Concentrating Large Volume Samples Directly Into Small Vials with Minimal Sample Loss...

Many laboratories are familiar with the problem of significant sample loss when faced with concentrating large volume samples and transferring the residue into a dedicated analysis support or storage format, such as a vial. In this article we report upon an innovative development that has enabled “automation” of the transfer step within a Genevac evaporator system thereby avoiding costly losses. The benefits of the SampleGenie™ technology are illustrated with 4 case studies.

Introduction
The SampleGenie™ comprises a specially designed glass funnel/flask that securely seals to a storage vial thus dramatically increasing its capacity - effectively creating a single volume, 2 stage container - and allowing a purified or other large volume sample to be evaporated directly into the sample vial and eliminating the transfer step. This assembly may be loaded into a Genevac evaporation system and concentrated using a stored method.

If an amorphous anhydrous powder is required at the end of this step then an HT series evaporator with LyoSpeed™ can be used to concentrate the large volume into the vial, and then automatically lyophilise the remaining volume to leave a powder in the vial. A concentration-only version is also available for users who require a small volume wet sample.

Figure 1 – SampleGenie
Selection of systems shown with arrangement sketch to right.

Key
Dark blue – flask
Red – seal
Light blue – vial
Green – adaptor

Applications that can benefit from SampleGenie technology include:

Medicinal Chemistry
Between synthesis and compound storage, transfer to the storage vial has either been done by scraping the dried compound into the storage vial or going through a process of resolublising and transfer using a liquid handling workstation and a further evaporation step. Both are time consuming and have potentially bad yields. Central to these approaches has been the Genevac HT and EZ-2 parallel evaporation systems. Using precise control they dry the compounds well but have not aided in their transfer to the desired storage vial.

Metabolism
In Metabolism Studies large volumes of solution containing a radioactive labelled test sample are often generated and must be dried and transferred to a small vial before counting to determine the quantity of sample present.
Environmental Analysis
In environmental analysis the concentration of sample is typically low, a few milligrams (mg) of sample per 100 milliliters (ml) of solution, whereas a purified sample may be as concentrated as 10mg/ml, or more. During concentration of environmental samples – scientists often encounter their samples coming out of solution and sticking to the flask before the volume is small enough to transfer to a vial. This is because once the organic solvent has evaporated, a non polar environmental molecule will not want to remain dissolved in the remaining aqueous solution, and will precipitate out.

CASE STUDY 1: Servier Research Drug Metabolism Group

The aim of this case study was to reduce the number of manual sample concentration steps prior to evaluation of the performance of drugs under test.

Methods
Two methods were used. 1. Evaporation of 40ml water to dryness (including concentrating then freeze drying the sample), 2. Evaporation of 40ml Acetonitrile to dryness. Recovery was assessed for the evaporation of a $^{14}$C labelled drug molecule in water and in acetonitrile.

Recovery From Water
40ml of water was spiked with approximately 100,000 dpm of the $^{14}$C labelled drug molecule. This solution was placed in the upper portion of a SampleGenie holder and the correct Genevac method was run. The sample, dry after 12 hours, was reconstituted into 2ml water. Recovery was then assessed.

Recovery From Acetonitrile
40ml of acetonitrile was spiked with approximately 100,000 dpm of the $^{14}$C labelled drug molecule and dried as before. The sample, dry after 4 hours, was reconstituted into 1ml methanol and recovery assessed.

Results
Recovery from 40ml Water was 92%
Recovery from 40ml acetonitrile was 93%
In a final visual experiment 50ml water was spiked with 100µl green food dye (figure 2) that was then evaporated to dryness using the ‘water’ method. All the green dye appears to be trapped in the base of the collection vial.

Figure 2 – Green food dye in water before concentration (shown left), and then concentrated into the vial (shown right)
Conclusions
The yield of >90% achieved by Servier Research using SampleGenie compares very favourably with standard methods. In addition, manual intervention and further evaporation required for transfer into the storage vial were eliminated so reducing the process time by 0.5-1 day in the typical purification laboratory. Although there was no XYZ coordinate programming involved, SampleGenie has effectively automated the liquid handling.

CASE STUDY 2: Leochimica Laboratories, Validation Laboratory, Italy

In evaluating SampleGenie – Leochimica Laboratories sought to improve their sample concentration methodology prior to environmental analysis.

Introduction
Leochimica Laboratories have a continuous development program to develop new methods for environmental analysis, covering food toxicology, beverages, occupational hygiene and safety. One such project was to evaluate the suitability of SampleGenie™ technology for concentrating large volumes directly into a 2ml GC vial, to help to eliminate potential sources of loss.

Use of the SampleGenie
In the analytical method under study – Leochimica typically concentrate samples in flasks of 60ml, 120ml, and 200ml volume. As the flasks are repeatedly reused a key consideration was the cleaning of flasks between different concentrations, and the elastomeric material which forms the seal between flask and vial. This study considers these issues, and evaluates the recoveries of a number of analytes when using the SampleGenie system.

Evaluation of the SampleGenie
The SampleGenie was evaluated using a Genevac EZ-2 evaporation system to determine the recovery of Polycyclic Aromatic Hydrocarbons (PAHs) and pesticide analytes following concentration from a large volume of solvent. Recoveries were compared to current methods. “Memory” effects of reusing the SampleGenie components were also evaluated.

Methodology
Standards of PAH mixture, Pentachlorobenzene and Hexachlorobenzene were prepared in two different solvents, Hexane and Dichloromethane (DCM). The concentration of these analytes (when concentrated from 50ml to 1ml) was calculated to be 0.1 mg/l PAH, 0.123 mg/l Pentachlorobenzene and 0.098 mg/l Hexachlorobenzene. A 50ml aliquot of each solution was transferred to a 60ml SampleGenie containers and to 60ml glass ASE vials.

Hexane Solution Concentration
The system was programmed to give highly concentrated, but not dried, samples in ASE vials.

DCM Solution Concentration
Samples were concentrated using progressive ramping pressure then stabilised until a highly concentrated result was achieved.

The recoveries in each vial were determined by GC-MS analysis.

Observations
The EZ2 & SampleGenie achieved highly consistent and reproducible concentration of standard solution in Hexane. Comparison of SampleGenie and ASE tubes shows that both are suitable for methods of evaporation at low final volume. However, SampleGenie permits use of the automatic end run facility on the Genevac evaporator, leaving the sample in the analytical vial ready for GC-MS analysis injection which represents a considerable work saving.
SampleGenie did not exhibit either cross contamination nor memory effects.

**Long Term Evaluation**
The above samples were used again to check the intra-run reproducibility after a period of 2 months.

**Methodology**
Standards in hexane and DCM were prepared as before and six samples were placed in the SampleGenie and evaporated as before, using automatic stop. Hexane samples took approx. 1 hour 20 minutes to stop, leaving approx. 1ml in the vial. The DCM solution took approx. 3 hours to stop, again leaving approx. 1ml in the vial. The samples were analysed by GC-MS.

**Conclusions**
Recoveries from the EZ-2 and the SampleGenie were highly consistent and reproducible, also for very volatile analytes. The “Inter run” comparison of the hexane Standard Solution evaporation confirms the excellent reproducibility and performance of the EZ-2 using the SampleGenie even in different seasons (winter and spring).

Comparison of SampleGenie and ASE tubes show that both these containers are suitable for methods of evaporation at low final volume. The SampleGenie offers additional benefit in that it permits use of the automatic end of run facility on the Genevac evaporator, and leaves the sample in the analytical vial ready for GC-MS injection.

The throughput of the system comprising the EZ-2 and the SampleGenie is lower than the one achieved by using ASE tubes: 8 versus 12 and longer concentration times. Nevertheless the SampleGenie offers the advantage of elimination of the need to transfer the residual volume from tube to vial. This delicate step requires working time and may decrease recovery. On balance, SampleGenie is favoured, particularly for use with delicate assays.

**Case Study 3. Laboratoire Départemental d’Analyses de la Drôme, Valence, France**

Laboratoire Départemental d’Analyses de la Drôme evaluated SampleGenie as a large volume evaporative sample preparation technique for samples containing volatile analytes.

**Introduction**
The impact of a new instrument on testing methods must be fully understood and the testing methods re-validated if necessary. Concerning sample concentration and evaporation technology the most important issue is sample recovery, especially for very volatile analytes. Laboratoire départemental d’analyses de la Drôme (LDA26) evaluated the Genevac Rocket evaporation system and its ability to prepare large volume samples containing volatile analytes.

**Rocket Evaporation System**
The Rocket evaporation system is a new parallel evaporation system which evaporates up to six flasks each containing up to 400ml in parallel and can run the SampleGenie™ style flasks.

**Evaluation of Rocket and Comparison to Existing Equipment**
Samples of the volatile environmental analytes were spiked (50mg/litre) into 100ml of a 50:50 volume:volume mixture of DCM and acetone. Samples were evaporated in SampleGenie flasks in the Genevac Rocket set at 35°C using the preset method, to leave just the drop of pentanol. The Rocket is designed to stop the evaporation automatically at this point. Following evaporation the samples were made up to 1ml with a 50:50 volume:volume mixture of water and acetonitrile adjusted to pH 2 and injected into a gradient HPLC system using a multi wavelength fluorescence
detector (HPLC-Fluor) for analysis. These results were compared to earlier work done at LDA26, using a TurboVap evaporator.

Conclusions
The Genevac Rocket with SampleGenie delivered enhanced recovery over the pre-existing methods of evaporation tested. Under vacuum, the solvents boiled at a low temperature keeping the samples cool. Other methods required running at 35°C.

Case Study 4. Genevac Applications Laboratory

From Genevac’s central applications laboratory in Ipswich, UK we report upon the development of a simple technique using SampleGenie to automate reverse phase HPLC fraction pooling, evaporation, and reformatting

Introduction
SampleGenie has been successfully implemented where samples are purified by reverse phase high performance liquid chromatography (HPLC). Normally, these HPLC samples or fractions would be dried before pooling, then redissolved in a low volume of solvent, such as dimethylsulfoxide (DMSO), transferred to the small vial, and dried to remove the DMSO.

Reverse phase HPLC sample fractions are often dissolved in a mixture of water and acetonitrile (or sometimes methanol), rather than a single organic solvent, or mixture of similar solvents. To prevent the sample precipitating out - 1,4-dioxane was added as it has a similar boiling point to water so that it did not all evaporate with the acetonitrile and it is miscible with water.

Co-solvent Addition Experiments
A series of experiments were done with the Genevac Rocket (figure 3) evaporation system equipped with SampleGenie flasks. In this study three standard compounds were used – Hydrocortisone, Cimetidine and Caffeine - selected to provide a range of differing solubilities and where they are most likely to elute in gradient reverse phase HPLC:

These trials were done in two fraction volumes, 100ml and 200ml, with 100mg and 200mg of the standard compounds used, respectively. The HPLC fraction method at 40°C was selected for all experiments. The recovery levels were between 97-100% collected in the vial.

Conclusion
The Rocket system with SampleGenie is a straightforward way to concentrate or dry large volume Reverse Phase HPLC samples directly into smaller vials. Addition of a moderate quantity of 1,4-dioxane is a simple and effective method of avoiding sticking of sample to the flask wall.

Full reports and the tabulated results from each set of tests and related references are available from Genevac upon request.

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Servier Research Case Study
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HPLC Fraction Pooling Case Study
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