

Dry samples improve resolution

in normal phase flash chromatography

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Chromatography Application Note AN15

Overview

Factors which affect the purification efficiency of flash chromatography include sample homogeneity and sample concentration, among others. When the compound and reaction by-products are over-diluted or dissolved in too polar a dissolving solvent, the compound peak broadens which leads to loss of resolution. This application note demonstrates that preparing dry samples using RediSep® solid load sample cartridges improves peak resolution over liquid injection techniques.

Results and Discussion

A CombiFlash® Companion® system with RediSep media cartridges was used for flash chromatography. The system's dual UV detection/monitor was used to gauge the effects of band broadening and overlapping peaks. The separation of a 280mg mixture of a phenolic ester and pyridine derivative was investigated. All separations were performed using RediSep 12g normal phase columns with a hexane/ethyl acetate mobile phase.

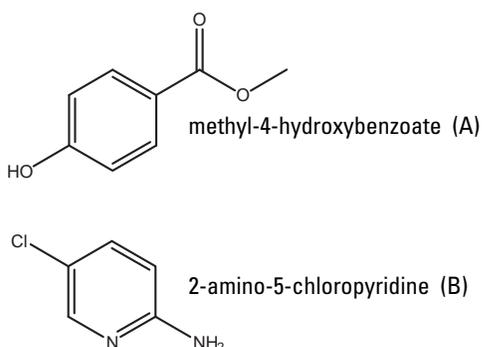


Figure 1 shows the chromatographic effect of dissolving the sample mixture and directly injecting it onto the column after equilibration. Figure 2 shows the chromatographic effect when the same dissolved mixture is injected through the injection port and followed by a chase solvent after column equilibration. Both techniques resulted in band broadening and overlapping peaks.

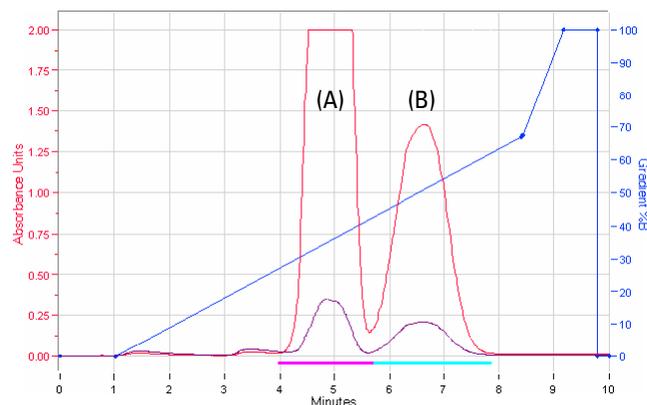


Figure 1: Direct loading on 12g normal phase column. Sample dissolved in 2mL of acetone loaded directly on column.

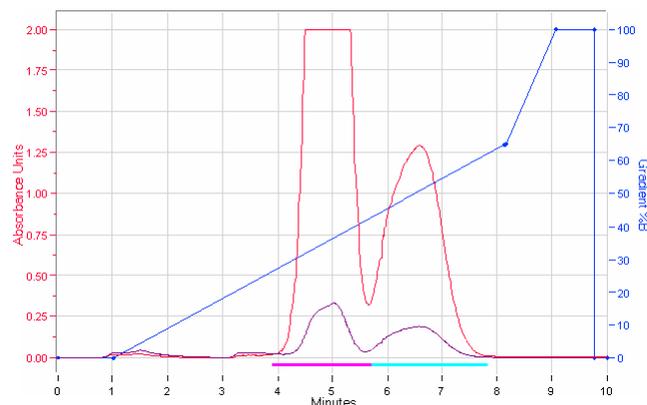


Figure 2: Liquid injection on 12g normal phase column. Sample dissolved in 2mL of acetone loaded through the injection port and followed by a 1mL acetone chase.

The same sample mixture was loaded onto a RediSep pre-packed 5g solid load sample cartridge. Placing the sample mixture and dissolving solvent in a solid load sample cartridge resulted in improved peak resolution and separation (Figure 3). Further improvement was evident when the dissolving solvent was removed by vacuum before placing the pre-packed solid load sample cartridge on the system (Figure 4).

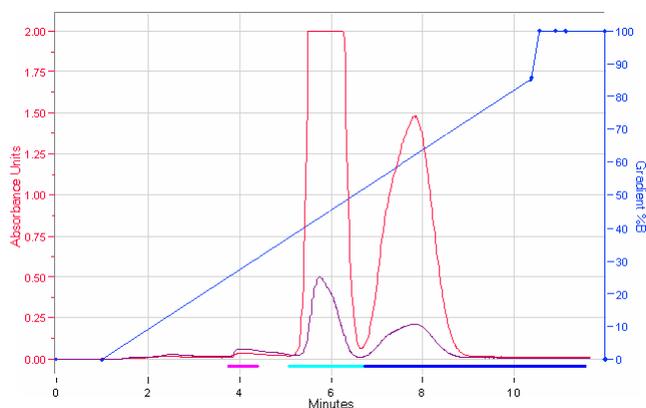


Figure 3: Wet solid load sample cartridge. Sample dissolved in 2mL of acetone and loaded in a 5g pre-packed normal phase silica cartridge. *Without removing acetone*, the sample was eluted on a normal phase column.

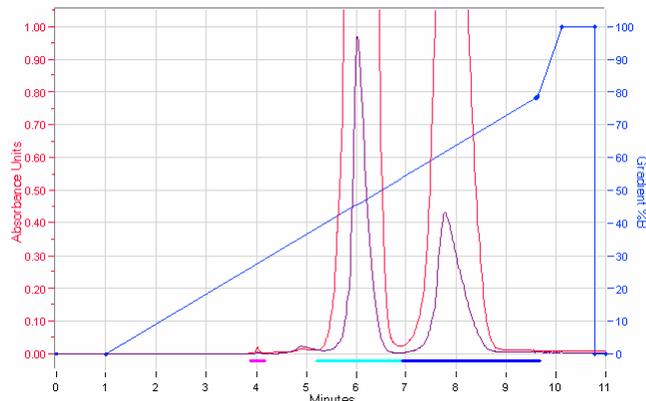


Figure 4: Dry solid load sample cartridge. Sample dissolved in 2mL of acetone and loaded in a 5g pre-packed normal phase silica cartridge. *After removing acetone by vacuum*, the sample was eluted on a normal phase column.

The same sample mixture was also adsorbed onto 300mg of Celite¹ and placed into an empty 5g solid load sample cartridge. Figure 5 shows improved peak resolution and separation, though less than that of Figure 4, due to the additional 5g of silica which enhances resolution by increasing the number of theoretical plates. (Note: Celite has no retentive properties.)

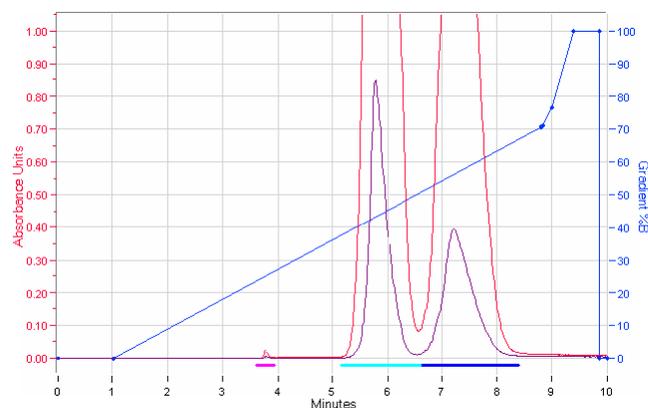


Figure 5: Solid load sample cartridge containing sample mixture on Celite. Sample dissolved in 2mL of acetone and adsorbed onto 300mg of Celite. Acetone was removed by vacuum and the adsorbed sample was placed in an empty 5g cartridge. Sample was then eluted on a normal phase column.

Experimental

Table 1: Typical method parameters

| | | |
|-----------------------|---|--------------------------|
| Instrumentation: | Teledyne Isco CombiFlash Companion System | |
| Column: | Teledyne Isco RediSep 12g Normal Phase Column | |
| Wavelength: | 254nm (detection), 280nm (monitoring) | |
| Sample: | 280mg mixture of methyl-4-hydroxybenzoate (140mg) and 2-amino-5-chloropyridine (140mg) | |
| Mobile phase: | Solvent A: Hexane | Solvent B: Ethyl acetate |
| Flow Rate: | 30mL/min | |
| Equilibration Volume: | 100mL | |
| Gradient: | PeakTrak's 12g default linear gradient was used until samples were off the column, then the run was shortened to increase throughput. | |

Conclusion

Volume overloading causes significant band broadening which degrades sample resolution. This can be quite evident when performing liquid injections. Dry sample loading has a dramatic enhancement over liquid sample loading as long as the polar diluent (acetone) is removed by drying to eliminate sample co-elution. The difference between the pre-packed silica and Celite is that one has an additional 5g of silica to improve compound and by-product separation while Celite has no retentive properties.

1. Celite is a registered trademark of Johns-Manville Corporation.

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