

Eliminate HPLC Sample Preparation Bottlenecks with High Speed Evaporation and Lyophilization



Innovative technology overcomes the challenges of lyophilizing HPLC samples containing organic solvents and accelerates sample preparation for high-throughput applications.

Lyophilization of HPLC fractions

Preparative reverse-phase high-performance liquid chromatography (HPLC) enables the isolation of highly pure compounds, and is widely used in chemical, pharmaceutical, biotechnology, and biochemistry industries. Most samples contain water and polar organic solvents such as acetonitrile, and require careful preparation to ensure accurate results. HPLC fractions are usually dried using a parallel sample evaporator, and during this process it is important to retain the quality and integrity of the sample. Utilizing a centrifugal evaporator to dry HPLC fractions offer many benefits with a few challenges. Alternatively, lyophilization (freeze drying) technology uses subtle conditions and is usually used to dry sensitive, biological samples. Due to the more open structure of the sample cake, a higher level of dryness may be achieved in this process. However the drawback to this technique is that it is time consuming. Here we discuss a methodology used to achieve the best of both worlds: a rapid process capable of producing thoroughly dried samples for improved resuspension and easier sample handling.

Evaporation, lyophilization, and the best of both worlds

Lyophilization is the process of removing solvents from a sample via sublimation, which is achieved with a low vacuum that boils water below its freezing point. Freeze drying HPLC samples containing water and solvents, such as acetonitrile, pose several challenges for lyophilization. Acetonitrile has a low freezing point (-65°C), and if this is not achieved during lyophilization, sample bumping and contamination may result. If acetonitrile accumulates in the ice trap, it can impair the vacuum and prevent lyophilization. In addition to the challenges faced in working with solvents, the lyophilization process itself is slow, requiring days to achieve the desired sample dryness.

Solvent removal may also be achieved by evaporation, whereby energy (heat) is applied and the liquid is vaporized to a gas and removed, leaving a dry product. Working with a centrifugal evaporator addresses many of the challenges associated with lyophilization, however this approach is not without its own limitations. Trifluoroacetic acid (TFA) is often added to the



Figure 1: SP Genevac HT Series 3i Evaporator

reverse phase HPLC mobile phase for its buffering capabilities, enhance retention, or to suppress certain ionic interactions. At times, centrifugal evaporation is insufficient to remove all of the TFA, due to sample interactions, and the residual could have detrimental effects on analysis. Further, this approach often produces a film-like dried sample on the bottom of the vial, making it difficult to re-suspend and weigh. In addition, fractions containing hydrophobic samples can crash out with the removal of the organic layer, to form a thin film on top, which could restrict the evaporation and may result in incomplete drying.

These challenges led to the development of a combined method, incorporating the speed and efficiency of centrifugal evaporation with the superior drying capability and improved sample integrity achieved with lyophilization. The method development was done using the SP Genevac HT Series 3i centrifugal evaporator, which consists of a robust oil-free vacuum pump, a low temperature, automatic defrosting and draining condenser and an intuitive user interface with user programmable methods.

The method shown below is a way to successfully freeze dry HPLC fractions with hydrophobic and hydrophilic compounds. It also accommodates fractions with varying gradients and volumes.

- **Stage 1** - Removal of a portion of acetonitrile by using Dri-Pure with the final pressure at full vacuum; then hold at full vacuum for a few hours to make sure
- **Stage 2** - Add heat for a limited time at full vacuum to speed up the sublimation

- **Stage 3** - Then continue to control the pressure at full vacuum with no heat until samples completely freeze dried

If the samples are completely water soluble, the organic layer can be removed first and then the aqueous layer can be concentrated before freeze drying. Method details are summarized below:

- **Stage 1** - Evaporate the acetonitrile using Dri-Pure and then control the pressure at 40mbar for a set time
- **Stage 2** - Concentrate the water at 8mbar to reduce the total volume
- **Stage 3** - Freeze the samples by reducing the pressure
- **Stage 4** - Add heat for a limited time at full vacuum to speed up the sublimation
- **Stage 5** - Then continue to hold the pressure at full vacuum with no heat until the samples are completely freeze dried

This method may be further adapted for aqueous samples:

- **Stage 1** - Concentrate the water at 8mbar to reduce the total volume
- **Stage 2** - Freeze the samples by reducing the pressure
- **Stage 3** - Add heat for a limited time at full vacuum to speed up the sublimation
- **Stage 4** - Then hold the pressure at full vacuum with no heat until the samples are completely freeze dried

Refined technology delivers superior results and high

throughput

Preventing processing bottlenecks during the lyophilization process is crucial for high throughput laboratories. Implementing a highly efficient instrument capable of rapid evaporation and lyophilization, accelerates sample preparation. The SP Genevac HT S3i evaporator enables high speed evaporation and lyophilization, reducing sample processing time from days to hours. This is achieved with a high-performance oil-free, vacuum pump, patented temperature control, and Dri-Pure anti-bumping technology to prevent sample loss and contamination. The system can accommodate a wide range of sample formats for various applications and the pre-programmed methods for commonly used solvents render the system easy to operate, even for occasional users. With the combination of speed and convenience associated with centrifugal evaporation, and the drying capabilities of lyophilization, the SP Genevac HT S3i produces a light, fluffy sample, fully lyophilized and ready for analysis in just hours.

To learn more about the SP Genevac HT Series 3i evaporator range, visit:

www.sp-scientificproducts.com/HT3/