



Application Note

Facilitating Filtration of Small Volumes: HPLC and UHPLC Sample Prep

Introduction

Filtrating small volumes of fluid (< 2 mL) with standard syringe filters can be difficult due to the volume remaining inside the filter after use. The fluid remaining inside the filter is typically referred to as “hold-up volume.” The hold-up volume for 25 mm syringe filters can sometimes be greater than 1 mL. This can make filtrating samples smaller than 2 mL very difficult, if not impossible, to filter.

There are three ways to reduce the hold-up volume in a syringe filter.

1. Use a 13 mm syringe filter designed specifically for small volumes instead of a 25 mm filter
2. Use a syringe filter that has a mini spike outlet
3. Run a post-filtration air purge on the syringe filter.

Pall Laboratory offers many 13 mm syringe filters with mini spike outlets for filtration of small volume samples. When used carefully with an air purge, samples as small as 25 μ L can be filtered with a hold-up volume as low as 5 μ L. To obtain the smallest hold-up volume possible when filtering small volume samples, the following methodology has been designed. Assuming samples will be analyzed by LCMS, a glass syringe will be used with a 13 mm Acrodisc MS syringe filter (PN MS-3301). 13 mm Acrodisc MS syringe filters are designed for low volume samples and low LCMS extractables.

Method

Procedure for obtaining minimal hold-up volumes when filtering samples < 100 μ L in volume.

1. Draw approximately 400 μ L of air into a 500 μ L glass syringe (luer-lock compatible). One example of an appropriate 500 μ L glass syringe is VWR (US) PN 60375-464 from Hamilton.
2. After drawing in air, draw sample to be filtered to the 25 μ L mark in the syringe, then attach the filter. Note: A hypodermic needle can be attached to the syringe prior to drawing the sample into the syringe to ensure maximum sample recovery. Samples larger than 25 μ L may be used.
3. Apply thumb pressure on the syringe to begin filtration; push liquid and complete volume of air through the filter. Note: Applying thumb pressure is recommended to ensure the lowest hold-up volume during air purge.

Utilizing this method, the average hold-up volume after filtering ten 25 μ L samples is 5 μ L. The data below represents ten samples tested (Ref. SLS Report Number 17690GT).

Sample Number	Sample Volume (µL)	Hold-Up Volume (µL)
1	25	5.6
2	25	4.4
3	25	5.2
4	25	3.5
5	25	4.5
6	25	5.1
7	25	5.8
8	25	4.3
9	25	5.7
10	25	5.8
Average:		5.0 µL

Conclusion

This procedure can make filtrating sample volumes as small as 25 µL easier with limited sample loss due to the hold-up volume of the filters.



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