

Drying of Problematic Purification Samples by Lyophilization in a Centrifugal Evaporator - with “Fluffy” Results



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Lyophilization is often the preferred method of sample drying because a high level of dryness is achieved with low residual solvent levels, and because of the light, powdery, ‘fluffy’ finish of the sample which enables the sample to be easily removed and weighed out. Nevertheless, there are many limitations of lyophilization.

In this study we will show how, by transferring the process to its centrifugal evaporators, Genevac has managed to change the rules and achieve the results that purification laboratories are searching for.

Introduction

For many years lyophilization, or freeze drying, has been used to dry samples in the laboratory. The technique is well researched and has become the method of choice for many researchers with a few samples to dry.

However, there are a number of potential drawbacks encountered with the conventional freeze drying apparatus, these include:

1. Samples must be prepared in a limited range of solvents, normally only water can be used
2. Volatile organic solvents and their mixtures cannot be used
3. The process is slow

Therefore researchers with many samples to process, or mixtures of solvents, say from preparative reverse phase HPLC separation containing water and acetonitrile, have turned to centrifugal evaporators. In other laboratories, compound handling for example, the aggressive nature of the organic solvents used renders a freeze drier unsuitable. Even in for these environments, state of the art centrifugal evaporators, such as the Genevac HT-Series, shown in Figure 1, have some limitations.

Problems with Purification

Purification laboratories receive samples dissolved in water and acetonitrile, with a low level of a modifier present, normally 0.1% TFA. Freeze drying to remove these solvents is fraught with difficulties: the acetonitrile requires either a very deep vacuum to freeze it, or a freeze drier which actively freezes the samples. Acetonitrile freezes at -65°C . If it is

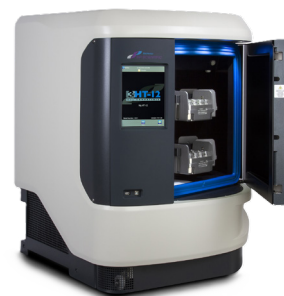


Figure 1 - Genevac HT Series 3i Centrifugal Evaporator

not frozen then bumping is inevitable resulting in sample loss and cross contamination. Secondly, acetonitrile in the cold trap will spoil the vacuum making lyophilization of the water almost impossible. Thirdly, this slow process is incompatible with reduced process times that industry wants. The centrifugal evaporator rapidly dries many different samples in parallel, without bumping.

However, users report difficulties with a few samples dried in a batch. Not all the TFA is removed and may damage the sample when in storage, and the compound may interact with the water boosting the boiling point. Residual solvent shows up in NMR analysis. Whilst occurring occasionally, the implications of picking a few samples by hand are prohibitive for automated laboratories; therefore the whole sample rack is reprocessed.

Lyophilization in a Centrifugal Evaporator

Samples prepared in water can be lyophilized in a centrifugal evaporator by pulling the best vacuum available, in the Genevac HT-Series 3i (Figure 1) with solvent resistant scroll pump, the ultimate vacuum is below 0.5mbar; more than adequate to freeze water. This is like freeze drying and slow.

Genevac developed a process whereby users evaporate some solvent using the speed of a centrifugal evaporator, then switch to a lyophilization mode when only a few millilitres of solvent are left. This took process time for 96 x 30ml fractions from 48 hours in a freeze drier, to 16 hours (overnight) in a Genevac HT-12, equivalent to only 10 minutes per 30ml sample sequentially. The effects of heating a sample during lyophilization were studied to determine if this gave a speed advantage.

Initially just water was used to develop the optimum conditions, and then with water and acetonitrile to simulate samples taken from HPLC. Lyophilization of Water

A stock solution of 0.01M Ibuprofen sodium salt in water was used as the standard sample. 15ml of solution was loaded into each of 48 20ml scintillation vials and placed dried in a Genevac HT Series evaporator under various conditions.

The actual evaporation method usually comprises up to four stages:

1. Concentration of the bulk of the solvent using fast evaporation
2. Cooling of the samples and sample holders, in preparation for
3. Freezing of the sample using deep vacuum
4. Lyophilising the residual solvent, with or without heat

In fact the freezing stage achieves cooling as well as freezing, therefore a separate stage 2 is not necessary. However, the freezing stage with no heat appears to be essential, as without it the samples dried normally and did not lyophilize, so it was evident that the sample had not frozen at all. Careful adjustment of heating time permitted a reduction in concentration time from 8 hours to 5.5 hours.



Figure 2 - Genevac 20ml scintillation vial holder.

Lyophilization of Water & Acetonitrile

Whilst the time savings for water are welcome, the issue remains of how to deal with solvent mixtures. An earlier stage, stage 0, in three parts, is introduced to remove the acetonitrile before concentration of the water.

1. Dri-Pure® – vacuum ramping and high rotor speed prevent bumping
2. Concentration – a 40mbar stage removing the acetonitrile without freezing the water (here acetonitrile boils at +2°C).
3. Draining the condenser – or residual acetonitrile will spoil the vacuum in later stages.

A 0.01M solution of Ibuprofen sodium salt was prepared in a 60:40 mixture of water and acetonitrile. Trials showed that the cooling stage was not required. With adjustment to achieve the correct balance of concentration and lyophilization. 48 x 15ml samples dried in 5 hours, equivalent to 6.25 minutes of sequential drying. Figure 3 shows the difference between a lyophilized result and traditionally centrifugally evaporated sample. The difference is stark, and the ease of resuspension is greater with the lyophilized sample, whereas the dried sample does not fully dissolve readily.”

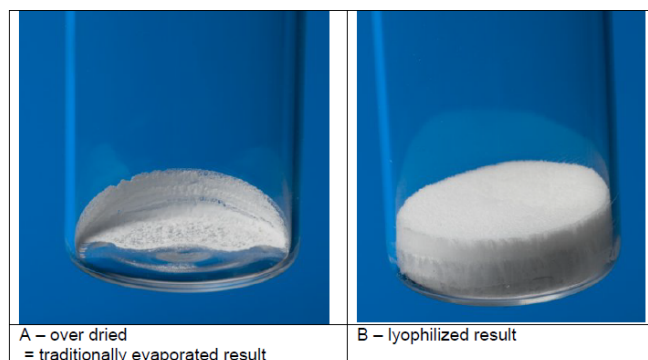


Figure 3 - Samples of the same mass prepared via different methods in Scintillation vials

Conclusions & Discussion

Lyophilization of the samples improved the ease of redissolving the sample post drying. The addition of heat during the lyophilization stage reduced the lyophilization time considerably. The cooling stage is not required, but the freezing stage is essential. When evaporating water and acetonitrile mixtures it is necessary to drain the condenser following acetonitrile evaporation and before evaporation and lyophilization of the water.

The HT-Series system had to be drained manually at the end of stage 1; some Genevac systems automate this facility eliminating the need for user intervention. The Genevac HT systems today incorporate Auto Defrost and Drain.

A full set of original trial data on the on the above plus the specific customer applications below is available upon request from Genevac.

Case 1. KuDOS Pharmaceuticals

Preparation of weighable samples from difficult to dry HPLC purification fractions

Following purification sample fractions are normally dried, resuspended in minimal solvent and the fractions pooled into a (small) sample container for storage. Analytical methods are often included, such as dry weight determination of yield and sub sampling for NMR analysis. Until recently, scientists at KuDOS Pharmaceuticals had been experiencing difficulties drying samples after purification. For KuDOS, typical stubborn samples form a gum or an oil which creates handling problems including:

- Incompletely dried samples resulting in the inability to determine the true weight.
- Residual solvent promoting faster compound degradation

The Solution

A new sample drying methodology was developed by KuDOS / AstraZeneca that overcomes both residual solvent and fraction pooling problems.

Fraction Processing and Pooling Flow

1. Dried fractions are redissolved in minimal water and methanol
2. Fractions are pooled into 20ml scintillation vials and dried
3. Samples are dissolved in minimal 1,4-dioxane – some stubborn samples present as gums or oils
4. Fractions are pooled into a barcoded, tared vial
5. Vials placed in sample holders for evaporation, and frozen in a –20°C freezer
6. Vials are lyophilized using Genevac HT-Series evaporator at full vacuum, normally overnight

Results, valid for >90% of previously intractable samples, are friable “fluffy” solids that can be easily handled and are fully dried. For the few samples that do not dissolve well in 1,4-dioxane, two methods have been successfully used:

1: Samples are redissolved in a small amount of methanol, (2-3ml), then water, (approx 10ml), is added, and the sample dried in the Genevac HT-Series using vacuum ramping

(Dri-Pure) to prevent bumping plus full vacuum to freeze the water.

Or 2: Samples are redissolved in a small volume of tertiary butanol (freezing at +25°C, ideal for lyophilization) and then dried at full vacuum.

Result: “Fluffy” solid or crystalline sample (dry and weighable).

Case 2. Novartis Institute for BioMedical Research

An easy way to protect samples from cross contamination during lyophilization

NIBMR reports production of perfectly lyophilized ‘fluffy’ samples, free from residual solvent and free from cross contamination. Incompletely dried samples resulting in the inability to determine the true weight.

Residual solvent promoting faster compound degradation.

Methodology

The following amino acids L-Asn, L-Asp, L-Arg and one macrolid xy were selected as model compounds for the lyophilization trials. The following range of solvent mixtures and volumes was developed and selected for samples based on the researched knowledge of sample solubility:

- 1,4-dioxane – 2-3ml
- tertiary butanol – 2-3ml
- water – between 1-1.5ml
- 1,4-dioxane and water (1:1) – approximately 2ml
- tertiary butanol and water (1:1) – approximately 2ml

Solvent volumes were measured using a 1.0ml syringe. Sufficient solvent must be used to enable good freezing – samples are being frozen using vacuum alone. Sample volumes were halved under vacuum and samples were completely frozen after twenty minutes.

Samples were lyophilized in 4 ml vials held in microplate holders that insulated the samples from too much heat.

Trials were carried out with and without a heating stage. Both settings produced lyophilized samples but occasionally some powder was seen to drift out of tubes and into the evaporation chamber.

Protection from cross contamination

Cross-contamination by fluffy samples that could have been blown around was avoided by restricting the vent inlet and covering the samples with pierced parafilm. Samples were tested using proton NMR and found to be free of residual solvent and cross contamination. Care is required when removing the film. Cross contamination can also be eliminated by applying a higher g-force.

Conclusions

Careful sample preparation, application of rotational speed and high vacuum produced products free from cross contamination.

Case 3. A UK Agrochemicals Research Centre

A method to drying stubborn samples from DMSO solutions

When handling new chemical entity (NCE) drug or agrochemical libraries and arrays it is common practice to dissolve each NCE in dimethyl sulfoxide (DMSO) making it possible to process them with liquid handling automation. Many samples form a gum, oil or waxy solid and do not truly dry, although this is essential or inaccurate conclusions may be drawn.

Work Flow

Samples are synthesised in small arrays, purified by reverse phase HPLC and then the purification fractions, containing water and acetonitrile, are dried. DMSO is added to redissolve samples. Those that are split between several tubes are pooled into a single vial and then dried before being submitted for storage.

Freeze-Drying Methodology

Samples are redissolved in a mixture of DMSO and water (that helps to keep the DMSO frozen during evaporation). The holder is frozen in liquid nitrogen and transferred to the Genevac evaporator for drying under vacuum.

Results

Seven out of nine samples dried fully although two only dried to a wax. The powdered nature of the resulting samples enables safe storage. Analysis by ¹H NMR showed samples to be free from residual DMSO and other solvents.

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