Introduction:

In many laboratories across a wide range of disciplines removal of solvent from dissolved sample is a routine, daily procedure. The majority of the methods employed to remove the solvent rely on the use of vacuum to achieve low temperature boiling, normally to prevent damage to a temperature sensitive sample. This article demonstrates the great importance of paying sufficient attention to closely monitoring the drying process and presents solutions to save time and prevent sample loss.

Principles of Vacuum Evaporation:

Vacuum assisted evaporators, be they a rotary evaporator, centrifugal evaporator, vortex evaporator or even a traditional freeze drier (or lyophiliser), rely on boiling the solvents away at low temperature. Low temperature boiling is achieved by pulling a vacuum on the samples, the boiling point of the solvents decreases proportionally to the pressure in the system, see Figure 1.

The pressure in the system controls the boiling point and therefore the temperature of the sample regardless of the temperature of the evaporator water bath or sample holders. In the case of the freeze drier a high level of vacuum is used so that the solvents begin to boil below their freezing point, causing the sample to freeze, thereafter the solvents sublime until dryness is achieved.

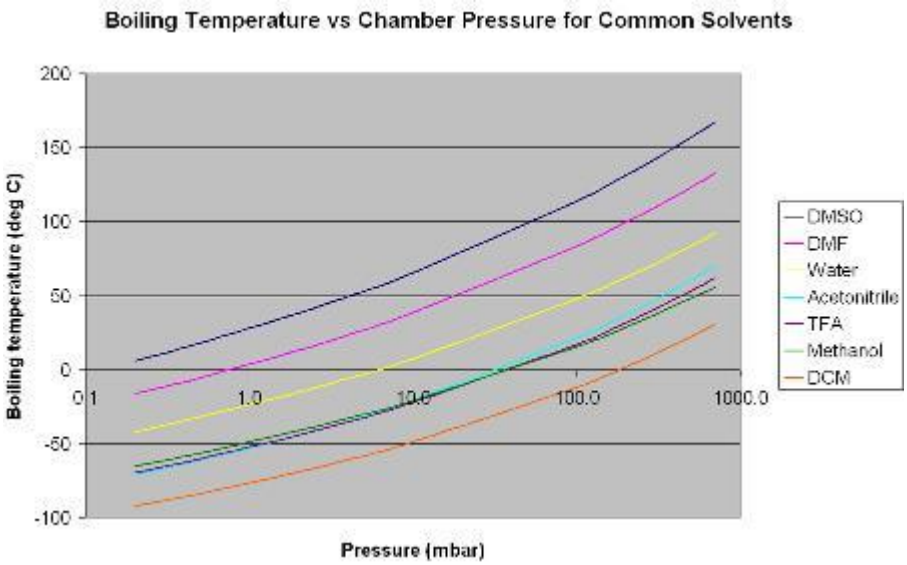

Figure 1 – Plot of boiling point vs. pressure for some common solvents.

It can be seen from Figure 1 that to achieve low temperature boiling of high boiling point solvents (e.g. DMSO or DMF) you need to achieve better levels of vacuum. Methanol may be boiled at –20°C using a pressure of 11mbar, however to boil DMF at –20°C would require a pressure of 0.15mbar which might be achievable, however with a typical vacuum pump it is not be able to boil DMSO at anything below about +6°C (although it freezes at +18°C).

Factors Affecting Speed of Evaporation:

Once a sufficient level of vacuum is achieved to cause the solvent to boil, there are two principle factors that affect the speed of evaporation that are the specific latent heat of vaporisation of the solvent and the effect of the dissolved sample.

The specific latent heat of vaporisation of the solvent is the amount of heat energy that is required to boil one unit of a

solvent. DMSO has a relatively low heat of vaporisation (603J/ml), whereas methanol requires 923J/ml, and water a massive 2441J/ml. Therefore, water takes more heat energy than DMSO or methanol to boil, and so the samples will normally take longer to dry.

The effect the dissolved sample has on boiling point of the solvent is highly variable. Some have little or no effect, others have a massive effect. The best known example is the use of common salt on the roads in winter, sodium chloride dissolved in water boosts the boiling point and most importantly in this example, drops the freezing point of water to approximately –15°C. The problem for most laboratories in assessing the likely impact of these factors is that the nature of their work is such that different and often unknown samples are processed each day.

Improving Throughput:

Efficiency savings are desirable in most laboratories, increases in throughput mean that more work can be achieved with the same resources contributing to savings.

Whilst the use of fixed length sample drying methods is commonplace a notable exception is the rotary evaporator. Research shows that typical rotary evaporator users attend the sample during the drying process to ensure that the operation is trouble free.

However this is not good use of operator time when multiple samples need to be dried. In such circumstances safe unattended solvent evaporation of multiple samples can be achieved with a system such as a Genevac EZ-2 centrifugal evaporator, see Figure 2.

The problem with fixed length drying methods is that the drying time often varies between solvents, and between samples. Therefore to ensure that every sample is dry users tend to overestimate the drying time. By automatically stopping the evaporation process when the samples are dry the user can gain significant time savings as is demonstrated below.

Figure 2 – Genevac EZ-2 Centrifugal Evaporator for Parallel Drying of Samples

Working with a laboratory that produces libraries of small molecules and undertakes many drying procedures daily, drying many different solvents, we studied the effect on average drying time when they switched from fixed time methods to using Genevac's automatic end of method detection software.

Despite the wide range of solvents used, the sample format is standard across all protocols, being up to 6mls of solvent in each of 24 vials per sample holder in a Genevac HT-12 series 2 evaporator. This study was trialed over two key locations where the solvent profiles differed significantly, see Figure 3. The change in average drying times is show in Figure 4.

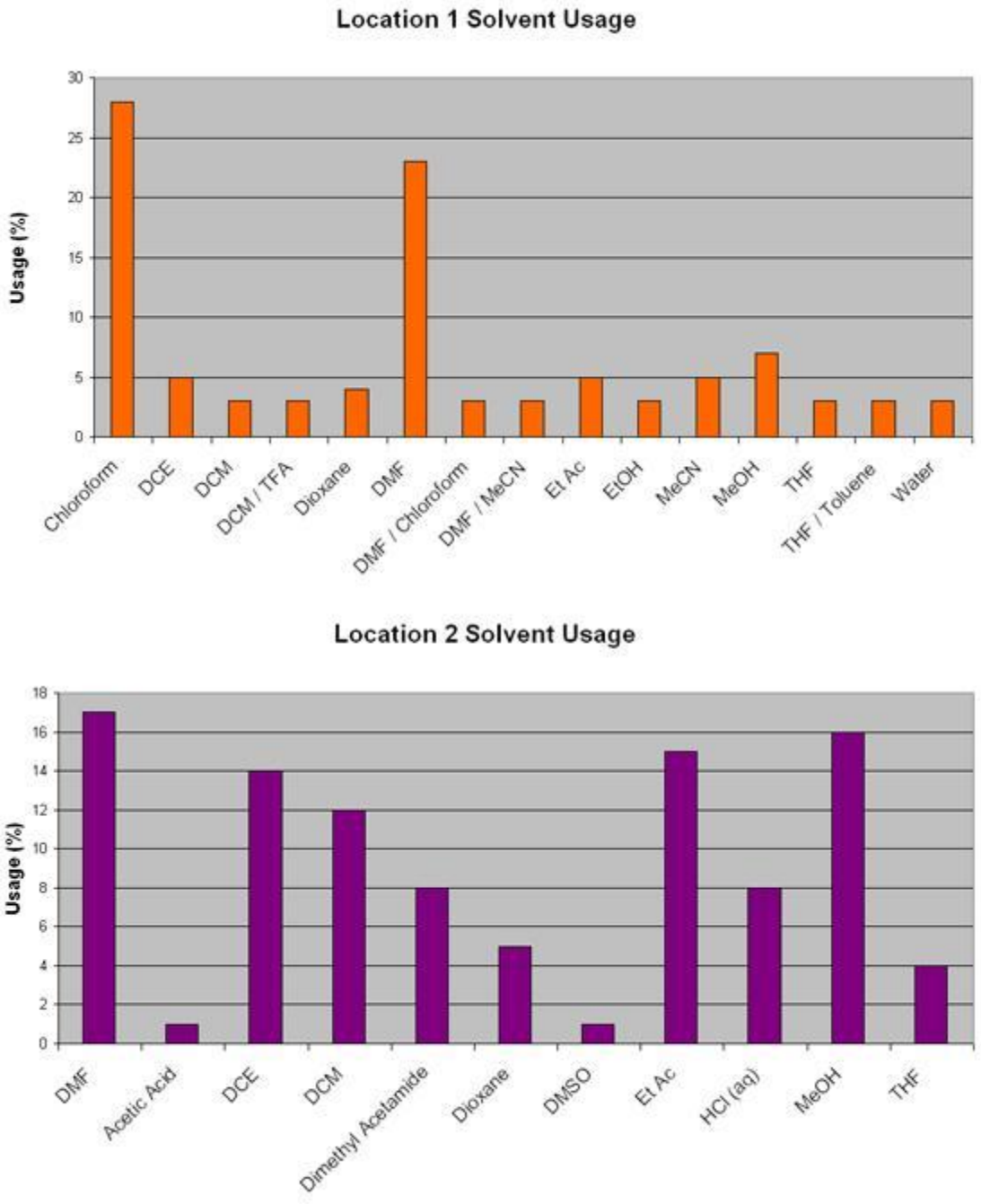

Figure 3 – Profile of solvents used in the two study locations

	Average Fixed Time Method	Average Auto Ended Method	Average Time Saving

Location 1	182 minutes	130 minutes	28%
Location 2	202 minutes	152 minutes	25%

Figure 4 – Change in Average Drying Times

The reduction in evaporation times achieved times was significant and has contributed to increased throughput and productivity.

Improving Yield, or, Reducing Sample Loss:

Most samples can become volatile under the right conditions. Generally the smaller the size of a molecule the easier it is to volatilise, this is especially true for organic molecules. A number of Genevac users have reported that they have observed loss of sample when the sample is of low molecular weight, approximately 300 and below, and/or has high volatility, for example a straight chain organic molecule with few side groups.

Genevac have studied the effects of this problem on the common pharmaceutical Ibuprofen in our applications laboratory using an HT-4X evaporator, see Figure 5. Ibuprofen has a molecular weight of 203 that is similar to many drug molecules or intermediates produced in drug discovery chemistry.

However, Ibuprofen may be far bigger than typical molecules studied within metabolism, ADME, DMPK or toxicology groups who are usually looking for drug fragments. Another growing area where this effect may have impact is among the laboratories testing for drugs of abuse, either as part of routine prisoner, sports or work place screening or within forensic science laboratories.

Figure 6 illustrates the molecular weights of some common recreational and sporting drugs of abuse demonstrating the potential problem for testers who might easily use a sub-optimal evaporation protocol resulting in loss of some of the drug or drug metabolite under investigation.

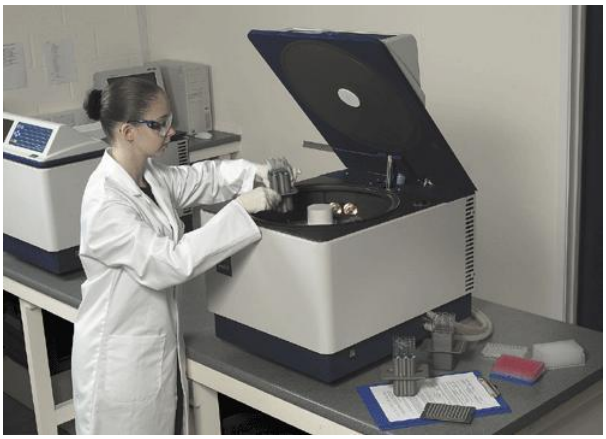

Figure 5 – HT-4X in Genevac Applications Laboratory

Cocaine	303
Ecstasy	193
Heroin	369
Methamphetamine Hydrochloride	186
Amphetamine Sulphate	368

Nandrolone Decanoate	429
Oxandrolone	306
Oxymetholone	332
Stanozolol	328

Figure 6 – Molecular Weights of some Common Drugs of Abuse
[source: http://pharmgkb.org]

In the Genevac applications laboratory we subjected dried samples of Ibuprofen to various levels of temperature and vacuum and recorded the weight loss of sample (Figure 7).

Conditions	Mean Ibuprofen Loss
10mbar, 50°C, 3 hours	5.8%
2mbar, 50°C, 3 hours	7.3%
1mbar, 50°C, 3 hours	9.4%
0.7mbar, 60°C, 3hours	24%

Figure 7 – Loss of Ibuprofen During Evaporation - Extrapolation of this data to show the potential sample loss is shown in Figure 8 below (note assumptions).

Facility	Average Avoidable Loss with Fixed time Method	Loss on Worst Case (High Boiling Solvent) Methods
Location 1	2.1%	7.9%
Location 2	2.0%	7.9%

Figure 8 – Sample Losses due to Over-Drying at Compound Supply Company
• Loss occurs due to sublimation after the solvent has evaporated, which is reasonable given that while samples are wet they will be boiling at temperatures well below 50°C.
• Loss to sublimation is proportional to time spent in the conditions.
• Losses are inversely proportional to log (pressure), which seems a good approximation

The data above shows the average loss figure. Whilst losses on a dichloromethane method at 50mbar are likely to be relatively low, those for a high boiling solvent such as DMF or DMSO method, where higher levels of vacuum and temperature have to be used, will be much higher.

Conclusions:

Fixed length vacuum drying methods typically are much slower than optimised procedures and with lower molecular weight organic molecules may lead to significant loss of the sample being dried. Compound loss is commonly due to sublimation of the dry compound following evaporation of the solvents it was dissolved in.

Preventing sample loss can be achieved by limiting the levels of vacuum and temperature used, however, this may have a knock on effect in increasing drying times. Use of the Genevac automatic end of run software can help prevent over drying and sample losses.

In our case study average evaporation times were reduced by 25% and 28%. Where a laboratory does not use a Genevac system it may be appropriate to revalidate the drying methodology and particularly the drying times used in the light of this study.