Removal of Non-volatile Solvents with Redi*Sep* Gold® C18Aq Columns

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Abstract

Samples are often dissolved in non-volatile solvents, such as dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), for experiments such as nuclear magnetic resonance (NMR) studies. Recovery of the sample from these solvents is difficult because of the solvent's high boiling point. Redi*Sep* Gold® C18Aq columns are useful for retaining the desired compound while removing the non-volatile solvent. The compound is then released using a solvent with a lower boiling point that is more easily evaporated. This method allows the recovery of valuable material for further experiments.

Background

Samples are dissolved in non-volatile solvents for analysis such as NMR; DMSO-d_6 is often used to dissolve polar compounds for NMR structure determination. This is because DMSO-d_6 has a simple NMR spectrum itself and freshly-opened ampoules do not contain hydrogen that can exchange with labile protons such as those on amines, alcohols, or phenols. These commonly used solvents are difficult to evaporate and in most instances, the compound is not recovered. DMSO boils at 189 °C and DMF, another common NMR solvent, boils at 153 °C under atmospheric pressure. These solvents are difficult to evaporate under vacuum and heating the mixture to speed evaporation may damage the compound of interest.

Many of these non-volatile solvents are soluble in water allowing compounds to be captured on a reverse phase resin, such as Redi*Sep* Gold C18Aq, and released into a volatile solvent, such as methanol, after washing the solvent used for the NMR experiment off the column. As NMR studies generally use purified compounds, there is no material left on the column after washing and the column can be used for this purpose several times.

The Redi*Sep* Gold C18Aq columns are well suited to this application because they resist phase collapse. NMR samples are generally dilute and the large volume of water used to dissolve the sample and non-volatile solvent can eventually cause phase collapse with other types of C18 (Figure 1).

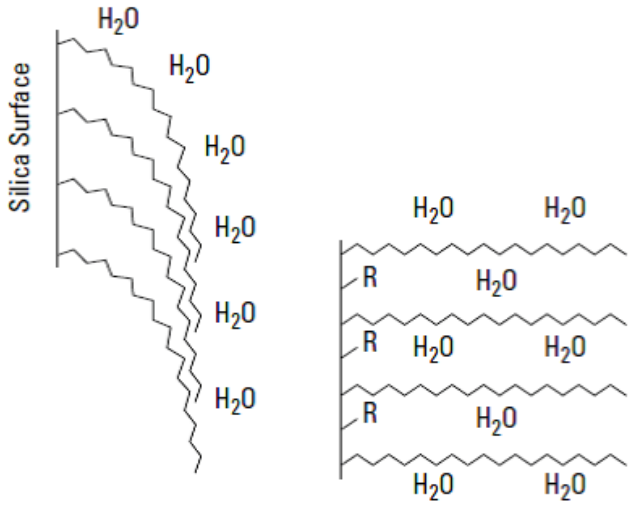

Figure 1: C18 chains are fully extended when organic solvent is present but undergo "phase collapse" (left) under highly aqueous conditions. Hydrophilic groups (right) such as those in RediSep Gold C18Aq columns reduce phase collapse.

Experiment and Results

A sample (95 mg) of epigallocatechin gallate was isolated and dissolved in DMSO-d6 for proton and carbon NMR analysis. A 5.5 g Redi*Sep* Gold C18Aq column (PN 69-2203-558) was conditioned by washing with 2 column volumes (CV) methanol, followed by 5 CV water. The 1 mL sample was removed from the NMR tube and the mixture dissolved in 5 mL water which was loaded on the column. The Combi*Flash* was run in manual control mode and the column was washed with 5 CV water to remove the DMSO. The column was then run with a step gradient to 100% methanol (Figure 2). The peak was monitored at 214 nm. The chromatogram shows the compound eluted in a single sharp peak. A linear gradient could be run if the compound required further purification. The sample load should be limited to 5% of the column weight to avoid sample "breakthrough". For example, a 5.5 g Redi*Sep* Gold C18Aq column would desolvate up to ~250 mg sample. The column injection and wash should be captured if there is any question whether the desired compound will adsorb on a C18 column so as to avoid possible sample loss.

1. Purification of Phenolic Flavonoids with Flash Chromatography, Silver, J.E.; Drooby, M.; Lewis, R.L. presented at the International Congress on Natural Products Research 31 July 2012

Conclusion

Using a Redi*Sep* Gold C18Aq column allows facile recovery of a compound from a non-volatile solvent. The example compound elutes from a C18 column at 30% methanol when run as a gradient. It dissolves in water, but it still adsorbed well onto the C18Aq column to allow removal of the DMSO-d_6. This represents a general method for isolating compounds from such solvents for further studies.

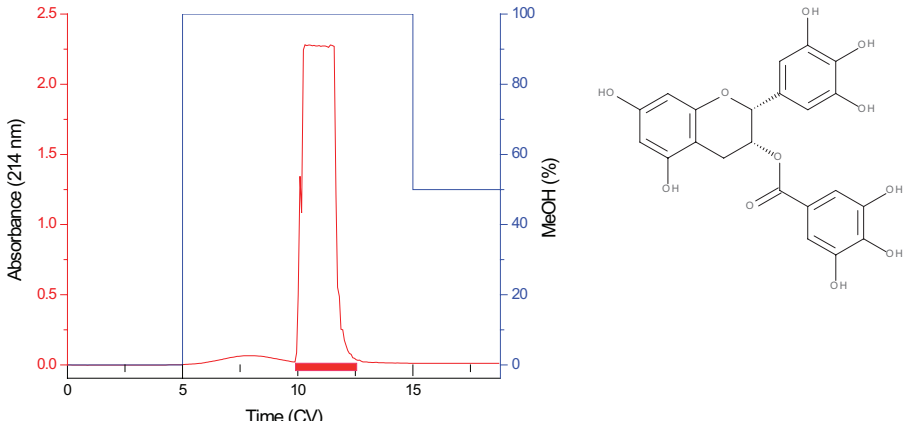

Figure 2: Elution of epigallocatechin gallate after removal of DMSO-d_6.

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