On-Column Dilution: A Technique to Load Samples Dissolved in DMSO

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Abstract

Peptide purification efficiency is often limited by resolution. Resolution from sample loading is decreased if samples need to be dissolved in solvents such as DMSO or DMF, particularly for water-soluble peptides. On-column dilution is a technique that allows samples to be dissolved in strong solvents such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) while maintaining resolution during preparative chromatography. The sample is "bracketed" between slugs of weak solvent. Air-gaps between the sample and weak solvent prevent precipitation of the sample during injection. A 300% increase in peptide sample loading is demonstrated.

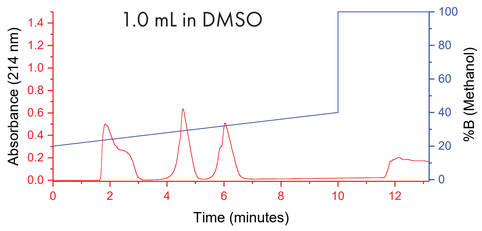
Background

Samples are ideally be dissolved in either the mobile phase, or a chromatographically weaker solvent for best chromatography. Dissolving samples in a stronger solvent may cause them to run down the column with the strong solvent, with little interaction with the stationary phase, causing reduced resolution and loading capacity. Even when peaks are resolved during the chromatography, some compound is often lost in the injection solvent peak.

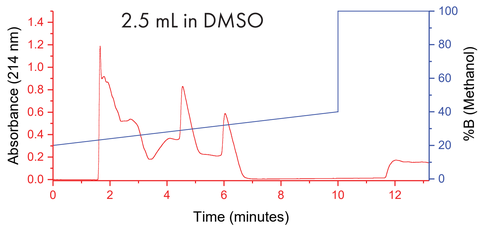
Chromatographers use solvents such as DMSO and DMF because nearly all compounds are soluble in them. At the same time, these solvents are weaker than the strong solvent used for reverse-phase chromatography, which limits the peak dispersion of non-polar compounds. Unfortunately, polar compounds show greatly reduced retention when dissolved in these solvents. Samples are dissolved in DMSO or DMF because it saves time compared to finding the optimal dissolution solvents. Many samples contain a mixture of polar and non-polar compounds for which DMSO or DMF are the best dissolution solvents. DMSO and DMF also permit a high concentration of sample to be loaded.

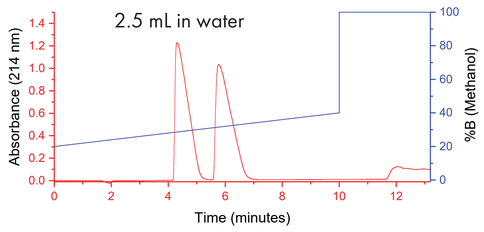
One method to inject samples in strong solution is via "At-column dilution" [1], where the weak and strong solvents are mixed at the head of the column. The sample, dissolved in DMSO or DMF, is injected into the strong solvent stream. At-column dilution can lead to precipitation on the column head under some conditions[2] particularly when the gradient needs to start in low concentrations of organic solvent, and the sample to be purified contains large amounts of hydrophobic material.

By using a weak solvent to sandwich the sample, the strong solvent is diluted sufficiently to allow the sample to be adsorbed and permit focusing of the peak. Although commonly used for analytical chromatography with a small sample volume, the compound precipitates at the interface between the strong and weak solvents leading to clogging and peak tailing when applied to a preparative system. Air gaps between the solvent and the sample prevent this mixing until the sample is on the column in "On-column dilution". This is a variation of the sandwich or "DMSO slug" described by Leister *et al*[3] except that the DMSO sandwiches are replaced with water.



Resorcinol and catechol (10 mg/mL each) dissolved in DMSO or water. The use of DMSO limits the injection volume and sample loading.

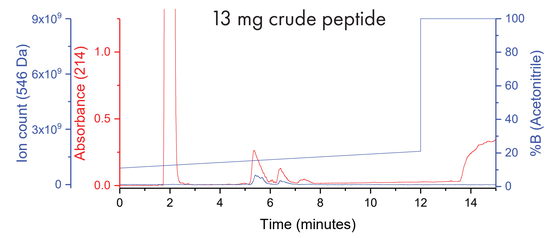




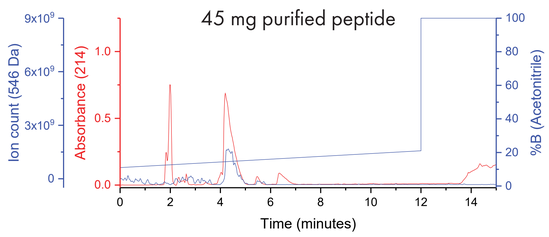
Tube cross section showing DMSO slugs with air gaps. On-column dilution replaces the DMSO with water. Precipitation is prevented because of the air gaps.

Experimental and Results

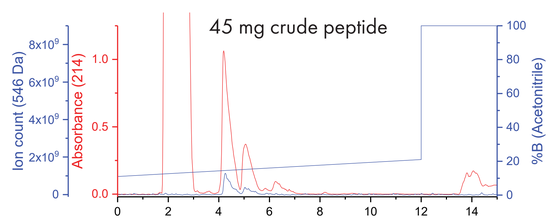
An ACCQPrep HP150 system (Teledyne ISCO PN 685230053) equipped with a PurIon L mass spectrometer (PN 685237084) with a 20x150 mm RediSep Prep C18 column (PN 692203810) was used for these runs. The system was equipped with a 5 mL sample loop. The peptide (H2H-ITTNP-OH, New England Peptide) was dissolved in 1.0 mL DMSO or water. For the On-Column Dilution run, the loop was filled with water. An air gap was made by injecting 1 mL water, 100 µL air, followed by the sample, followed by another 100 µL air. Finally, 1 mL water was injected. The purification gradient was determined using the Focused Gradient Generator feature in the ACCQPrep.



Direct injection of peptide dissolved in DMSO.



Injection of peptide dissolved in water. Peptide was pre-purified on a flash system prior to final purification on the ACCQPrep system.



On-column Dilution injection of peptide dissolved in DMSO.

Conclusion

The use of On-column Dilution allowed increased loading of the peptide–the same loading possible as a sample dissolved in water. The higher loading increases throughput by ~3 fold in this example, saving time and solvent while still providing pure product. It was found that the 100 µL water plugs on either side of the sample needed to be increased to at least 1.0 mL to sufficiently dilute the injection solvent. The loop size can also be a limiting factor since the loop needs to hold the sample, air gaps, and water plugs on either side (3.2 mL for these experiments). No precipitation was observed in these runs, and no replumbing of the pumps was required.

References

[1] Blom, K.F. Two-Pump at-Column-Dilution Configuration for Preparative Liquid Chromatography-Mass Spectrometry. *J. Comb. Chem.* **2002**, *4*, 295–301

[2] Blom, K.F.; Glass, B.; Sparks, R.; Combs, A.P. Preparative LC-MS Purification: Improved Compound-Specific Method Optimization. *J. Comb. Chem.* **2004**, 6, 874–883.

[3] Leister, W.; Strauss, K.; Wisnoski, D.; Zhao, Z.; Lindsley, C. Development of a Custom High-Throughput Preparative Liquid Chromatography/Mass Spectrometer Platform for the Preparative Purification and Analytical Analysis of Compound Libraries. *J. Comb. Chem.* **2003**, *5*, 322–329.

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