | 1.0 |
| Recovery yields: | chromone product (A): 99% | dihydroxybenzoic acid product (B): 99% |

Conclusion

The RediSep SAX column is a multi-purpose scavenger column which can be used for the separation and isolation of either acidic compounds or neutral compounds.

Luer-lok is a registered trademark of Becton, Dickinson and Company Corporation

RediSep and CombiFlash are registered trademarks, and Companion is a trademark of Teledyne Isco, Inc.

All other brand and product names are trademarks or registered trademarks of their respective holders.

Last modified November 8, 2012

Teledyne Isco

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

Teledyne Isco is continually improving its products and reserves the right to change product specifications, replacement parts, schematics, and instructions without notice.

