

## Application Note

# The Smplicity™ Filtration System Streamlines Chromatography Sample Preparation

## Abstract

Recent innovations that have accelerated chromatographic separation, such as ultrahigh performance liquid chromatography (UHPLC) and monolithic chromatography columns, are turning up the pressure on sample preparation processes to keep pace. It is important that analytical researchers choose a sample preparation system that matches the speed and throughput requirements of their separation and analysis. The Smplicity Filtration System from EMD Millipore is an easy-to-use, high throughput alternative to syringe filters when preparing samples for analytical separations such as HPLC and UHPLC. Up to eight samples—even those with high viscosity or particulates—can be simultaneously vacuum-filtered in seconds. Our data show that the Smplicity system provides increased speed, ease of use, and reduced waste generation compared to syringe filtration.

## Introduction

Sample preparation prior to analysis helps to bring a sample to a format that is compatible with the analytical technique. Sample preparation also helps to reduce complexity of the sample by selectively removing interfering impurities from the matrix and thereby concentrating the analyte prior to analysis. A typical sample for HPLC / UHPLC needs to be particle-free and completely soluble in the solvent compatible with the chromatography system.

One of the simple sample preparation techniques that can be used prior to HPLC / UHPLC is microfiltration through membranes. Microfiltration refers to use of membrane filters with pore sizes between 0.1 and 0.45  $\mu\text{m}$ , which enables removal of particulate impurities prior to analysis. For samples containing higher loads of particles or larger particles, a pre-filter membrane with a larger pore size can be used, followed by final filtration.

Since filtration is a very simple sample preparation technique, it is one of the most neglected steps in sample prep optimization. Not enough attention is paid to filtration and the choice of filtration devices and

materials, leading to inconsistency and even failure of the downstream analysis.

Sample filtration prior to HPLC is most commonly performed using syringe filtration. This is a serial, manual process, involving filtration of one sample at a time. Syringe filtration can also be very labor intensive depending on the sample type; samples which are difficult to filter (such as particle-laden or viscous samples) require even higher pressures to filter, which can lead to fatigue. Syringe filtering one or two samples a day may present a mere inconvenience, but filtering large numbers of samples at a time can lead to severe fatigue, musculoskeletal pain or repetitive stress injuries.

To simplify the process of sample filtration and reduce the monotony of sample preparation prior to HPLC, EMD Millipore's Smplicity sample preparation system enables filtration of up to 8 samples directly into standard HPLC vials (12 x 32 mm) using vacuum-driven filtration. This avoids multiple transfers that are sometimes necessary with syringe filtration, simplifies workflow and reduces time and fatigue associated with syringe filtration.

## Methods

For all experiments, samples were filtered either through hydrophilic polytetrafluoroethylene (PTFE) Millex® syringe filters with a 10 mL syringe, or through 0.45 µm hydrophilic PTFE Millex Smplicity™ filters using the Smplicity filtration system.

The Smplicity workflow consists of the following steps:

- Make sure that all the components of the vacuum system are available and connected to a vacuum source.
- Put vials in the vial tray.
- Place the clear lid over the vial tray.
- Open doors for actual number of samples / vials.
- Place filters over openings.
- Add sample to the filter funnels. Repeat this process for all the samples. Make sure that openings that do not have sample are closed.
- Turn on the vacuum using the lever on the left side of the Smplicity system.
- After filtration is complete, turn the vacuum off and wait for the vacuum to bleed off prior to removing the filters and clear lid.

To evaluate ease of use, time savings, manual force required for filtration, and solid waste generation, 13 users were observed and timed as they filtered 24 samples using both the Smplicity system and syringe filtration. Approximately half the subjects operated the Smplicity system first, and the other half used syringe filters first. Users then rated their subjective experience of force required on a scale of 1 to 10. At the end of the testing period, solid waste from each process was collected, weighed, and placed into 10 in x 10 in x 10 in boxes to evaluate relative volume.

## Results

### Filtration Speed

The filtration speed for three different samples with varying composition and viscosity was evaluated and results with % relative standard deviation (RSD) are shown in Table 1. Deionized water was the least viscous, and therefore displayed the fastest filtration. Human plasma exhibited intermediate filtration speed, while the highly viscous Pepto-Bismol® solution was the slowest. Filtration times listed are the averages of eight replicate samples.

Sample (1.5 mL)	Viscosity (cP)	Filtration Time (Sec.)	% RSD
DI Water	1.0	2.4	4.2
Human Plasma (1:3 Dilution)	1.6	29.8	8.0
Pepto-Bismol (1% in Water)	7-10	82.8	10.9

Table 1. Filtration speed of the Smplicity system depends on the viscosity of the sample.

To compare the speed of the Smplicity system with syringe filtration, either four or eight 1.0 mL samples of 1% Pepto-Bismol were filtered by the 13 users. On average, the Smplicity system accelerated four-sample filtration by 26%, and it accelerated eight-sample filtration by 35% (Figure 1).

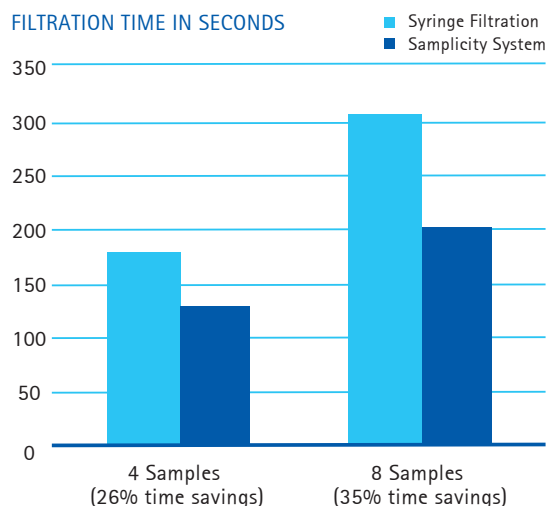


Figure 1. Overall time savings using Smplicity filtration system compared to syringe filtration

### Ergonomic Benefits

The Smplicity system saves significant amounts of time by enabling users to process multiple samples at one time. Further, the system provides ergonomic benefits by enabling steps to be completed in batch format ("assembly-line style") rather than repeated serially.

Figure 2 illustrates that, although both filtration workflows involve seven different tasks, the Smplicity system enables clustering of identical tasks together and involves only a single filtration step, thereby streamlining and accelerating the preparation of multiple samples. One of the biggest ergonomic benefits of using the vacuum-driven Smplicity system is that no manual force is needed to filter samples. This was clearly shown by user ratings of force requirement for syringe filtration vs. the Smplicity filtration system (Figure 3).

## Sequential vs. Batch Processing

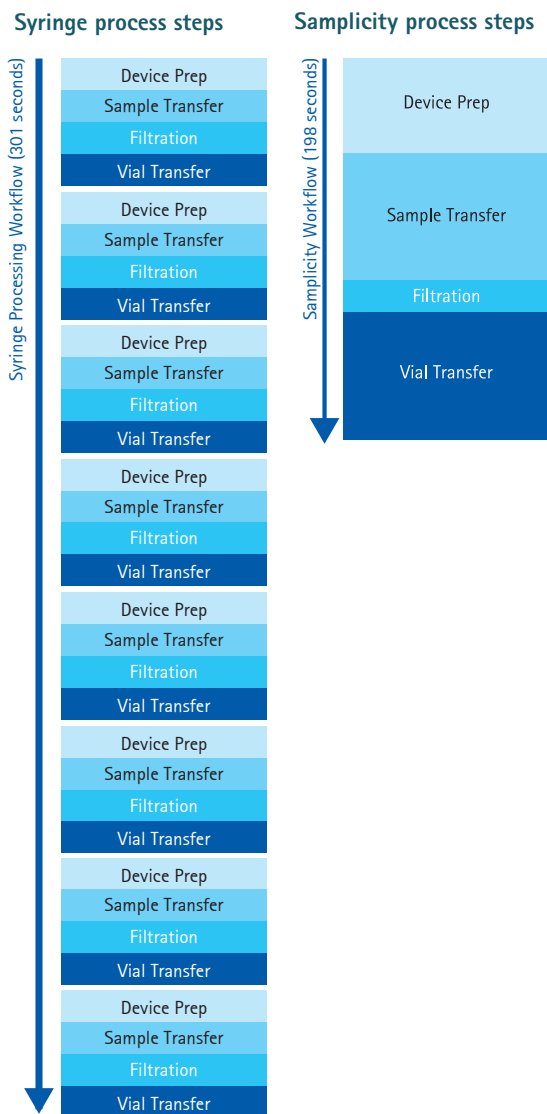


Figure 2. Streamlined, batch processing of eight samples using the Sampilcity system (right) provides ergonomic benefits and time savings over sequential syringe filtration of eight samples (left).

### Waste Reduction

Waste disposal represents a significant cost, both in time and monetary resources, to laboratories performing larger scale sample preparation. Using the Sampilcity system results in a 33% reduction in waste weight and a 66% reduction in waste volume, and eliminates the need to segregate syringe waste (Table 2).

	Sampilcity system	Syringe filtration	% Reduction in Waste
Weight (g)	2827.5	4232.9	33.2%
Volume (in <sup>3</sup> )	1000	3000	66%

Table 2. Comparing the weight and volume of waste generated by the Sampilcity system compared to syringe filtration.

### FORCE REQUIRED

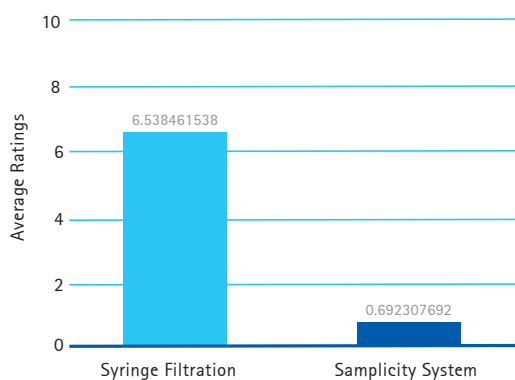


Figure 3. Average ratings of manual force required to operate syringe filters vs. the Sampilcity filtration system. 1.0 mL samples of 1% Pepto-Bismol were filtered by 13 users, either through hydrophilic polytetrafluoroethylene (PTFE) Millex syringe filters with a 10 mL syringe, or through 0.45 µm hydrophilic PTFE Millex Sampilcity membrane filters using the Sampilcity filtration system.

### No Cross-Contamination

One of the major concerns when processing multiple samples at one time is cross-contamination of filtrates. To assess signal cross-talk between filter positions, samples containing 0.005% fluorescein in 80:20 (v/v) acetonitrile:water were filtered using every other filter position in the Sampilcity system, with alternate filter positions being used to filter solvent alone. Fluorescence signal was measured by spectrophotometry (Figure 4). The results clearly showed that there was no cross-contamination between various positions on the Sampilcity system.

Filter Position	Fluorescence Intensity	
1	40564	19
2	21	42320
3	40034	18
4	16	42499
5	40982	18
6	20	45390
7	43158	23
8	19	45432

Figure 4. Evaluating the possibility of cross-contamination between filter positions on the Sampilcity system. In row 1, filter positions 1, 3, 5, and 7 were used to filter the fluorescein-containing samples. In row 2, filter positions 2, 4, 6, and 8 were used to filter the fluorescein-containing samples.

### HPLC Vial Compatibility

Chromatographers use a variety of 12 X 32 mm standard HPLC vials for collecting and storing prepared samples. Although these vials have the same external dimensions, their internal dimensions vary to take into account types of closures available (snap, screw or crimp) as well as inserts put into the vials to reduce the sample volume required. The Sampilcity system was tested with six of the most commonly available HPLC vials and worked well with all these vial types. One of the serendipitous advantages of the Sampilcity system was that, unlike syringe filtration, no air bubble was trapped in the maximum recovery vials.

## Conclusion

By accelerating sample preparation and providing ease of use, reduced waste generation, and ergonomic benefits compared to syringe filtration, the Smplicity filtration system has the potential to increase the productivity and data quality generated by liquid chromatography. In particular, laboratories preparing multiple samples for each HPLC or UHPLC experiment and performing multiple experiments a week or more can benefit the most from adopting the Smplicity system.



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