Application Note

Multisample filtration of small volumes using the Samplicity® system

Introduction

With advances in high performance liquid chromatography (HPLC) and ultra-high pressure liquid chromatography (UHPLC), analytical separations have become more and more sensitive, meaning that smaller and smaller sample injections are sufficient to provide required data. At the same time, the use of small-volume samples has become more common, as researchers seek to maximize the number of analyses performed using limited sample amounts. As a result, the amount of sample available for chromatography has been frequently reduced. Even just a few years ago, 1 – 2 mL samples were typically available for chromatography; however, today's researchers are often limited to samples that are less than 500 µL. To accommodate the decreased sample volume, chromatographers need to use HPLC vials which have inserts in them (as in Figure 1) so that small samples can be reproducibly injected into an HPLC system.

Even though the amount of sample available for chromatography is reduced, the sample still needs to be filtered to ensure that it is particle-free before it is injected into the HPLC system. When filtering a few samples at a time, syringe filters are commonly used. Syringe filters are available in various sizes, enabling filtration of very small sample volumes without losing significant sample to hold-up.

Syringe filters prove less efficient for users typically filtering 10 – 100 samples a day (65 – 70% of users). For these users, the Samplicity® multifiltration system facilitates sample preparation by enabling vacuum-driven filtration of up to eight samples directly into standard (12 x 32 mm) HPLC vials. The Samplicity® system has been used widely to filter 300 µL – 2 mL samples into HPLC vials without inserts.

In this application note, we present data showing that the Samplicity® system can be used for filtration of smaller volume samples into HPLC vials containing inserts.

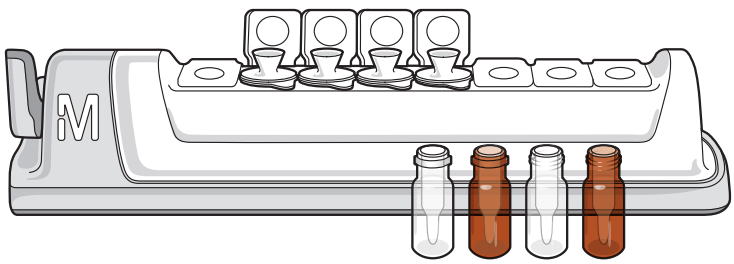

Figure 1. Samplicity® System with HPLC vials containing small-volume insert.

Materials and Methods

HPLC vials:

Table 1. Vials with small volume inserts tested for performance in the Samplicity® filtration system

Vial Information	Vendor	Catalogue No.
12 x 32 Qsert™ Vial, Amber screw cap vial cap and LectraBond™ preslit PTFE/silicone septa	Waters	186001131c
12 x 32 Deactivated, Qsert™ Vial screw thread cap and LectraBond™ PTFE/silicone septa	Waters	186001126DV
Qsert™ Vial snap cap vial with cap and PTFE septa	Waters	186001127
Verex™ Vial, Snap, µVial i3 (Qsert™) Clear, w/ Patch	Verex	AR0-3625-12
Interlocked Vial/Insert 300 µL Amber, 11 mm Crimp or Snap Ring	Restek	22439
Interlocked Vial/Insert 300 µL Clear, 11 mm Crimp or Snap Ring	Restek	22433
Polypropylene plastic snap-top vial, 300 µL, with cap and bonded PTFE/silicone septa	Waters	186002642

Other materials used:

- Samplicity® filtration system (Cat. Nos. SAMPSYSGR or SAMPSYSBL) with Millex Samplicity® Filters (0.2 µm pore size, Cat. No. SAMPLG001)
- Milli-Q® water
- HPLC grade acetonitrile
- Pipettors and pipette tips

General experimental protocol:

1. Filter 100, 200 or 300 µL of sample into preweighed vials as shown in Figure 2.
2. Observe filtration into the vial.
3. When filtration into vials is observed to be complete, weigh the vials and determine recovery of the sample.

Effect of vacuum pressure on filtration speed and sample recovery

We also examined the effect of vacuum pressure on filtration performance, and found no correlation. Filtration tests were conducted at 14 – 22 inches Hg (474-745 mbar) vacuum pressure and there was no impact of vacuum pressure on filtration into the vial (data not shown). All the sample recovery experiments were therefore conducted at 22 (maximum possible) inches Hg (745 mbar) vacuum pressure as measured by the gauge.

Pipetting technique for maximizing recovery of small sample volumes

When pipetting small volumes for filtration using Samplicity® system, make sure that the sample is pipetted below the cross bar at the bottom of Millex Samplicity® funnel. This will ensure that the sample will be filtered as soon as vacuum is applied and that the system does not air-lock. An air lock will completely prevent all filtration.

Pipetting technique is even more crucial when handling aqueous samples because of their high surface tension. If the sample is not pipetted below the cross bar, the surface tension may cause the sample to bubble up on top of the cross bar, leaving the filter dry. In contrast, organic samples exhibit reduced surface tension, allowing samples to pass easily below the cross bar.

To prevent air locking and sample retention on top of the cross bar, stream the sample slowly along the sides of the funnel with the pipette tip touching the side of the funnel.

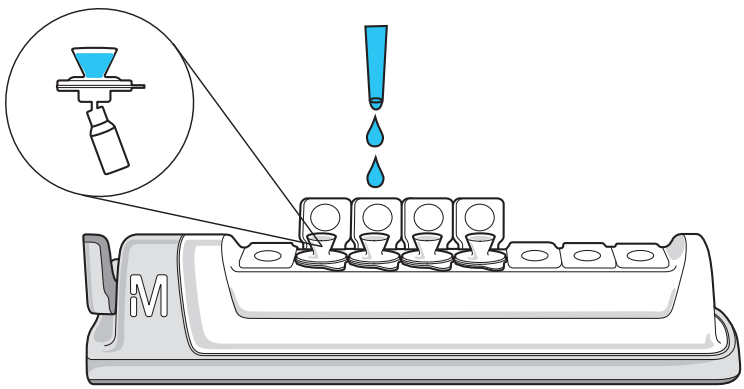

Figure 2. Operation of the Samplicity® Filtration System. Millex Samplicity® filters are placed over the openings. HPLC vials are installed underneath the openings, where they are seated at the optimal angle for filtrate recovery (inset). To avoid air-locking the filter, the sample (shown in blue) is pipetted directly into the center of the funnel, not down the side.

Results

Filtration of Milli-Q® water using 0.2 µm filters

Using 0.2 µm filters, we obtained consistent filtration of 100 µL samples. For all the types of vials tested, greater than 70% recovery was obtained when filtering 4 or more samples at the same time (Table 2).

Volume Filtered	% Recovery	% RSD	Vendor	Vial Cat. No.
100 µL	73	10	Waters	186001131c
100 µL	73	11	Waters	186001126DV
100 µL	78	14	Waters	186001127
100 µL	76	7	Verex	AR0-3625-12
100 µL	79	2	Restek	22439
100 µL	82	2	Restek	22433
100 µL	82	5	Waters	186002642

Table 2. Sample recovery rates after Samplicity® filtration of 100 µL Milli-Q® water into different vials containing inserts. Four or eight samples were simultaneously filtered for each vial type. For each measurement %RSD (relative standard deviation) was calculated by dividing the standard deviation by the mean.

Filtration of acetonitrile using 0.2 µm filters

The smallest volume of acetonitrile that could be consistently filtered was 300 µL. When filtering 100 or 200 µL of acetonitrile sample, the vacuum pressure caused the solvent to spray into the system, which resulted in the sample spilling outside the vial. When filtering 300 µL of sample, no sample spillage was observed, and all of the filtrate was consistently collected in the HPLC vials.

We thus used 300 µL acetonitrile samples and the indicated vials to test recovery using the Samplicity® system (Table 3). We achieved recoveries of around 50% in every case.

The lower rate of acetonitrile sample recovery was expected due to the increased hydrophobicity of this solvent. Acetonitrile tends to fully wet the PTFE membrane, thereby spreading onto the entire membrane surface. As a result, a large portion of the sample is held up by the membrane after filtration, leading to reduced sample recovery. Membrane wetting is a slower process for aqueous samples; therefore, higher sample recovery is possible.

Volume	% Recovery	% RSD	Vendor	Vial Insert Cat. No.
300 µL	48	6	Waters	186001131c
300 µL	45	9	Waters	186001126DV
300 µL	52	5	Verex	AR0-3625-12
300 µL	49	7	Restek	22439
300 µL	52	4	Restek	22433
300 µL	48	10	Waters	186002642

Table 3. Sample recovery rates after Samplicity® filtration of 300 µL acetonitrile samples into vials containing various different inserts. Four or eight samples were simultaneously filtered for each vial type.

Discussion

Our data show that filtration of aqueous samples as small as 100 µL into any 12 x 32 mm HPLC vial containing inserts can be efficiently performed using the Samplicity® system, with good recovery rates (greater than 70%). We have also shown that acetonitrile, and presumably other organic solvents, can be filtered using the system into these low-volume inserts, although larger starting volumes (300 µL) are required. The vials mentioned in this report were the only ones tested during this study, and it is possible that more vials containing low volume inserts can be used for low volume filtration with the Samplicity® system. As more chromatographers turn to small-volume samples for their analytical separations, they are likely to find that the Samplicity® filtration system provides an ergonomic alternative to syringe filters, increases sample preparation throughput and maintains quality of downstream results.

Ordering Information

Description	Catalogue No.
Samplicity® Filtration System	SAMPSYSGR or SAMPSYSBL
Millex Samplicity® Filters, 0.2 µm	SAMPLG001

To Place an Order or Receive Technical Assistance

In Europe, please call Customer Service:

France: 0825 045 645
Germany: 01805 045 645
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
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