Application Note

The Samplicity™ System Delivers High-Performance Multi-sample Filtration While Streamlining Preparation of Chromatography Samples

Abstract

The Samplicity™ filtration system has helped researchers streamline chromatographic sample preparation in order to match the speed and throughput requirements of their analytical separations such as high/ultrahigh performance liquid chromatography (HPLC /UHPLC). An easy-to-use alternative to syringe filters, the Samplicity™ system enables simultaneous vacuum filtration of up to eight samples. Even difficult-to-filter samples with high viscosity or particulates can be processed in seconds. Our data show that the Samplicity™ system facilitates preparation of chromatography samples with high recovery, low extractables and very low passage of particulate impurities.

Introduction

Sample preparation prior to analysis helps to bring a sample to a format that is compatible with the analytical technique, reduces sample complexity, removes interfering impurities from the matrix and thereby concentrates 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.

Due to its simplicity and effectiveness, membrane filtration is a common sample preparation technique; however, 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 chromatography 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.

Simplifying sample preparation, the Samplicity™ 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.

The Samplicity™ system involves filtration of samples through Millex Samplicity™ filter units. Here, we show performance data for these filter units, demonstrating filtration with low hold up volume, high sample volume recovery, high analyte recovery (by mass), and low extractables. Furthermore, we show that Millex Samplicity™ filter units provide accurate particle retention with respect to reported pore size and high sample volume recovery for volatile solvents.

Methods

For all experiments, samples were filtered either through hydrophilic polytetrafluoroethylene (PTFE) Millex® syringe filters (Merck Millipore Catalogue No. SLCR025NK) with a 10 mL syringe, or through 0.20 µm or 0.45 µm hydrophilic PTFE Millex Samplicity™ filters (Merck Millipore Catalogue Nos. SAMPLG001 or SAMPLCR01) using the Samplicity™ filtration system (Merck Millipore Catalogue Nos. SAMPSYSGR or SAMPSYSBL).

Determination of holdup volume and sample volume recovery

HPLC vials and Millex Samplicity™ filters (0.20 µm filters and 0.45 µm filters) were preweighed using an analytical balance. Four Samplicity™ base units were used for filtration. Milli-Q® water (2 mL) was applied to all filters and vacuum was applied until visual inspection revealed that filtration was complete. Vials were reweighed. The bottom of each filter was wiped using a laboratory wipe and the filters were weighed.

Determination of analyte recovery

Four drug tablets (ranitidine, loratadine, ibuprofen and acetaminophen) were dissolved in their respective dissolution media (900 mL) for 24 hours with constant stirring at room temperature. 1.5 mL of the resulting drug suspensions were pipetted into 1.5 mL microcentrifuge tubes and centrifuged at high speed in a microcentrifuge. Samples clarified by centrifugation were analyzed by UV spectroscopy, and absorbance recorded. These samples were considered to represent 100% recovery. All samples were then filtered using the Samplicity™ filtration system using either 0.20 µm or 0.45 µm Millex Samplicity™ filters. Filtrates were analyzed by UV spectroscopy, and analyte concentrations determined and % recovery determined in comparison to centrifuged sample.

Extractable analysis using HPLC-UV

Three solvents (Milli-Q® water, methanol (LC-MS grade), and acetonitrile (LC-MS grade) were filtered through 0.20 µm and 0.45 µm Millex Samplicity™ filters for each solvent. 1 mL of solvent was applied to each filter and filtrates were collected in vials. These vials were then replaced with new vials, and an additional 1 mL of each solvent was applied to the same filters and filtered into the second set of vials. Samples and starting solvents were analyzed by reversed phase HPLC using C18 column run under gradient condition (0 – 100 % acetonitrile) followed by UV detection (214 and 254 nm) .

Particle retention analysis

Polystyrene latex particles (0.5 µm, Sigma Aldrich Cat. No. LB5 or 0.3 µm, Sigma Aldrich Catalogue No. LB3) were diluted to a 0.005% suspension in Triton® X-100 and absorbance measured at 272 nm. 1.5 mL of each particle suspension was added to 0.20 µm or 0.45 µm Millex Samplicity™ filters and vacuum applied. The absorbance of each filtrate (as well as a Triton® X-100 blank) at 272 nm was measured by UV spectroscopy and particle retention calculated.

Sample recovery filtering volatile solvents

1 mL of each solvent (acetonitrile, tetrahydrofuran and acetone) was filtered through Millex Samplicity™ filters (0.45 µm) into preweighed vials. Immediately after filtration (Time 0), vials were weighed and placed back into the system. After 10 minutes of additional vacuum (Time 10), the vials were weighed again. Recoveries were calculated based on the initial weight of solvent. The typical holdup volume of the filter varied between 70-100 µL; therefore, losses of 7-10% were expected.

Results

The holdup volume of a filter can affect the volume of sample recovered in the filtrate. We showed that Millex Samplicity™ filters had average holdup volumes of less than 70 µL, indicating that, for most applications involving filtration of 1-2 mL volumes, the volume of sample lost to holdup is not a significant loss (Table 1).

Pore size	Holdup volume, µL	%CV	Volume filtered in vial, µL	%CV
0.45 µm	68.759	13.082	1905.731	0.730
0.20 µm	62.790	12.663	1907.771	1.174

Table 1. Millex Samplicity™ filters feature holdup volumes less than 70 µL (N = 32). Experimental error is reported as percent coefficient of variation (%CV).

Next, we tested the percent recovery (by mass) for four different analyte types, to simulate the preparation of samples during dissolution testing, a common procedure during the development of pharmaceutical products. We found that, for all four analytes tested, Millex Samplicity™ filters provided recovery between 96-100% (Table 2).

Pore size	Analyte	Average percent drug recovery after filtration	%CV
0.45 µm	Acetaminophen	99.52 %	1.22
	Loratadine	95.88 %	0.91
	Ranitidine	96.5 %	0.76
	Ibuprofen	100.12 %	0.13
0.20 µm	Acetaminophen	99.9 %	1.64
	Loratadine	96.16 %	0.74
	Ranitidine	99.17 %	0.52
	Ibuprofen	99.99 %	0.12

Table 2. High recovery (96–100%) of drug analytes after filtration using the Samplicity™ system. Experimental error is reported as %CV. N = 16.

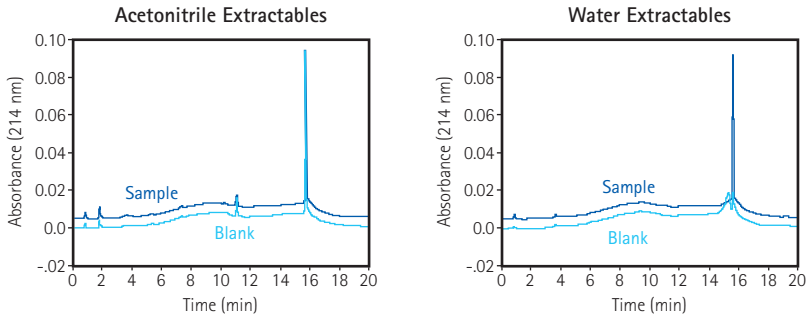
The Samplicity™ system saves significant amounts of time by enabling users to process multiple samples at once. Further, the system provides ergonomic benefits by enabling steps to be completed in batch format (assembly-line style) rather than repeated serially. As a result, the Samplicity™ system requires only 7 steps for filtration of 4 samples, while syringe filtration requires 24 steps for the same 4 samples (Table 3).

Filtration System Used	Number of steps for filtration of 4 samples
Syringe Filter + Syringe	24
Samplicity™ System	7

Table 3. The Samplicity™ filtration system requires fewer steps to filter 4 samples than syringe filtration.

It is important that sample preparation methods do not introduce additional impurities into the sample, particularly for sensitive downstream analyses. Analytical results can be confounded by extractable impurities in the sample, which can come from a filter, filter housing, or from the filter manufacturing process. Chemical compatibility between the liquid being filtered and the filter system is important to minimize the extractables present in the filtrate. We tested several solvents (acetonitrile and water shown in Figure 1) for their potential to extract impurities from Millex Samplicity™ filters. Because these filter membranes are composed of hydrophilic PTFE, which has broad chemical compatibility, few impurities were leached into either solvent (Figure 1). Extractables were analyzed by UV spectroscopy at 214 nm. Typically, the lower the wavelength of absorbance detection used, the higher the chance that the extractable impurities will be visible.

A. Extractables from 0.45 µm filters



B. Extractables from 0.20 µm filters

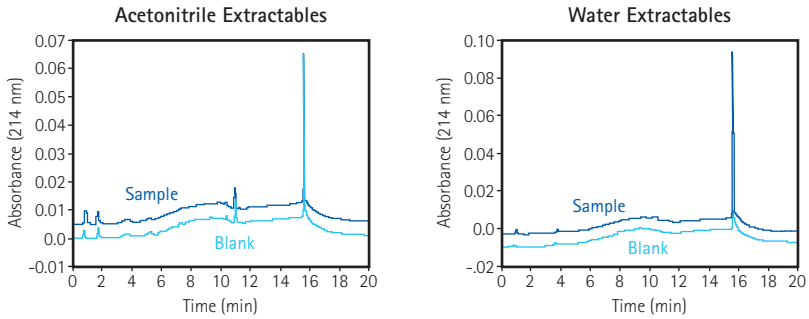


Figure 1. Solvents filtered through Millex Samplicity™ filters (HPLC traces labeled "sample") contained few extractables when compared to blank solvents (HPLC traces labeled "blank"). Neither acetonitrile (left) nor water (right) extracted significant impurities from 0.45 µm (top) or 0.20 µm (bottom) filters.

A measure of membrane filtration performance is the degree to which the filter prevents particles larger than its pore size from passing into the filtrate. We challenged 0.45 µm filters with 0.5 µm diameter particles and 0.20 µm filters with 0.3 µm particles. As shown in Table 4, both membranes caused retention of almost all particles – 95% retention for 0.20 µm Millex Samplicity™ filters and 100 % retention for 0.45 µm filters.

Pore size	Average % retention of particles	%CV
0.45 µm	101.82%	1.90
0.20 µm	94.46%	4.67

Table 4. Millex Samplicity™ filters retained latex microspheres with high (95–100%) efficiency. Experimental error is reported as %CV.

Certain analytical methods require that the concentration of analyte in the sample be maintained during sample filtration. Particularly for these cases, it is important to minimize evaporation of solvent. To examine the recovery of volatile solvents after filtration by the Samplicity™ system, we measured % recovery of the solvent immediately after filtration (Time 0) as well as after 10 minutes of exposure to vacuum following filtration (Time 10). For all three volatile solvents tested, percent recovery was not significantly altered after prolonged exposure to vacuum (Table 5).

	% Recovery		%CV	
Solvent	Time 0	Time 10	Time 0	Time 10
Acetonitrile	88	89	1%	1%
Tetrahydrofuran	92	94	3%	4%
Acetone	99	96	2%	5%

Table 5. Percent recoveries of three volatile solvents were unchanged after exposure to vacuum for ten minutes. Experimental error is represented as %CV.

Conclusion

By streamlining sample preparation while still providing high performance filtration, the Samplicity™ filtration system has the potential to increase productivity and data quality generated by liquid chromatography. Samples filtered using the Samplicity™ filtration system will benefit from low extractables and efficient particulate retention, rendering this sample preparation method compatible with sensitive downstream analysis, including highly sensitive analytical separations and mass spectrometry. Furthermore, the high analyte recovery, low holdup volume and good recovery of volatile solvents afforded by the Samplicity™ system translate into the preservation of particularly precious samples, saving time and cost of obtaining the starting materials for chromatography.

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