Evaluation of the Genevac Rocket Evaporation System

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Introduction

The process of taking fractions from preparative chromatography, evaporating off the solvent and retrieving the purified sample in an appropriate solid form has always been a challenge. Traditionally at UCB a rotary evaporator has been used to remove the mobile phase (usually a mixture of ammonium formate buffer and either acetonitrile (MeCN) or methanol (MeOH)) then the dry sample is re-suspended in a mixture of water (H_2O) and MeCN to transfer it from the large round bottomed flask to a smaller vial in which the sample is freeze dried. The freeze dried sample is then transferred from this vial into a tared, barcoded vial ready for submission into the corporate collection. The multiple transfer steps inevitably reduce the amount of sample recovered (approximately 10 % of sample is lost) but also raise safety concerns about the exposure to, often "fluffy", freeze dried solids.

Rocket Evaporation System

Aims of the Evaluation

The use of the Genevac Rocket was considered as an alternative method of drying down fractions. The Rocket allows parallel evaporation of six samples and, by using the SampleGenie, fractions of up to 250 mL in volume can be dried down into the tared submission vials eliminating the transfer steps responsible the safety concerns and potential loss of sample.

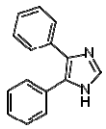
An evaluation of the Rocket was undertaken to test its suitability as part of the preparative chromatography process. Issues that needed to be considered included the time and efficiency to dry down fractions from both H_2O / MeCN mixtures and H_2O / MeOH mixtures and the average recovery of sample from the fractions into the vial.

Test Compounds

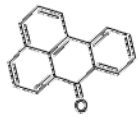
The two test compounds used in this evaluation were 4,5-Diphenylimidazole (4,5-DPI) and Benzanthrone.

4,5-DPI is routinely used by UCB to test the performance and recoveries of the LC and LC-MS preparative chromatography systems.

Benzanthrone is a neutral compound which exhibits low aqueous solubility.



4,5-DPI



Benzanthrone

Method Details

100 mg of test sample was dissolved / suspended in 100 mL solvent in the SampleGenie. These samples were run using the "HPLC Fraction" method (default settings 60 °C, 60 minute final stage). The average run time for this method was 5 hours. After this first evaporation step a large amount of solid sample remained in the neck of the SampleGenie (see Figure 1) so the sample was re-dissolved in 50 mL MeCN and run using the "Low BP" method (45 °C). The average run time for this method was 1 hour.

Figure 1. Benzanthrone remaining in the SampleGenie after 1st drying step (left) and 2nd drying step (right)

In an attempt avoid a 2nd drying step the addition of 1,4-dioxane to the fractions prior to drying was investigated (as described by Abeysena and Darrington[1])

The addition of 1,4-dioxane to the fractions showed an average recovery of 53 (± 6) %, much lower than would be acceptable for the processing of preparative chromatography fractions. Initial results suggested that the two stage drying process works better for 4,5-DPI than the addition of 1,4-dioxane. Further investigation would be required as this finding may be compound specific.

1 Development of a Simple Technique to Automate Reverse Phase HPLC Fraction Pooling, Evaporation, and Reformatting, Genevac

Results

		Recovery (%)	
Test Compound	Mobile Phase	After 1st Dry	After 2nd Dry
4,5-DPI	1:1 H_2O / MeCN	16 ± 2	78 ± 6
4,5-DPI	1:1 H_2O / MeOH	22 ± 10	78 ± 15
Benzanthrone	1:1 H_2O / MeCN	48 ± 19	94 ± 3
Benzanthrone	90:10 H_2O / MeCN	84 ± 10	98 ± 1
Benzanthrone	10:90 H_2O / MeCN	38 ± 4	67 ± 8

Discussion

The overall recoveries from the Rocket are comparable to recoveries obtained through the traditional fraction dry down process.

The recovery of 4,5-DPI was lower than that of Benzanthrone which suggests that the recovery may be sample dependent.

Recoveries after the 1st drying step were poor but improved significantly with the 2nd drying step. Even with the addition of this 2nd step, 6 x 250 mL of fractions can comfortably be processed during a working day allowing more fractions to be processed overnight.

Varying the composition of the H_2O and MeCN showed differing recoveries. Recovery was greater from 90 % H_2O than from 90 % MeCN (98 % compared to 67 %).

The solid form of the samples from the Rocket was less "fluffy" than samples processed on the freeze drier making future weighing of samples less hazardous.

Conclusion

The Genevac Rocket Evaporation System allows parallel drying of up to six samples directly into submission vials, negating the need for transfer steps which result in exposure to dry powders and loss of sample.

The ability to process up to six samples simultaneously, the improved form of the compound and the ability to dry directly into the submission vial are benefits which make the Rocket an attractive alternative to manual processing of fractions.