

## Optimal Evaporation of Purification Fractions Using a Genevac Series II Centrifugal Evaporator

If you're carrying out HPLC purification, then at some point you'll need to dry a solution of Acetonitrile/Water or Methanol/Water. This is actually one of the most complicated solvent mixtures to dry, and many users, unaware of the best way to do it, take far longer than they need to, to dry their solutions. Genevac Series II Evaporators have special features that enable you to get the job done quickly and effectively while all the time providing the unique Genevac "SampleGuard" protection that prevents your sample ever exceeding a safe temperature. The techniques described below apply equally to Acetonitrile/Water or Methanol/Water.

The first complication is that Acetonitrile/Water is prone to bumping. This is relatively easily dealt with - simply enable the unique Genevac Dri-Pure™ system to prevent bumping entirely.

The second complication is that water freezes at 0° C. Water at 6 mbar boils at approx. 0° C, so any solvent mix including water should always be evaporated at > 6 mbar or else the water will freeze and all subsequent solvent removal will be by sublimation which is very slow. (Genevac always recommend 8 mbar to give a good safety margin).

That is all very well if the solvent is just water, but with Acetonitrile (ACN), the problem is more complicated. ACN at 8 mbar has a boiling point of < -20 deg C, so simply running the evaporation at 8 mbar can actually result in some of the water freezing as a result of the ACN's "heat-hungriness" as it boils off. Once a significant amount of ice is present, the solution is slow to conduct the heat required for boiling, and so the drying takes **much** longer.

You could run the whole evaporation at a higher pressure (e.g. 40 mbar), in which case the ACN's boiling point would be > 0 and the water would not freeze. But then after the ACN was gone, the water would dry very slowly and the net effect might be that the long runtime would be no better than at 8 mbar.

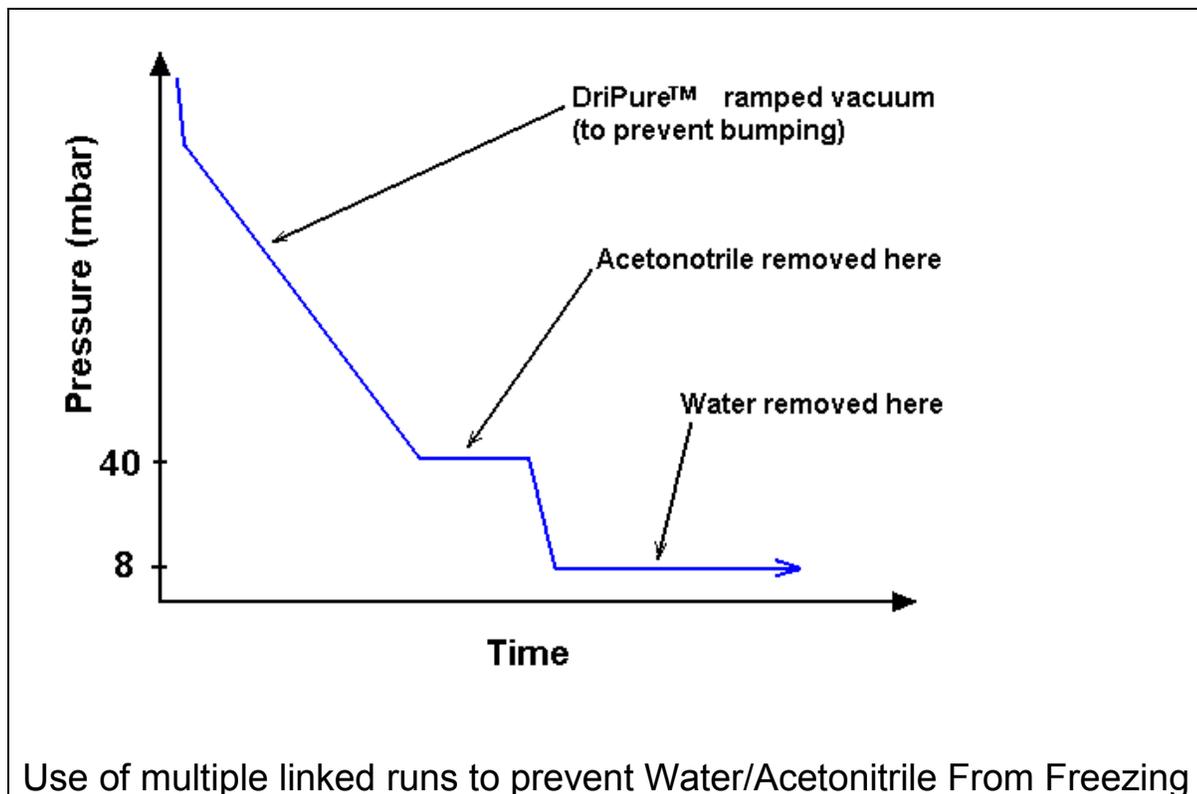
### The Two-Stage Technique

The secret is to split the drying process into two parts, with quite different drying conditions in each part. One part is tailored to removing the ACN (without freezing the water). The second part is tailored specifically to removing the remaining water at the optimal speed (but still without freezing the water). The two different parts are "linked" automatically so that one runs after the other is completed. All Genevac HT Series II Evaporators offer this facility.



## How it Works in Practice

The idea is to write a multi-stage program to achieve the following pressure profile.



Exactly how this is achieved depends on the version of Genevac control software you have.

To find out which software version you have, watch the LCD screen immediately after turning the Evaporator on. There will be a blue panel displayed, and this tells you (among other things) the version of control software.

Another way to tell is that older software versions do not refer on screen to Dri-Pure™ but instead to "Vacramp".

### NOTE 1

If your machine is actually a Series I instrument then the facilities described in this application note are not available to you. But you might like to read the separate application note entitled "Pressure Control Upgrade for a Genevac HT Series I", found elsewhere on the website.

### NOTE 2

For Methanol/Water mixtures, use 45 mbar rather than 40mbar.

**But that's enough talk. Let's see how it's actually done in practice on your instrument:**

## Faster Drying of Purification Fractions

### Version 1.12 Software

You will need to create 2 different program stages and link them together. The first stage will have the following properties.

Cool Heat Enable Pressure	100mbar
Chamber Heat	Wait to heat, 20 deg C
SampleGuard™ temperature	whatever your sample's max permissible temperature is.
Vacuum control type	Dri-Pure™
Ultimate Vacuum level	40 mbar
Heat off time / End of Run time	See later in this document

This stage must be linked directly to the following stage.

The 2<sup>nd</sup> stage will look something like this.

Cool Heat Enable Pressure	100mbar
Chamber Heat	Wait to heat or cool, temperature set to the same as SampleGuard™.
SampleGuard™ temperature	whatever your sample's max permissible temperature is.
Vacuum control type	Controlled Pressure
Ultimate Vacuum level	8 mbar
Heat off time / End of Run time	See later in this document.

This stage might be linked to yet another stage (at 2 mbar) if a small number of samples are still not dry at the end. Look at the "ask the experts" section of the website for more information about this.

## Faster Drying of Purification Fractions

### Versions of control software earlier than 1.12

The difference with versions before 1.12 is that you cannot select a ramp and an ultimate pressure in one stage. So to create the same effect, you will need to create 3 different program stages and link them together. The first stage will have the following properties.

Cool Heat Enable Pressure	100mbar
Chamber Heat	Wait to heat, 20 deg C
SampleGuard™ temperature	Doesn't matter what you set (lamps are off during ramp)
Vacuum control type	Vacuum Ramp
Heat off time / End of Run time	0:45

The 2<sup>nd</sup> stage will look like this.

Cool Heat Enable Pressure	100mbar
Chamber Heat	Wait to cool, temperature set to the same as SampleGuard™.
SampleGuard™ temperature	whatever your sample's max permissible temperature is.
Vacuum control type	Controlled Pressure
Ultimate Vacuum level	40 mbar
Heat off time / End of Run time	See later in this document.

The 3<sup>rd</sup> stage will look like this.

Cool Heat Enable Pressure	100mbar
Chamber Heat	Wait to heat or cool, temperature set to the same as SampleGuard™.
SampleGuard™ temperature	whatever your sample's max permissible temperature is.
Vacuum control type	Controlled Pressure
Ultimate Vacuum level	8 mbar
Heat off time / End of Run time	See later in this document.

This stage might be linked to yet another stage (at 2 mbar) if a small number of samples are still not dry at the end. Look at the "ask the experts" section of the website for more information about this.

## Faster Drying of Purification Fractions

### "How long should each stage be?"

Your Genevac applications engineer can calculate these times for you in many cases, but with non-Genevac sample holders it will be easier to find the times by experiment as follows.

Load the evaporator with a typical batch, and fit a 2<sup>nd</sup> SampleGuard™ probe. Insert this second probe into a representative sample. (For example, if you typically run gradients from 10% ACN to 90% ACN, put the probe in a 50/50 sample).

Start an evaporation run as described above, but with the 40 mbar stage set very long (e.g. 50 hours) so that it will run and run. Watch the sample temperature carefully after the vacuum ramp finishes. While ACN is still being evaporated the solution will be at approximately 3° C. When the ACN runs out and the water starts to boil, you should see the temperature rise to approximately 30° C †. Note down the time (the onscreen display will show you the time taken by this stage so far). This is how long you should ideally be running this stage in future.

Now for the water. It is possible to edit the parameters for this current stage while it is still running. Set the vacuum down to "Controlled pressure, 8 mbar" (which will be just as if the machine had finished the "ACN" stage and jumped to the subsequent "water" stage) and then watch the sample temperature (which should fall to a few degrees and then stay there while the water is removed). Eventually this will rise to whatever your SampleGuard™ temperature was set to (which indicates the water has all been removed and your samples are almost dry). Now you know what length of time to program the 8 mbar stage to.

Finally you may want to drop the pressure even lower (perhaps 2mbar) to get the samples completely dry. This means adding yet another program stage. The length of this stage cannot be calculated - it must be found by trial and error.

If you have any problems with this approach, contact your Genevac Applications Specialist by sending an email to [applications@genevac.com](mailto:applications@genevac.com)

Ensure that you include the following details

- What sample holders you are using
- Exact solvent mixture
- Overall solvent volume
- Your maximum permissible sample temperature
- Which model of Genevac instrument you are using (and whether it has "high powered lamps" or not).

† If your SampleGuard™ temperature is set below 30° C then the temperature will not rise this high.