Acetonitrile as a Replacement for Methanol in Normal-phase Flash Chromatography

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Introduction

When it comes to polar organic compound purification, many chemists turn to normal-phase flash chromatography often utilizing dichloromethane and methanol as the eluting solvents. While this can work, it often can be challenging to optimize due to methanol's high polarity and protic chemistry.

Some of the issues are well-chronicled including:

- Difficulty creating methods from TLC
- Silica instability
- Challenges creating shallow, low methanol concentration gradients

In my research, I have found that acetonitrile, because if aprotic nature, can often replace methanol as the polar modifier in DCM-based solvent systems and in this poster I will show an example where this is true.

Experimental protocol

Reagents and Materials

Solvents used in study included: dichloromethane, methanol, and acetonitrile (all from Reagents, Inc., Charlotte, NC).

Reagents included methyl paraben, butyl paraben, and 4-methyl-4(5)-nitroimidazole (all from Aldrich Chemical)

Silica TLC plates (Biotage) were used for solvent scouting and method development.

Biotage® SNAP Ultra (10 g) flash cartridges were used for this study.

A Biotage Isolera™ Dalton system was used for purification.

Normal-phase Flash Chromatography

Those familiar with normal-phase liquid chromatography know that methanol and acetonitrile fall into different selectivity groupings (methanol in group 2 and acetonitrile in group 6). This selectivity group difference can influence the separation between any pair of compounds. Likewise, methanol and acetonitrile also have different polarities relative to silica (methanol is more polar) which influences compound retention, Table 1.

Table 1. Strengths of solvents used in this study

	DCM	MeOH	MeCN
Strength	0.32	0.70	0.50

TLC was performed to evaluate which DCM-MeOH and DCM-MeCN solvent ratios provided the best separations. The TLC data defined the flash purification linear gradient methods.

Results and discussion

Two mobile phase blends, one for each solvent system, were found to separate the three-component mix. The blends were created to provide an equivalent solvent strength, which is important to ensure similar separation duration. The solvent ratios compared were 10% MeOH in DCM and 20% MeCN in DCM, each with a blend strength of 0.36.

Strengths of solvent blends are calculated by multiplying the volume fraction of each solvent and its solvent strength and adding them together.

To calculate the solvent strength for 10% MeOH in DCM…

$$0.1 \times 0.7 = 0.07$$

$$0.9 \times 0.32 = 0.29$$

$$0.07 + 0.29 = 0.36$$

The TLC data (Figure 1) clearly shows very different selectivity patterns. With DCM-MeOH, the nitroimidazole compound elutes closer to the parabens than with DCM-MeCN, indicating a better separation with DCM-MeCN. In both TLCs the parabens separate but elute with high Rf values which can complicate the separation and reduce loading capacity. The imidazole compound elutes with the same Rf, 0.34.

Using the TLC data linear gradients were created based on each blend. The DCM-MeOH TLC data defined a gradient of 2-20% MeOH in DCM. The DCM-MeCN TLC data defined a 5-40% MeCN in DCM gradient.

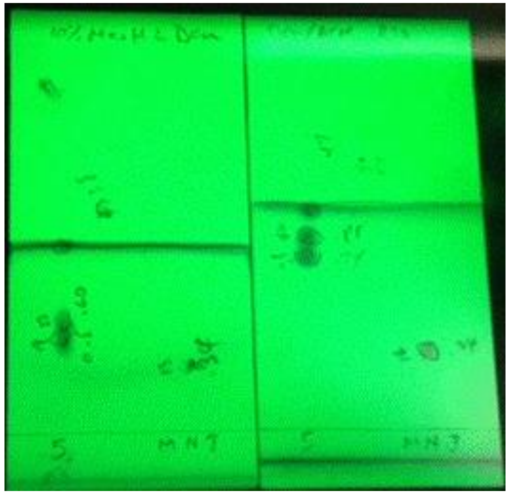

Figure 1. *TLC solvent blend comparison of 10% MeOH-DCM (left) and 20% MeCN-DCM for the separation of butyl paraben, methyl paraben and 4-methyl-4(5)-nitroimidazole. Each solvent blend strength was identical (0.36) insuring similar chromatographic durations.*

DCM-MeOH Flash Chromatography

Although the TLC method using 10% MeOH in DCM provided a separation of each of the three sample components the separation of the parabens (Rf 0.6 and 0.5) failed using flash chromatography with both parabens co-eluting just after 4 column volumes, Figure 2.

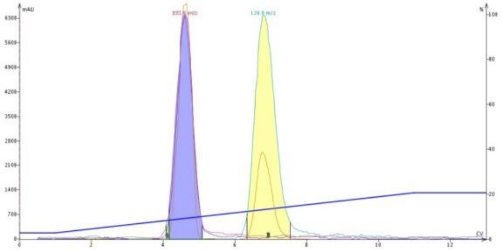

Figure 2. *DCM/MeOH separation on silica based on TLC results. The blue peak contains both methyl and butyl paraben which did resolve on TLC with the same solvents.*

The flash chromatography results are quite typical of the challenges associated with DCM-MeOH elution solvents, primarily an inability to create useful flash purification methods based on TLC results.

DCM-MeCN Flash Chromatography

Switching the polar solvent from protic methanol to aprotic acetonitrile alters both solvent strength and selectivity as seen with the TLC data in Figure 1. The two parabens elute at higher Rf values but do separate with Rf values of 0.85 and 0.74 (far from ideal) while the imidazole elutes with an Rf of 0.34.

When the TLC data were converted to a linear gradient, the results resembled the TLC with both parabens separating from each other and the imidazole, Figure 3.

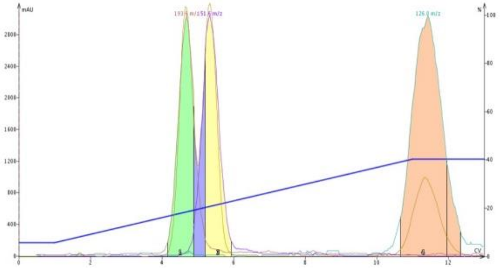

Figure 3. *DCM/MeCN separation on silica based on TLC results. With aprotic acetonitrile as the polar modifier in the gradient the parabens are separable from each other and from 4-methyl-4(5)-nitroimidazole (pink).*

Conclusions

The choice of polar modifier can play a critical role in the success of a flash purification. While methanol is inexpensive, it can be challenging to use with normal-phase chromatography due, primarily, to its protic chemistry, which competes strongly for adsorptive silanol groups on silica.

Selecting an alternative polar solvent such as aprotic acetonitrile can simplify and improve compound purification.

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