Development of a simple technique to automate reverse phase HPLC fraction pooling, evaporation, and reformatting.

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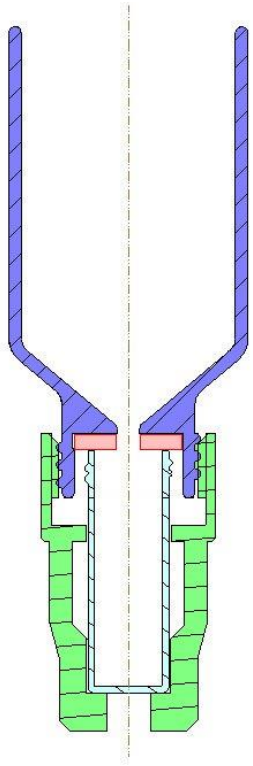

Figure 1 SampleGenie in section

Introduction

SampleGenie™ was introduced by Genevac as a simple item of laboratory "automation" to help scientists who work with, or store their dried samples in, small vials, but where their sample is initially dissolved in a large volume of solvent. SampleGenie (Figure 1) is essentially a glass flask to which a smaller vial can be attached, an inert seal ensures the integrity of the joint [1].

SampleGenie has been successfully implemented within both medicinal chemistry laboratories and dedicated purification groups where samples are typically purified by reverse phase high performance liquid chromatography (HPLC). Samples elute from the chromatography column in volumes larger than the average storage vial. Normal practice would be to dry the sample or fractions before pooling, and then re-dissolve in a minimal volume of solvent, such as dimethylsulfoxide (DMSO), transfer to the small vial, and dry to remove the DMSO. SampleGenie allows for the direct evaporation of large volumes (up to 250ml) directly into a vial of choice.

SampleGenie has been developed for use with Genevac HT and EZ-2 evaporators as well as the new high speed Rocket™ Evaporation System[2].

Early in the development issues were found with reverse phase HPLC fractions where a proportion of the samples stuck to the glass of the flask rather than fully entering the vial. This paper discusses the causes of this problem and the development of a solution that delivers more acceptable sample recovery.

Data from areas of applications outside of reverse phase HPLC has shown very high levels of sample recovery in the vial; two such being environmental analysis[1,4] and metabolic studies[3], where recoveries in excess of 90% or 95% were reported. For most scientists these are acceptable as any method of drying and transfer to a vial will have some losses. Losses can be minimised by washing out the flask, as could be done in this case, however this may reduce the gain in efficiency obtained by using SampleGenie.

Figure 2 – Rocket Evaporation System

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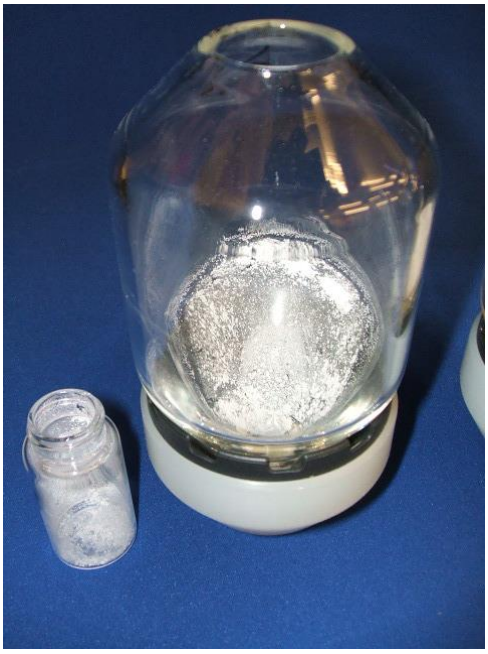

Figure 2 – Flask showing sample dried to the sides

Importantly, with reverse phase HPLC fractions the sample is usually dissolved in a mixture of water and organic solvent (acetonitrile or methanol), whereas, samples in the other applications are normally in a single organic solvent (or mixture of similar solvents). Also, in environmental or metabolism analysis the concentration of sample is typically low, a few milligrams (mg) of sample per 100 millilitres (ml), whereas a purified sample may be as concentrated as 10mg/ml, or more.

With high concentratrations samples come out of solution before the volume is small enough to occupy only the vial. Evaporative drying is typically concentration until dryness, and at the point where the solution becomes saturated the dissolved sample will crash out, and stick to the flask (Figure 2). Compounding this, in vacuum evaporation, the organic solvent evaporates more quickly than the water in a fraction. Once all the organic solvent has evaporated, non-polar molecules which are insoluble in water, will also crash out.

Solutions

A range of solutions were considered and evaluated, these can be subdivided into two categories, either, make the flask 'non-stick', or prevent the sample crashing out in the first place.

One 'non-stick' solution considered was to end terminate the free silanol groups on the glass molecules, typically with a silane. Several commercially available treatments where tried, however, none of these coatings were that successful, the samples still crashed out, and still adhered to the side of the flask. The use of the silane treatment did help move all the sample closer to the neck of the flask but none was considered adequate. In addition, none were permanent, in that they were removed over time by washing of the flask. The flasks were also coated with a Teflon® type material, however, those providing this material had difficulty in ensuring the adhesion between the coating and the glass, and delamination was a risk. This solution was dismissed.

The solution showing the most promise was that of preventing the sample crashing out in the first place by the addition of a solvent which would keep the sample in solution. Any such solvent would have to have a similar boiling point to water so that it did not all evaporate with the acetonitrile. Two were considered, toluene and 1,4-dioxane. Initial tests with toluene failed because it is immiscible with water, whereas 1,4-dioxane was much more successful.

Figure 6 – Flask showing minimal sample sticking with added 1,4-dioxane

Co-solvent Addition Experiments

A series of experiments were carried out using the Genevac Rocket evaporation system with SampleGenie. In this study three standard compounds were used, each was selected for a different solvent range based on solubility and where they are most likely to elute in gradient reverse phase HPLC:

Acetonitrile range	Test substance
70-80%	Hydrocortisone
50-60%	Cimetidine
20-40%	Caffeine

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 (Table 1)

Test No	Fraction Composition			Dioxane addition (mls)	Recovery			Test Substance
	Volume (mls)	MeCN %	H_2O %		%	End mass	Start mass	
R1396	100	20	80	0	98.9	0.1049	0.1037	Caffeine
R1396	100	20	80	10	97.7	0.1024	0.1	Caffeine
R1396	100	20	80	20	99.6	0.1076	0.1072	Caffeine
R1424	100	30	70	0	99.7	0.1016	0.1013	Caffeine
R1424	100	30	70	20	100.2	0.1048	0.105	Caffeine
R1397	100	40	60	0	100.3	0.1057	0.106	Caffeine
R1397	100	40	60	20	99.1	0.1037	0.1028	Caffeine
R1397	100	40	60	30	93.5	0.1021	0.0955	Caffeine
R1432	100	50	50	40	100.3	0.1015	0.1018	Cimetidine
R1432	100	50	50	20	105.4	0.1004	0.1058	Cimetidine
R1389	100	60	40	30	97.6	0.1046	0.1021	Hydrocortisone
R1432	100	60	40	20	103.2	0.1009	0.1041	Cimetidine
R1432	100	60	40	30	104.7	0.105	0.1099	Cimetidine
R1424	100	70	30	20	99.2	0.1092	0.1083	Hydrocortisone
R1424	100	70	30	30	102.0	0.1022	0.1042	Hydrocortisone
R1384	100	80	20	0	101.3	0.1023	0.1036	Hydrocortisone
R1384	100	80	20	5	99.3	0.1052	0.1045	Hydrocortisone
R1419	200	20	80	30	106.1	0.2051	0.2177	Caffeine
R1419	200	20	80	20	102.4	0.1945	0.1991	Caffeine
R1246	200	20	80	0	98.7	0.1028	0.1015	Caffeine
R1246	200	20	80	20	101.2	0.1082	0.1095	Caffeine
R1426	200	40	60	0	101.9	0.104	0.106	Caffeine
R1426	200	40	60	20	99.8	0.1065	0.1063	Caffeine
R1432	200	50	50	50	99.2	0.1962	0.1946	Cimetidine
R1432	200	60	40	50	108.2	0.1946	0.2105	Cimetidine
R1369	200	70	30	50	88.3	0.1059	0.0935	Hydrocortisone
R1405	200	80	20	50	95.8	0.1921	0.184	Hydrocortisone

Table 1 – Results of experiments done with Rocket Evaporation System and SampleGenie

The amount of 1,4-dioxane needed depends on the volume of the vial and the amount of water in the sample. To determine how much 1,4-dioxane is required to add, first select the sample with the least amount of acetonitrile and, depending on the volume, select the corresponding amount of 1,4-dioxane from the look up table, figure 5. Although 1,4-dioxane (BP 101°C) has a similar boiling point to water, because of the low latent heat it will evaporate quicker. So, hydrophobic compounds will need more care to avoid sticking to the flasks.

		Fraction Volume	
		100ml	**200ml**
Fraction % Organic	90	-	-
	80	15-30	30-50
	70	20-30	50
	60	20-30	50
	50	20	50
	40	0-20	0-20
	30	0-20	0-20
	20	0-20	0-20
	10	-	-

Figure 5 – Look up table of 1,4-dioxane addition for fraction handling

Conclusion

SampleGenie is a straightforward way to concentrate or dry large volume samples directly into smaller vials using the revolutionary Rocket evaporation system. To overcome the potential problem of dissolved samples crashing out of solution, sticking to the glass flask, and therefore not getting into the vial, the addition of a moderate quantity of 1,4-dioxane is a simple and effective method. This work has been summarised in a look up table to assist users determine how much 1,4-dioxane is required to add for different fractions.

References

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Acknowledgements

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