Life Sciences

Application Note

How to Facilitate Filtration of Small Volumes for HPLC and UHPLC Sample Prep

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Introduction

Filtration of 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 the "hold-up volume." The hold-up volume for 25 mm syringe filters can be in excess of 1 mL and can make filtration of 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 Corporation 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 µL can be filtered with a hold-up volume as low as 5 µL. The following methodology is designed to provide guidance to obtain the smallest hold-up volume possible when filtering small volume samples. We will assume the samples will be analyzed by LCMS so we will use a glass syringe and a 13 mm Acrodisc® MS syringe filter (PN MS-3301) designed for low volume samples and low LCMS extractables.

Methods

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

1. Draw approximately 400 µL of air into a 500 µL glass syringe (luer-lock compatible). One example of an appropriate 500 µL glass syringe is VWR (US) PN 60375-464 from Hamilton.
2. After drawing in air, draw sample to be filtered to the 25 µL 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 µL may be used.
3. Apply rapid thumb pressure on the syringe to begin filtration. Push liquid and complete volume of air through the filter. **Note:** A rapid application of thumb pressure is recommended to ensure the lowest hold-up volume.

Using this method, the average hold-up volume after filtering ten 25 µL samples is 5 µL. Following are the data for the 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 mL

Conclusions

In summary, this procedure can be used to help facilitate the filtration of sample volumes as small as 25 µL with limited sample loss due to the hold-up volume of the filters.

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