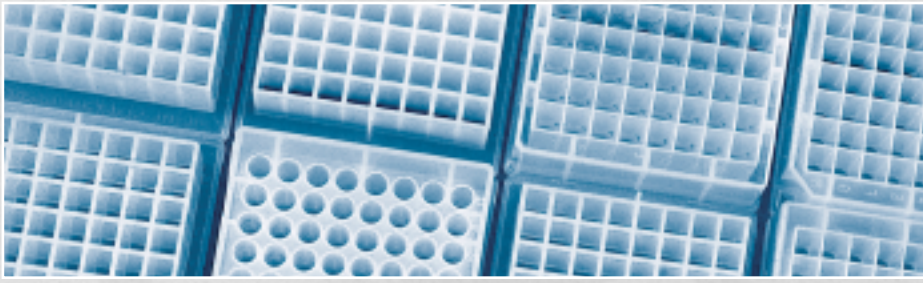
Dri-Pure

Sample Integrity Protection System



An Evaluation by GlaxoWellcome

Taking Technology Further

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Dri-Pure Evaluation

Vacuum centrifugation has become recognised as the technology of choice for organic solvent evaporation in chemistry both in high throughput hit generation labs, and more recently in lead optimisation where parallel synthesis is being employed.

The first generation of machines that became available provided the basic function of evaporation. However it became increasingly clear that they lacked the necessary control of the process that was required to perform the evaporation whilst maintaining the integrity of the required dried sample (which is obviously of critical importance to the drug discovery process).

We at GlaxoWellcome have been using the Genevac range of evaporators for several years. Their versatility has led to applications in a wide range of areas, involving evaporation following both synthesis and purification, in a variety of tube formats (from microtitre plates to 50ml test tubes) and with a wide range of solvents and solvent mixtures.

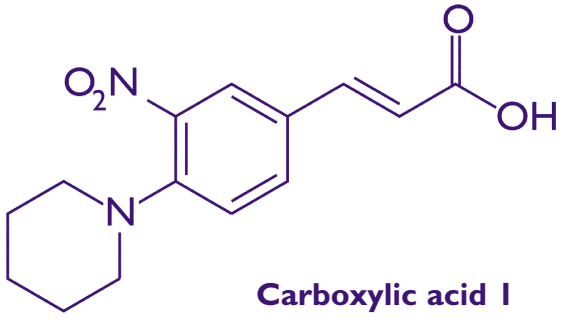
We became concerned over a period of time that we may be experiencing cross contamination problems during the evaporation process. This was indicated by the gradual build up of a brown deposit on the inside of the chamber, and the occasional observation that supposedly empty tubes or wells of a microtitre plate contained traces of sample following evaporation. It seemed that certain solvent mixtures were more prone to problems, in particular (not surprisingly) mixtures containing DCM.

The underlying cause of the problem appeared to be the lack of control whilst applying vacuum with this low boiling (40°C) solvent, particularly at or below the pressure at which DCM boils at room temperature (~450mbar). This results in "bumping" of the solvent mixture from the sample container, leading to a transfer of some of the sample into adjacent containers.

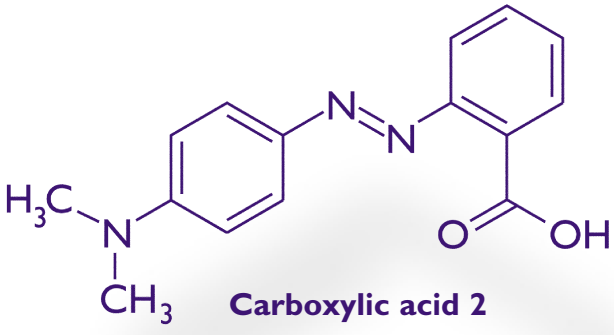
Working in close collaboration with Genevac has allowed the development of the Dri-Pure control technology. This provides a controlled ramping down of the vacuum over an extended time period (approx. 45 mins), and this together with an increase in the speed of the rotor (from an ultimate speed of 1400rpm to around 1900rpm) has allowed the problem to be resolved.

The following study has been undertaken at GlaxoWellcome to demonstrate the potential effects of cross contamination and the effectiveness of Dri-Pure technology at eliminating it.

Two different compounds were selected for the study, chosen to be representative of typical samples that we are routinely handling. These compounds have a high colour density at low concentrations, which would enable any cross contamination to be easily observed.



Carboxylic acid 1



Carboxylic acid 2

The study was performed in 96 (deep) well microtitre plates, as this was considered to be the highest risk situation for cross contamination, because of the close proximity of the sample wells. Three different manufacturers plates were evaluated, square well with rounded bottom, square well with conical bottom and round well, to compare the effect.

All solutions were prepared at 2mg/ml concentration. 1 column (8 wells) per plate was filled with 0.5ml of a solution of acid 1 or acid 2, the remaining wells being left empty to allow easy visualisation of any cross contamination.

3 commonly encountered solvent combinations were evaluated

1:1 dichloromethane:methanol

Typically produced from chromatographic or SPE purification applications

1:1 ethyl acetate:40-60 petroleum ether

Typically produced from chromatographic or SPE purification applications

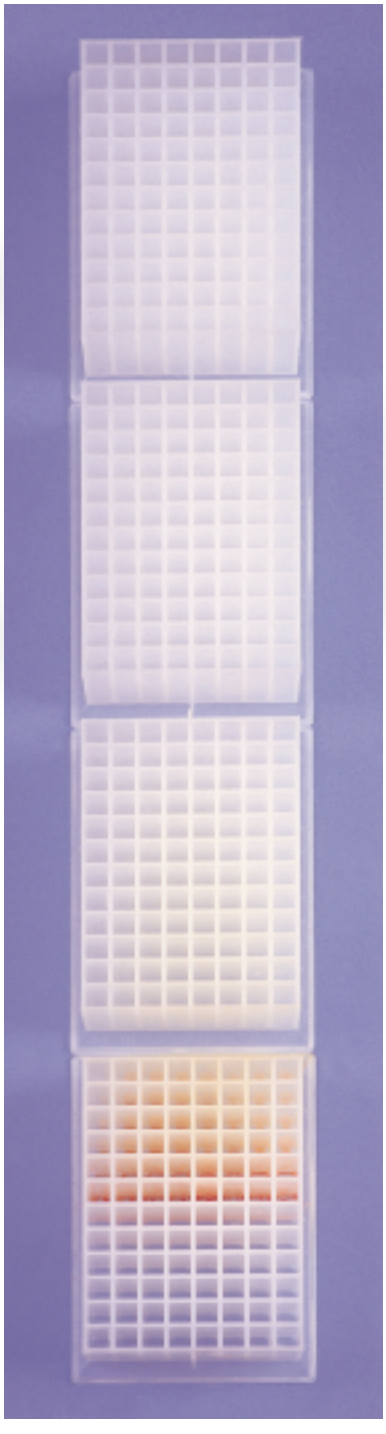
1:1 acetonitrile:water

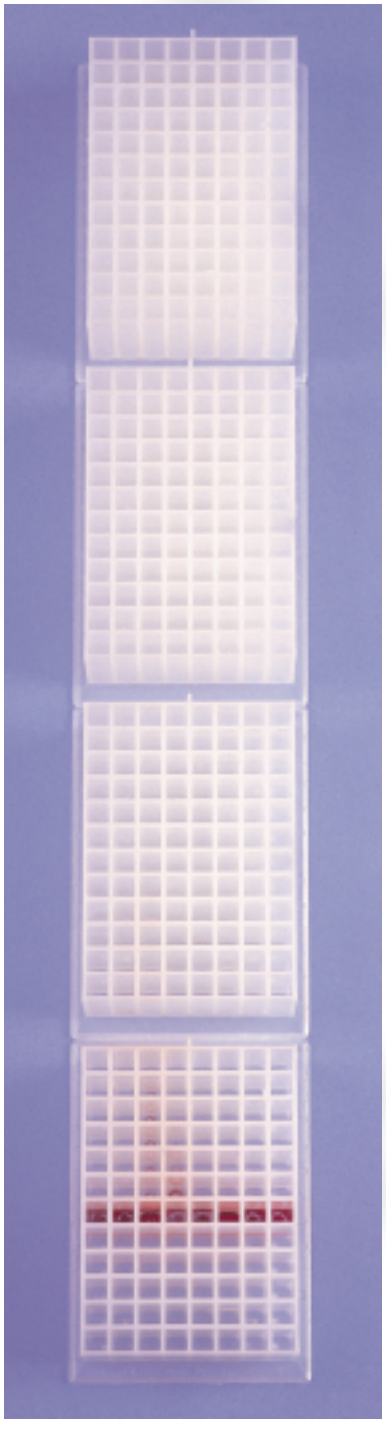
Typically produced from HPLC purification applications

Comparisons were made for each solvent combination with each of the two carboxylic acids, after evaporation under standard conditions and using Dri-Pure.

1:1 dichloromethane:methanol

Standard Conditions (No Dri-Pure)





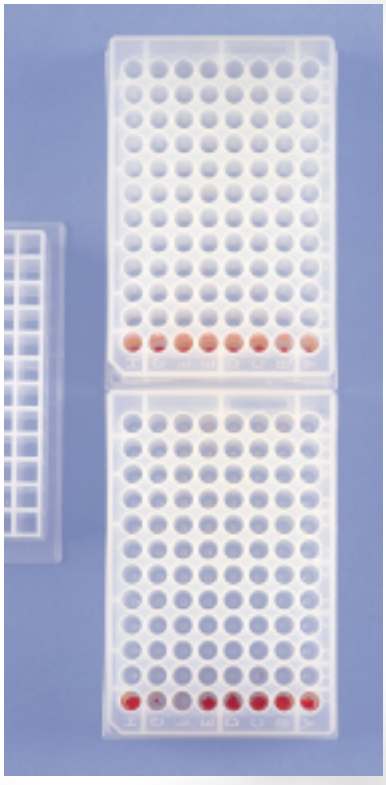
Substantial levels of cross contamination were observed with this solvent mixture, with three levels of effect.

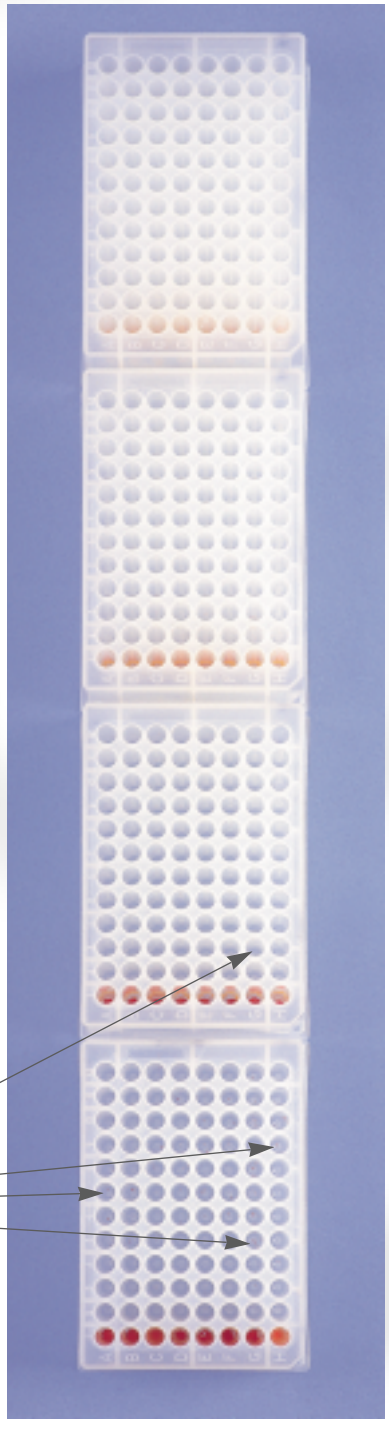
1. Sample Travel

After evaporation, the dried sample could be observed at the bottom of wells adjacent to the original sample location (which should have been empty).

The sample travel was always observed to be linear (horizontal) and the amount of contamination varied from run to run. In worst cases, sample was seen to "hop" into up to 10 adjacent wells from a single sample well. Less severe cases were observed with only 1 or 2 of the 8 wells bumping , and only hopping into up to 2 adjacent wells.

1. Sample Travel





2. Surface Contamination

This effect is less severe, and cross contamination probably hasn't occurred, but is still undesirable.

3. Patches

Cross contamination of samples is likely to occur with this effect.

2. Surface Contamination

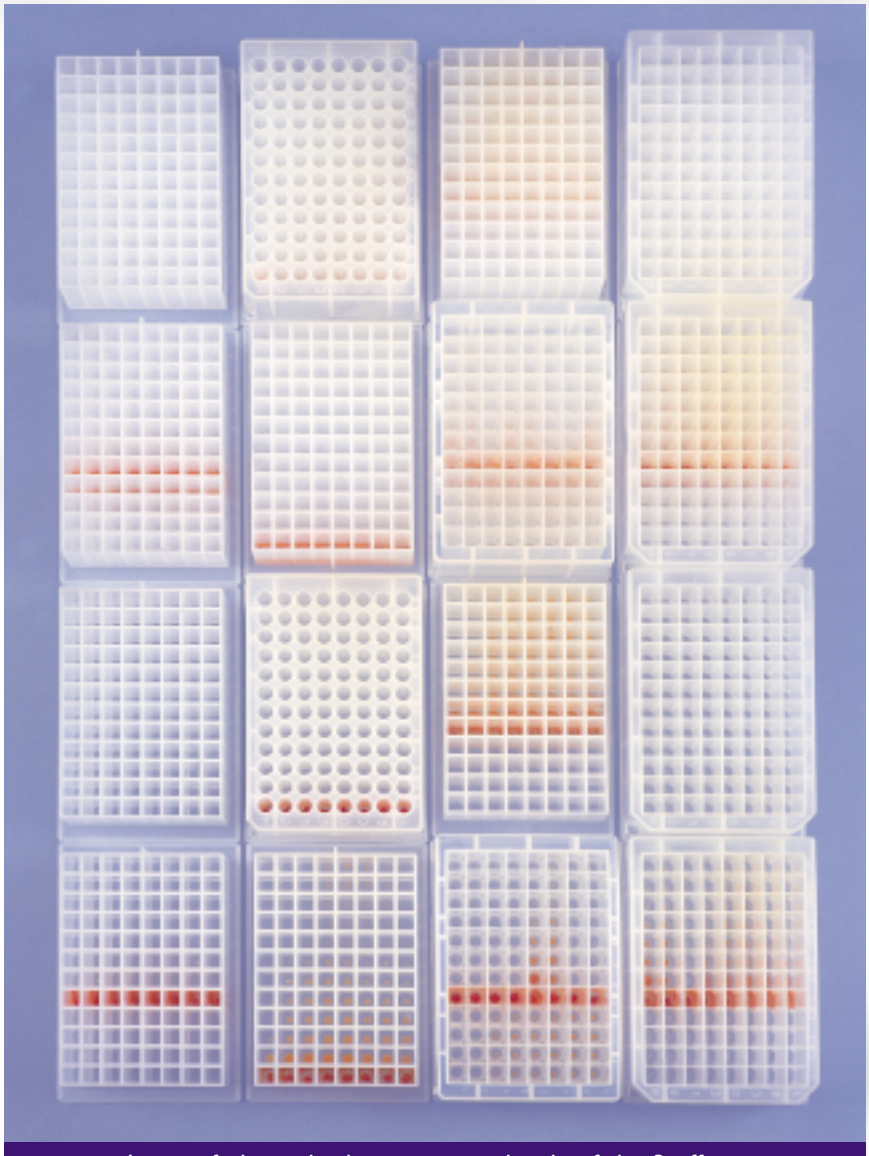
After evaporation, a fine coating of the dried sample could be observed on the top face of the microtitre plate, but there was no visual evidence of sample in any of the empty wells.

3. Patches

After evaporation, small, intense patches of sample could be observed randomly distributed within wells and over the top face of the microtitre plate.

Over a large number of runs we have observed varying levels of each of these 3 effects, and sometimes combinations of the three. The variation appears to be independent of the sample type (acid 1 or acid 2), or the specific style of the microtitre wells.

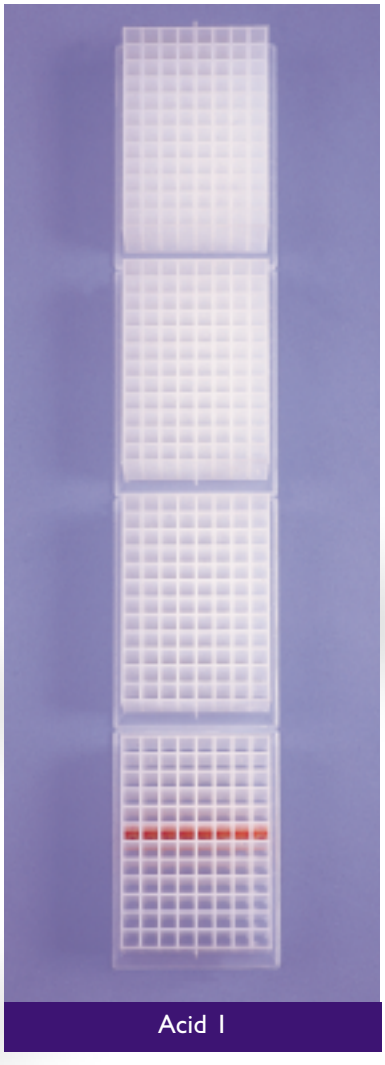
Array of plates



Array of plates displaying varying levels of the 3 effects

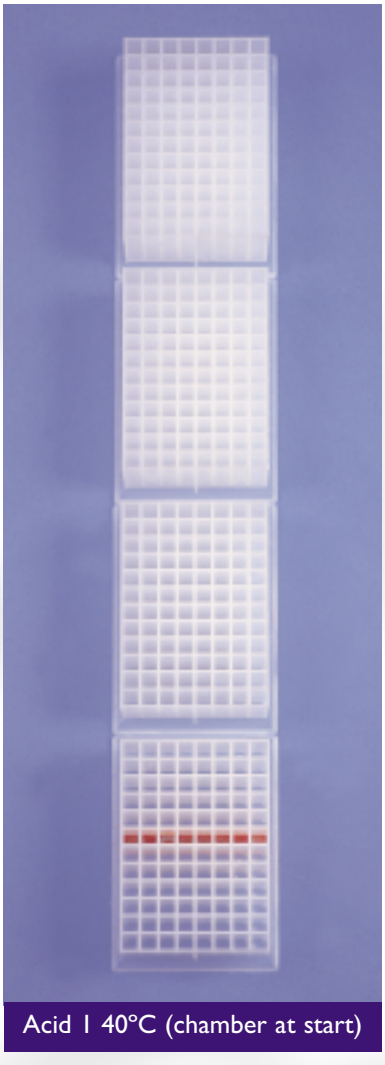
Evaporation with Dri-Pure

The testing was repeated, keeping all conditions as comparable as possible to those above, with the Dri-Pure technology being employed. A rotor speed of 1740 rpm was used, and the vacuum ramped down from 450mbar to maximum vacuum over 45 mins. This was followed by an evaporation time of 60mins. On each occasion, all dried samples have been completely retained within their original well in the microtitre plate.



Acid 1

Acid 2

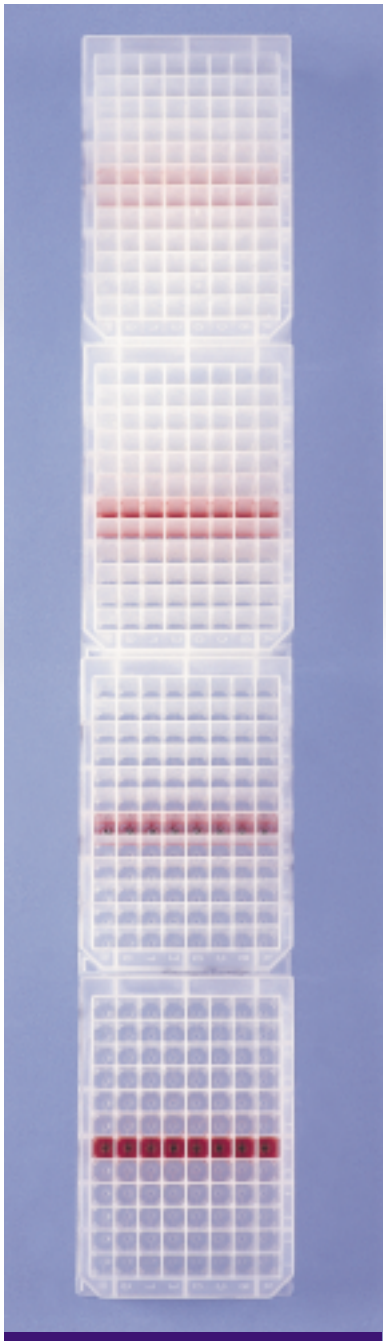


Acid 1 40°C (chamber at start)

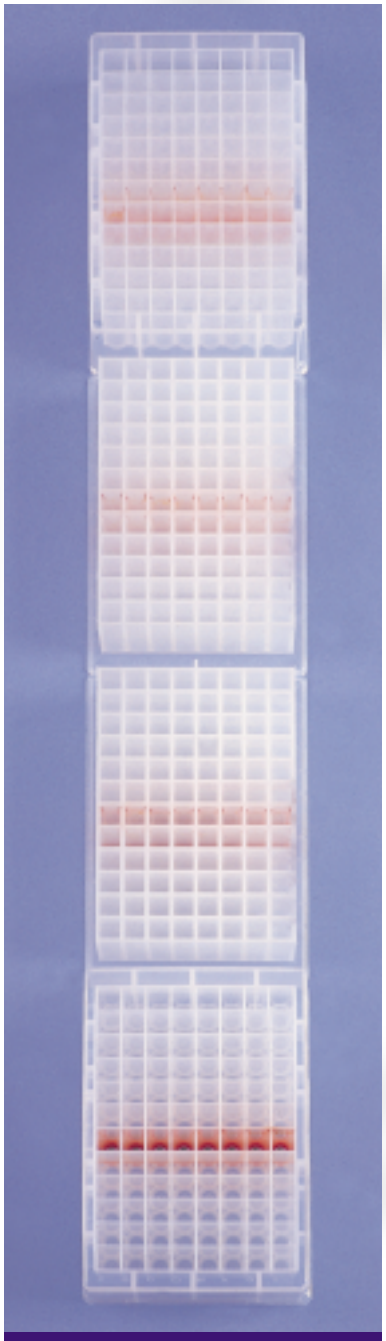
A worse case situation was also evaluated, where the chamber was preheated to 40°C (conditions typically found in a real working situation where the machine had previously been in recent use with a high boiling solvent). Using the same run conditions with Dri-Pure activated there was again complete retention of the samples in their original wells.

All evidence collected to date has indicated that using Dri-Pure eliminates any bumping, and therefore cross contamination with this solvent mixture,

1:1 Ethyl Acetate: 40-60 petroleum ether



Acid 2
Standard conditions (No Dri-Pure)



Acid 2
Using Dri-Pure technology

No cross contamination of samples was observed with this solvent mixture, however an interesting effect was seen with the square well plates. Following evaporation of solutions of acid 2, dried sample was present in a discrete band around the inside of each well, approximately ¾ of the way up the well. There appeared to be a "corner effect" as each band showed higher travel of the compound in each of the 4 corners.

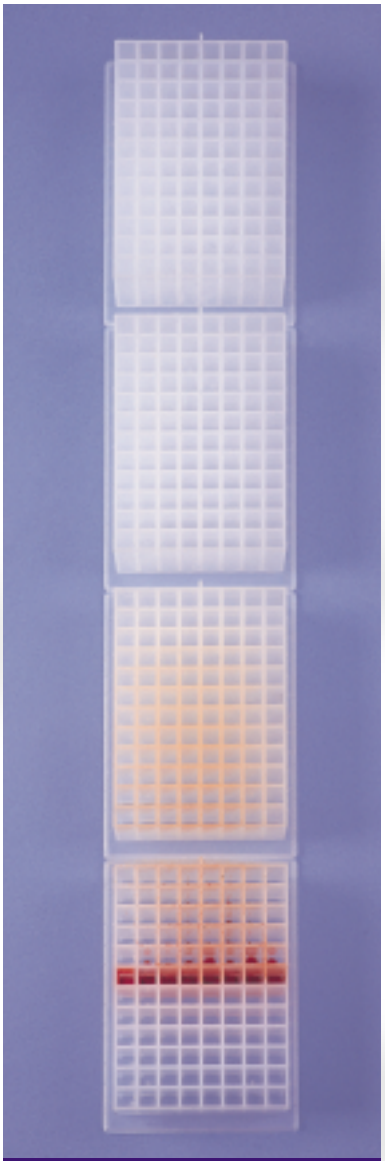
Closer investigation revealed that this corner effect was a result of solvent "creep" up the corners before the samples were placed in the evaporator. Application of the Dri-Pure technology with this solvent combination therefore gave no improvement towards eliminating this particular effect.

1:1 Acetonitrile : water

Standard Conditions (No Dri-Pure)

Only square well, rounded bottom plates were investigated for this part of the study.

Substantial levels of cross-contamination were observed with this solvent mixture, with both acid 1 and acid 2. This showed similar characteristics to the linear (horizontal) "sample travel" observed with 1:1 DCM : methanol, with "hopping" in up to 10 adjacent wells.

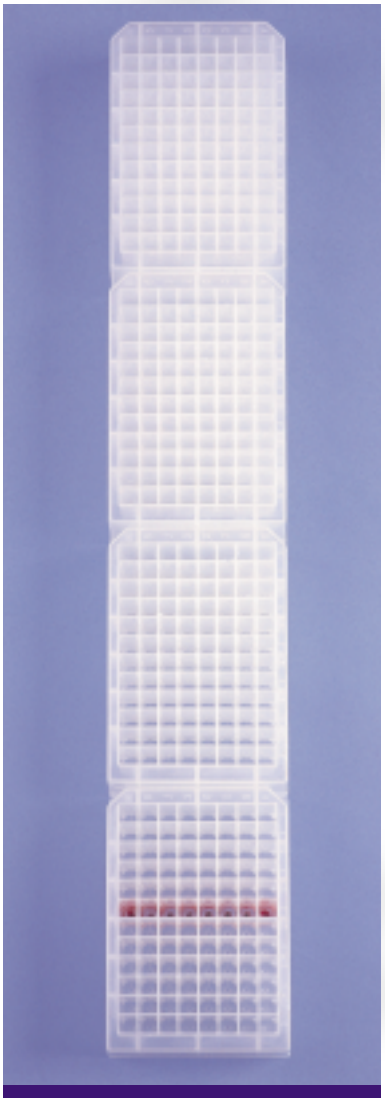


Standard conditions
(no Dri-Pure)

Evaporation with Dri-Pure

The testing was repeated, keeping all conditions as comparable as possible, with the Dri-Pure technology being employed. A rotor speed of 1740rpm was used, and the vacuum ramped down from 450 mbar to maximum vacuum over a period of 45 mins. This was followed by an evaporation time of 90 minutes.

All dried samples were again completely retained within their original well.



Using Dri-Pure technology

All evidence collected to date has indicated that using Dri-Pure eliminates any bumping of this solvent mix, therefore avoiding potential cross contamination.

Conclusions

Bumping of solvent mixtures during centrifugal evaporation has been demonstrated to be a real problem. The use of strongly coloured compounds adjacent to empty wells has enabled us to clearly see the risk of cross contamination from one well to the next, which may not have been obvious with a full plate of samples under real conditions. The fact that these plates of compounds have often come to the centrifuge direct from a purification process adds to the severity of the problem.

At least 2 combinations of solvent mixtures have been prone to the problem, and this is almost certainly not the limit.

The exact cause and mechanism of the bumping has yet to be determined, but all indications to date point to the fact that it can be readily overcome by applying two simple modifications, an increased speed of rotation, together with a carefully controlled application of the vacuum. The new generation of Genevac evaporators, incorporating this Dri-Pure technology will undoubtedly lead to significant improvements in the quality of samples generated within our labs.

This study was carried out by Martyn Deal at GlaxoWellcome Research Laboratories, Stevenage, UK