Mobile Phase Preparation for UHPLC: Membrane Filtration Method Affects System Performance and Leaching of Extractable Impurities

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UHPLC/UPLC® is a revolutionary chromatography technique that is gaining wide acceptance among researchers due to improved resolution, shorter chromatographic runs, and the capability for doing fast method development. The presence of sub-2 µm particles in UHPLC columns provide these benefits but also poses challenges in sample and mobile phase preparation. Particulate impurities in the sample or mobile phase can cause backpressure buildup in the UHPLC system, causing system failure. In fact, most UHPLC instrument vendors recommend filtration of mobile phase using 0.2 µm filters, but there is a lack of data showing the benefits of filtration. In this article we describe the filtration of mobile phases through syringe filters of varying pore size and membrane type, followed by analysis by UHPLC and mass spectrometry. Our results clearly indicate that filtration of mobile phase components using the optimal membrane filter will help protect UHPLC systems from particulate impurities that may clog and shut down the system, increase the sensitivity of detection, and improve the accuracy of quantitation.

Introduction

Sample throughput and separation efficiency in chromatography is improved by decreasing the size of the particles packed in the column, as predicted by the van Deemter equation (van Deemter, 1956):

$$H = A + \frac{B}{\mu} + C \bullet \mu$$

In this equation, H is the plate height and µ represents the velocity of the mobile phase. H is therefore inversely proportional to separation efficiency. To increase sample throughput, velocity (µ) should increase while minimizing H.

The constants A and B are diffusion terms, and C reflects the movement of the analyte between the mobile and stationary phases. A, B, and C all contribute to wider analyte

peaks in the HPLC profile, limiting resolution. Decreasing the particle size of the stationary phase makes both A and C smaller, increasing resolution and decreasing H. As a result, H is less sensitive to increases in velocity, allowing for high sample throughput without compromising peak resolution (Thompson, 2006). In fact, resolution improvements of up to 50% (compared to standard HPLC separations) are routine (Swartz, 2005).

UHPLC columns packed with sub-2μ m particles create high system backpressure that far exceeds the limits of traditional HPLC systems. To reap the benefits of this new column technology, manufacturers have developed instruments capable of running at pressures much higher (up to 15,000 psi) than standard HPLC systems (Swartz, 2005).

The advantages that an UHPLC system can bring to an analytical lab include a general increase in productivity, reduced method development time, more data from small samples, and a 3-10 fold decrease in solvent usage and disposal costs. Most methods developed on traditional HPLC systems can be transferred to a UHPLC system without much difficulty. However, UHPLC, with its smaller particle sizes, lower interstitial void volumes, decreased column diameters and higher flow rates, presents scientists with new challenges (Dong, 2007). Columns filled with very small particles are more susceptible to premature plugging by particulates. UHPLC Column life is generally shorter than that of traditional HPLC columns.

Clean mobile phase components (buffers and solvents) are the key to addressing some of the challenges associated with UHPLC technology. To minimize system failure and maximize system performance, instrument manufacturers recommend using ultra-pure water and filtering mobile phase components through 0.2 ìm membrane filters (Waters Corporation, 2007). Poor water quality and unfiltered buffer salts result in particulates in the mobile phase. Particles can cause increased backpressure, column clogging and eventual system shutdown.

Simple filtration provides a fast and economical means of preparing samples and mobile phases for optimal UHPLC results. A precut disc filter that costs about $1.50 is a far better place to collect damaging particulates than a high priced column (~ $ 400) critical to the operation of sensitive and expensive UHPLC equipment.

Membrane Microfiltration

Microfiltration is the process of removing particles or biological entities in the 0.025 μm to 10.0 μm range from fluids by passage through a microporous medium such as a membrane filter. Because membrane filters, unlike depth filters such as glass fiber filters, have precisely defined pore sizes, contaminants can be quantitatively retained (Meltzer, 1987). Membrane disc filters are therefore routinely used to remove

particulate contamination from solvents prior to chromatographic analysis, and are well suited to preparing mobile phases for UHPLC.

Although, theoretically, microporous membranes should retain all particles greater than the reported pore size, the true retention properties depend upon both the physicochemical characteristics of the membrane, as well as pore size uniformity. Most membrane pores have unequal sizes, which are statistically distributed. This, together, with variations in membrane construction, tortuosity, and electrostatic interaction with particles, results in retention cutoffs that are not absolute (Hernandez et al, 1996).

In this study, we wanted to determine the effects of variations in retention by membrane filters on UHPLC performance. We determined the percent retention of various microporous membranes, and then used the same membranes to filter a typical UHPLC mobile phase (1:1 Water : Acetonitrile). We measured the increase in UHPLC system backpressure upon running this filtered mobile phase through a UHPLC system.

Extractables and Leachables

Another factor affecting downstream sample analysis is the presence of extractables and leachables. Extractables are soluble impurities that leach out of the filter into the sample or mobile phase during filtration. High levels of these impurities typically are leached into samples and mobile phases that are chemically incompatible with the filter. The presence of these extractable impurities leads to higher background during sample analysis, thereby reducing the sensitivity of detection and quantitation.

For example, if extractable impurities co-elute with the analyte of interest in downstream UHPLC/LC-MS analysis, quantitation of that analyte will be inaccurate. In cases where the extractable impurities are well separated from analyte of interest, resulting chromatographic or spectrometric peaks may be difficult to explain and usually vary from sample to sample.

Materials and Methods

10 % Polystyrene latex suspension (Cat # LB3) and Triton-X 100 was obtained from Sigma-Aldrich. Acetonitrile for chromatography was obtained from Merck.

Determination of nano-particle retention efficiency

A 0.005% suspension (v/v) of 0.3 μm latex particles was prepared by diluting a 10% polystyrene suspension with a 0.1% solution of Triton-X 100 in water.

Particle size distribution was determined by laser light diffraction technique As can be

seen in figure 1, the latex suspension includes particles of diameters ranging from 0.2 to 0.34 μm.

We measured the UV absorbance at 272 nm of a dilution series of latex particles to create a standard curve relating UV absorbance to particle concentration. Then, we filtered the latex suspension through various syringe filters and measured the UV absorbance of the filtrate. We compared this value to the absorbance of the unfiltered suspension to determine the percent retention of each membrane type. Each measurement was repeated three times, and the mean, standard deviation and coefficient of variation were determined.

UHPLC experiments

Milli-Q™ water and acetonitrile were filtered through polypropylene, nylon, polyvinylidene fluoride (PVDF), or polytetrafluoroethylene (PTFE) membrane filters. Acetonitrile could not be filtered through PVDF due to chemical incompatibility. Filtered water and acetonitrile were then mixed 1:1 (v/v) to create a mobile phase for UHPLC. Chromatography was performed using an ACQUITY UPLC® (Waters Corporation) system with a Acquity UPLC BEH- **C18** column (2.1 mm X 100 mm, 1.7 μm).

The system was run for 600 minutes at a flow rate of 0.25 mL/min, and backpressure was monitored continuously using the system.

Mass spectrometry

LC-MS grade acetonitrile was filtered through the respective syringe filters. The first and second 1 ml filtrate fractions were collected. Extracts from 5 different filters were pooled to obtain enough extract volume for analysis.

Extractable analysis was done using diffusion studies directly into the mass spectrometer. The flow rate was 20 μl/min and analysis was carried out for 5 min. The mass spectrometer used was an API 2000 Triple Quadrupole MS system (ABI/SciEX). The mass spectrometer was operated in electrospray ionization mode (ESI) and the polarity used was positive. Average mass spectra were collected for M/Z of 100-1000. Between sample analysis, the mass spectrometer was cleaned by infusing LC-MS grade acetonitrile.

Results and Discussion

Retention of particles by microporous membranes

Of the four different 0.2 μm membranes tested for retention of latex particles in suspension, nylon, PVDF and PTFE membranes all retained more than 95% of

particles in the suspension. Polypropylene membrane, however, retained only 79% of the particles (table 1). Although the particle size distribution (figure 1) shows that many particles in the suspension are between 0.2 and 0.3 μm in diameter, variations in polypropylene membrane pore size, as well as variations in the particle size, may cause latex particles to pass through polypropylene membrane.

Effect of membrane filtration of mobile phase on UHPLC system backpressure

In figure 2, we present data showing the benefits of filtration of mobile phase through 0.2 μm membrane filters on the performance of a UHPLC system. Of the various membrane filters evaluated, hydrophilic PTFE provided the best filtration performance as indicated by the lowest backpressure increase in a UHPLC system—in fact, backpressure actually decreased after 600 minutes of run time. The hydrophilic polypropylene (PP) filter was unable to retain particulate impurities present in the solvents as indicated by highest backpressure gain of all the filters studied. This behavior was consistent with its poor percent retention of latex particles as measured in table 1. Nylon and hydrophilic PVDF filters showed an intermediate performance in terms of backpressure increase.

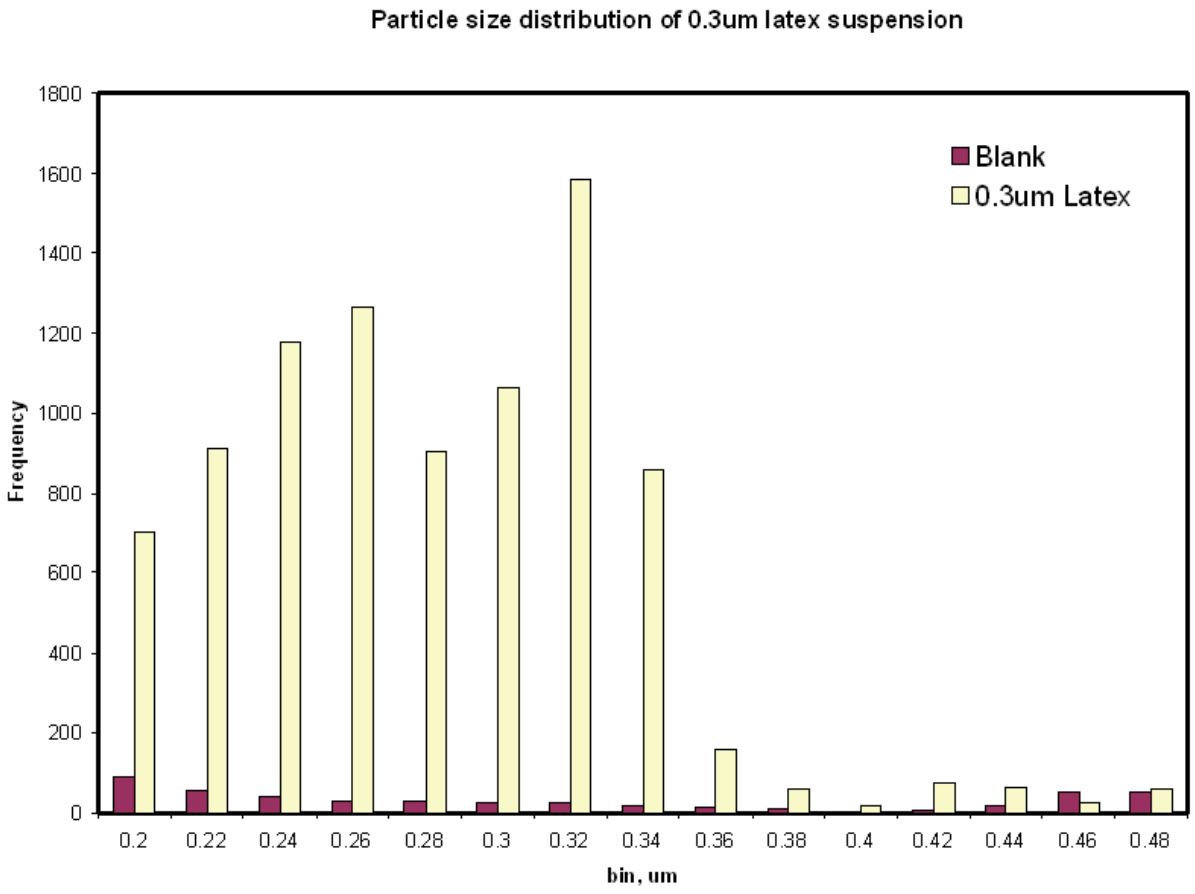

Figure 1: Particle size distribution of 0.3 μm latex suspension, measured by Laser Light Scattering (Liquilaz ® Particle Counting Spectrometer, LS200)

Table 1: Percent retention efficiency for filtration of 0.005% latex suspension (0.3 μm) through 0.2 μìm syringe filters

	0.2 μm nylon	0.2 μm PTFE	0.2 μm polypropylene	0.2 μm PVDF

Average Retention Efficiency	95.06	97.57	78.64	97.91
Std. deviation	4.53	1.70	1.88	2.63
% CV	4.76	1.74	2.39	2.38

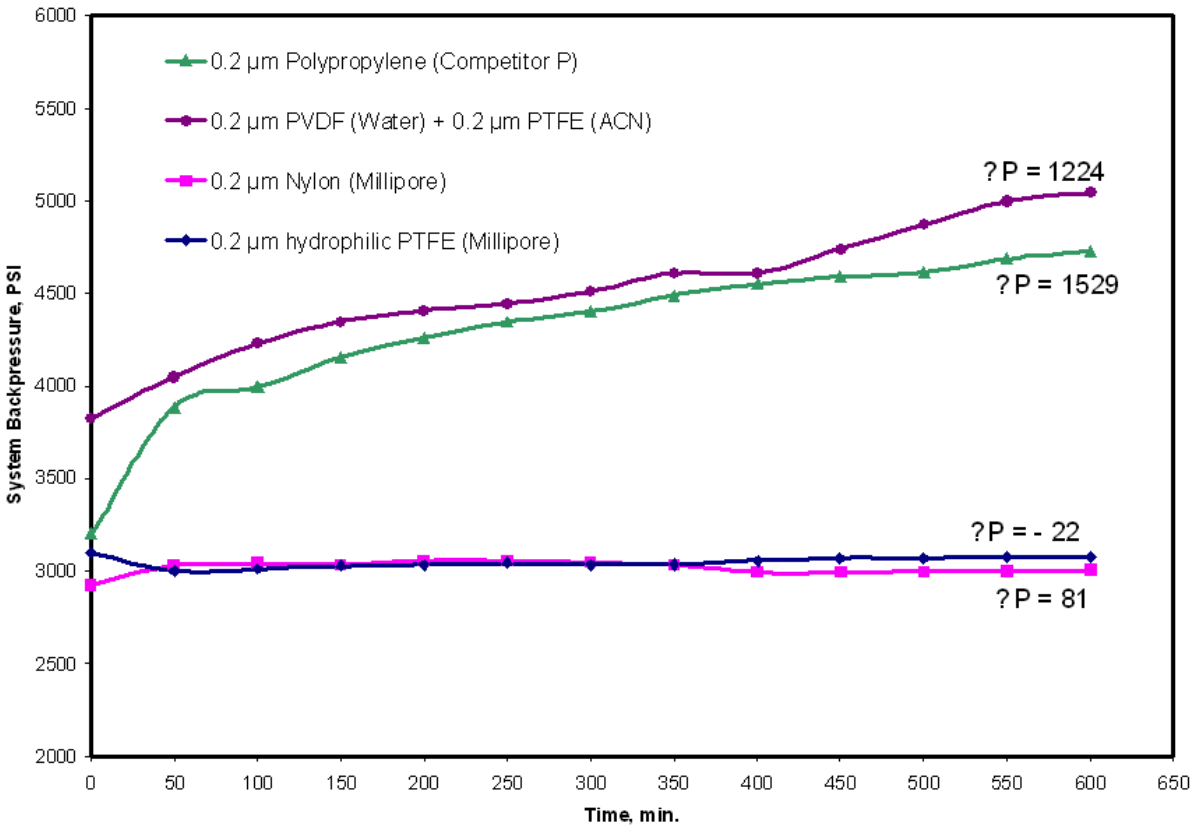

Figure 2: Change in system backpressure depends on filtration membrane used to prepare UHPLC mobile phase. Water and acetonitrile were passed through polypropylene, PVDF, PTFE or nylon syringe filters (membranes indicated in legend), then used 1:1 (v/v) to prepare the mobile phase for UHPLC. The system was run at 0.25 mL/min for 600 min with backpressure recorded every 50 min. ÄP represents total change in backpressure after 600 min.

Effect of filter composition on leaching of extractable impurities

Extractable impurities leached from syringe filter membranes were detected by MS analysis of acetonitrile filtered through two different membranes (Figures 3 and 4). The data clearly show that the sample filtered through hydrophilic PTFE Millex filters

contains dramatically lower levels of extractables compared with samples filtered through non-Millipore syringe filters with polypropylene membrane.

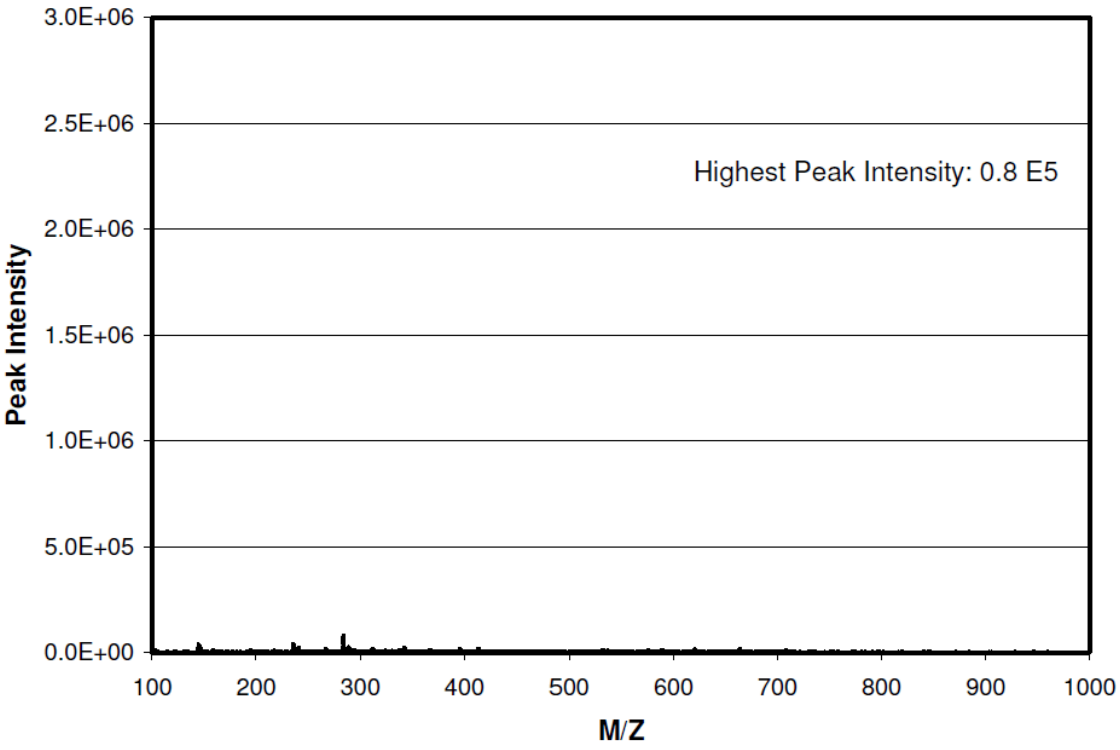

Figure 3: Mass spectrometry detects few extractable impurities from Millex syringe filter containing 0.45 µm pore hydrophilic PTFE membrane.

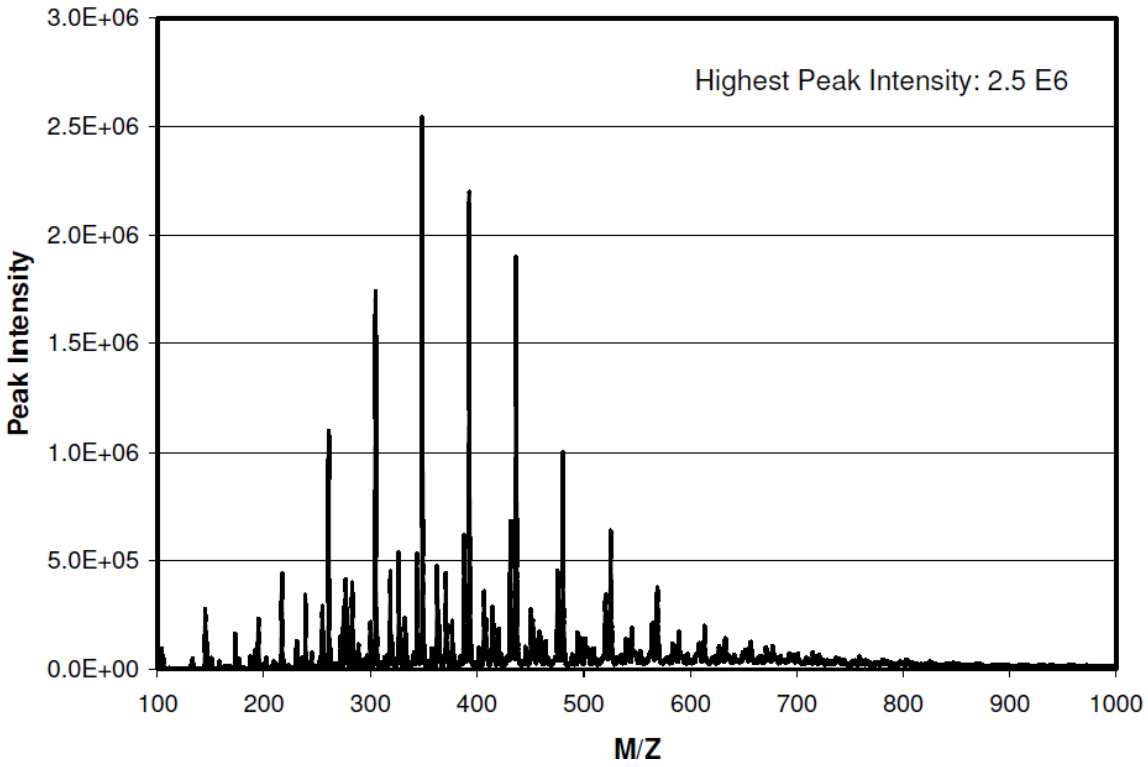

Figure 4: Mass spectrometry reveals extractable impurities from syringe filter containing 0.45 µm pore polypropylene membrane (non-Millipore).

Summary

Using membrane filtration techniques to purify mobile phase as well as samples is an easy way to achieve the highest levels of UHPLC system performance. However, given the wide range of physicochemical properties of filters and UHPLC solvents, one filter type is unlikely to be optimal for all applications. Carefully choosing the correct filters will maximize retention of damaging particulates and minimize leaching of extractables, enabling any laboratory to benefit from increased throughput and resolution provided by UHPLC.

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