Can Strong Solvents Like DMSO and DMF be Used as Injection Solvents in Reversed-Phase Flash Chromatography?

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Introduction

Synthesis reactions are often conducted using DMSO and other highly solvating solvents (NMP, DMF) because their high boiling points allow for higher reaction temperatures. Retrieving the synthetic products from these solvents through evaporation, however, is nearly impossible and back extraction can be challenging.

Chromatographic purification should be an ideal method but because of these solvents' physical properties (density, viscosity, and high UV cutoff) many chemists shy away from them fearing high pressures or diminished separations.

In this poster we show the results of using both DMSO and DMF as dissolution solvents and provide guidelines for using them successfully in reversed-phase flash purification.

Experimental Protocol

Reagents and Materials

Reagents used in the study included: methanol, dimethyl sulfoxide, N,N-dimethylformamide, deionized water, acetone, naphthalene, 1-nitronaphthalene, 3,5-dibenzyloxyacetophenone, butyl paraben, and methyl paraben, all from Sigma Aldrich (Milwaukee, WI).

A Biotage® SNAP Ultra C18 flash cartridge, 12 g, was used for this study.

Test Mix Preparation

A 5-component crude mix of naphthalene, 1-nitronaphthalene, 3,5-dibenzyloxyacetophenone, butyl paraben, and methyl paraben were combined and dissolved in both DMSO and DMF at ~1 g each/5-mL solvent. These were used as a stock solutions from which several serial dilutions were made.

Chromatography

For reversed-phase chromatography a water-methanol gradient was used (Table 1). Serial dilutions - 1:1, 1:2, and 1:5 - were tested as was the stock solution. All injection volumes delivered approximately 100 mg of the test mix.

Table 1. Chromatographic conditions

Instrument	Isolera™ One SV
Solvent A	Water
Solvent B	Methanol
Equilibration	55% B for 3 CV
Gradient	55% B for 2 CV
	55%-90% B in 10 CV
	90% B for 1 CV
	100% B for 3 CV
Cartridge	Biotage® SNAP Ultra C18, 12g
Flow rate (mL/min)	20 mL/min
Test mix load	Stock 0.1 mL 1:1 dilution 0.2 mL 1:2 dilution 0.3 mL 1:5 dilution 0.6 mL
Collection wavelengths	λ-All (200-400 nm)
Monitoring wavelengths	254 nm, 280 nm

The first purifications were performed with the stock solutions. The injection volume for each stock solution was 0.1 mL providing a load of 100 mg. The chromatographic results for each solvent show a decent separation but with some peak tailing and without baseline resolution (Figure 1).

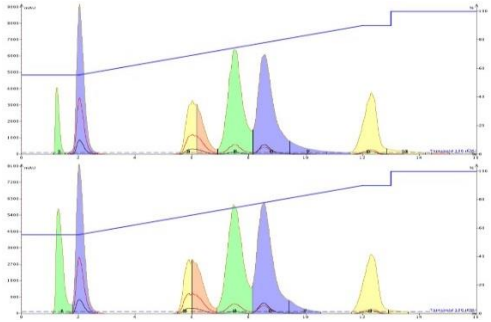

Figure 1. Purification of each 5-component stock solution. DMSO (top) and DMF (bottom) shows similar separation quality but fail to fully resolve the three middle compounds.

After injecting 0.1 mL of each stock solution each of the serial dilutions was also evaluated. Both the 1:1 (0.2 mL injection) and 1:2 (0.3 mL injection) dilution runs show better separations than the stock solution results (Figures 2 and 3). It is also interesting to note the lack of separation loss between the dissolution solvent peak (first green peak) and the first eluting compound with increased injection volume.

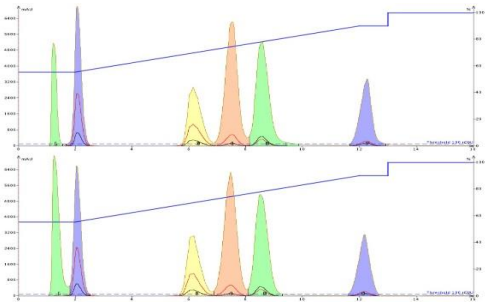

Figure 2. Purification of the 1:1 DMSO (top) and DMF (bottom) dilutions of the stock samples, injection volume was 0.2 mL. In both cases the chromatography actually improved versus the stock solution results.

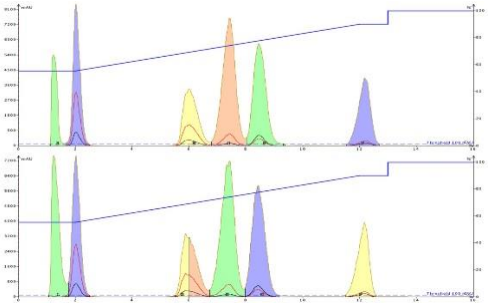

Figure 3. Examples of 1:2 DMSO (top) and DMF (bottom) dilutions. Injection volume was 0.3 mL. These results indicate no resolution or retention loss with increased injection volume.

The 1:5 dilution data show a departure from the 1:1 and 1:2 dilution results. While the DMSO chromatography shows no change the DMF separation displays severe peak fronting and resolution loss (Figure 4).

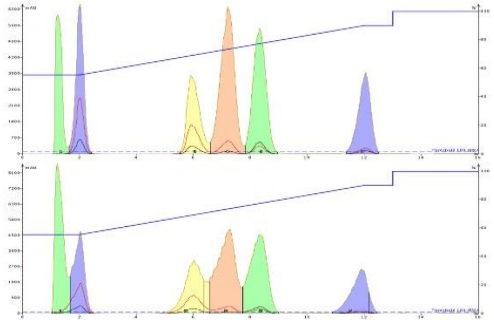

Figure 4. Chromatography of 1:5 dilutions of stock solutions with DMSO (top) and DMF (bottom) shows a volume limitation with DMF but not with DMSO. With DMF the peaks are displaying volume overload not present with the DMSO injection. Injection volumes were 0.6 mL for each.

Results and Discussion

The data clearly show DMSO and DMF are suitable dissolution solvents for reversed-phase flash. However, it is interesting to see that chromatographic performance is maintained using DMSO with increasingly larger volumes but that DMF has volume limitations. It is believed that the much lower log P for DMSO (-2.03)[1] vs. DMF (-1.01)[2] is the reason for the better performance. Though these solvents are very polar, they do have the capability to solvate a broad polarity range of compounds. We surmise that DMSO and DMF solvation capabilities "wet" the C18 surface so well that the sample components can be loaded in a tight band in high concentration. However, the stock solutions at 1g/mL may be too concentrated, even with a small 0.6% injection volume, to get optimal separation between all compounds. Larger injection volumes of diluted solutions provided optimal loading and resulting chromatography.

Conclusion

DMSO and DMF are suitable injection solvents for reversed-phase flash purification. DMSO shows it can be loaded in larger volumes (up to 0.05 mL/g of C18 media or 3.5% of a column volume) without affecting chromatographic separations or carrying compounds with it.

[1] Gaylord Chemicals DMSO MSDS #GCC1-11, October 30, 2013
[2] Sigma-Aldrich DMF SDS version 4.11, May, 25, 2015

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