

Choosing the Best Syringe Filters for Biological Sample Filtration

Application Note

Proteomics & Protein Sciences

Authors

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Abstract

Agilent Captiva Premium PES syringe filters are evaluated and compared to other suppliers' PES and PVDF syringe filters for the risk of losing protein during filtration. Captiva Premium PES syringe filters demonstrate low protein binding with excellent recovery for biological samples, especially for 'sticky' proteins at low concentrations. In addition, an assessment of filter extractables on LC/MS shows that Captiva Premium PES syringe filters provide excellent chemical cleanliness and do not introduce observable chemical contamination through the filtration process.

Introduction

Filter filtration is a common method for preparing and sterilizing biological samples to remove impurities and micro-organisms. However, using syringe filters could cause loss of significant amounts of biological materials due to unwanted protein binding with the membrane or introduce unexpected interferences to the samples from the syringe filter. Therefore, low protein binding and cleanliness are important features of a syringe filter's performance. Polyethersulfone (PES) and polyvinylidene fluoride (PVDF) membranes are typically used for biological sample filtration and are claimed to provide very low protein binding. In this study, PES and PVDF membranes were evaluated and compared. A group of common proteins was used for the protein binding evaluations, including BSA, myoglobin, ovalbumin, cytochrome C, and thyroglobulin. After protein samples were filtered through syringe filters, the protein samples were evaluated using HPLC/UV by comparing samples with or without filtration for their monomer, dimer, or aggregates peaks. In addition, syringe filter cleanliness was evaluated by filtering membrane-compatible solutions and monitoring the filtrate with LC/MS under positive and negative modes.



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Experimental

Five proteins were selected for the filtration protein binding evaluation: albumin from bovine serum (BSA), myoglobin from horse heart, ovalbumin from chicken egg, cytochrome C from bovine heart, and thyroglobulin from bovine thyroid. These common proteins provided a range of hydrophobicities and molecule sizes, from hydrophobic myoglobin to hydrophilic thyroglobulin. Table 1 shows the important physical properties of the proteins.

Chemicals and reagents

All proteins were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium phosphate dibasic (Na_2HPO_4) and sodium phosphate monobasic (NaH_2PO_4) salts were also from Sigma Aldrich. Acetonitrile (ACN) and methanol (MeOH) were from Honeywell B&J (Muskegon, MI, USA).

Solutions and standards

To avoid any potential binding during protein solution storage, all the samples were prepared fresh in plastic tubes and used only for the day. For each protein, a 0.5 mg/mL solution was prepared by dissolving protein powder into Milli-Q water. For further evaluation of myoglobin at different concentrations, a 2 mg/mL myoglobin stock solution was made and then diluted in series to make 0.1, 0.2, 0.5, and 1 mg/mL samples.

Sodium phosphate stock solutions (0.5 M of Na_2HPO_4 and NaH_2PO_4) were prepared by dissolving salt powder into the appropriate volume of Milli-Q water. Vigorous stirring was

applied to make sure the salts were fully dissolved. A 0.5 M phosphate buffer, pH 7.0, was then made by combining the Na_2HPO_4 and NaH_2PO_4 stock solutions at 4:1, and then adjusting the pH to 7.0 with NaH_2PO_4 stock solution. This 0.5 M phosphate stock solution was subsequently used for further dilution to 150 mM phosphate buffer, pH 7.0, which was used as the mobile phase for protein analysis with LC.

A 30:70 MeOH/water (v/v) solution was prepared by combining 30 mL MeOH with 70 mL Milli-Q water. This reagent blank was used to spike the internal standard and to evaluate filter extractables.

Sample filtration

For the protein binding test, approximately 2 mL of freshly prepared protein sample was withdrawn into a 10 mL syringe. Approximately 1 mL of air was left inside the syringe. A syringe filter was then attached to the syringe. The syringe plunger was compressed steadily and slowly to force the protein samples through the syringe filter. The flowed-through protein samples were then injected for LC/UV analysis. Filtered sample was compared to unfiltered sample for the filtration recovery assay.

For the extractables test, approximately 2 mL of 30:70 MeOH/water blank was filtered through the syringe filter. The filtrate was injected for LC/MS analysis under positive and negative modes, and filtered sample was compared to unfiltered sample chromatographically.

Table 1. Selected protein physical properties [1-5].

Protein	BSA	Myoglobin	Ovalbumin	Cytochrome C	Thyroglobulin
Molecular weight (kDa)	66.5	17.2	45	12	660
Number of amino acids	583	154	386	105	~ 5000
Isoelectric point (pI)	4.7	7.1	4.5	9.6	4.5

Instrumentation

For the protein binding test, an Agilent 1200 Series SL HPLC system was used. The extractables test was conducted on an Agilent 1290 Infinity UHPLC combined with an Agilent 6150 Single Quadrupole MS System. Table 2 lists the instrument, column, and parameters used for the protein test, and Table 3 lists the instrument, column, and parameters for the extractables test. Table 4 lists the experimental supplies used for both tests.

Table 2. Instrument, column, and parameters for the protein test.

HPLC:	Agilent 1200 SL Series
Column:	Agilent Bio SEC-3, 300Å, 7.8 × 100 mm, 3 µm (p/n 5190-2512)
Mobile phase:	150 mM Phosphate buffer, pH 7.0
Flow rate:	1.0 mL/min, isocratic
Injection volume:	6 µL
Total run time:	8 min
Detector:	DAD SL, wave length = 214 nm

Table 3. Instrument, column, and parameters for the extractables test.

UHPLC:	Agilent 1290 Infinity LC System
Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Mobile phases:	A: H ₂ O + 0.01% formic acid (FA) B: Acetonitrile + 0.01% FA
Flow rate:	0.5 mL/min, gradient
Total run time:	5 min plus 1 min post run
Gradient:	Hold at 30% B for 1 min, then ramped to 90% B in 3 min, and hold at 90% B for 1 min
Injection volume:	8 µL
Internal standard:	50 µg/mL Naproxin
MS:	Agilent 6150 Single Quadrupole LC/MS System
Source:	ESI with Agilent Jet Stream Technology (AJS-ES)
Capillary voltage:	4,000 V
Nozzle voltage:	2,000 V
Drying gas flow:	12 L/min
Drying gas temp:	250 °C
Nebulizer pressure:	35 psig
Sheath gas flow:	3.0 L/min
Sheath gas temp:	150 °C
Mass range:	100 – 1350 <i>m/z</i>
Fragmentor:	150 V (pos), 80 V (neg)

Table 4. Experimental supplies from Agilent Technologies, Inc..

Vials:	Amber, write-on spot, 100/pk (p/n 5182-0716)
Vial caps:	Blue, screw cap, 100/pk (p/n 5182-0717)
Syringe:	10 mL, 100/pk (p/n 9301-6474)
Syringe filter:	Captiva Premium PES, 0.2 µm, 15 mm (p/n 5190-5096) and 25 mm (p/n 5190-5098)
pH meter:	Agilent 3200P pH Meter (p/n G4391A)

Results and Discussion

Protein chromatograms

All of the proteins were run individually on the Agilent Bio SEC-3 column. The chromatograms are shown in Figure 1. In general, they all showed good peak shape. For BSA, myoglobin, and ovalbumin, baseline separation between dimer and monomer was achieved, and so the recovery of dimer and monomer were evaluated separately. Cytochrome C is monomeric, and this was confirmed with a neat single peak on its chromatograms, and so recovery evaluation of cytochrome C was based on monomer only. For thyroglobulin, partial separation between dimer and monomer was obtained. Dimer and monomer of thyroglobulin cannot be integrated accurately; therefore, the recovery evaluation was based on the aggregate peak.

Figure 1 also shows comparison chromatograms of filtered protein samples with the corresponding unfiltered samples. Excellent filtration recoveries were achieved for all 5 proteins. Filtration recovery (FR) was calculated based on Equation 1. Detailed filtration recovery is discussed later.

Equation 1

$$\text{Filtration recovery \% (FR)} = \frac{\text{Peak area of analyte from filtered sample}}{\text{Peak area of analyte from unfiltered sample}} \times 100\%$$

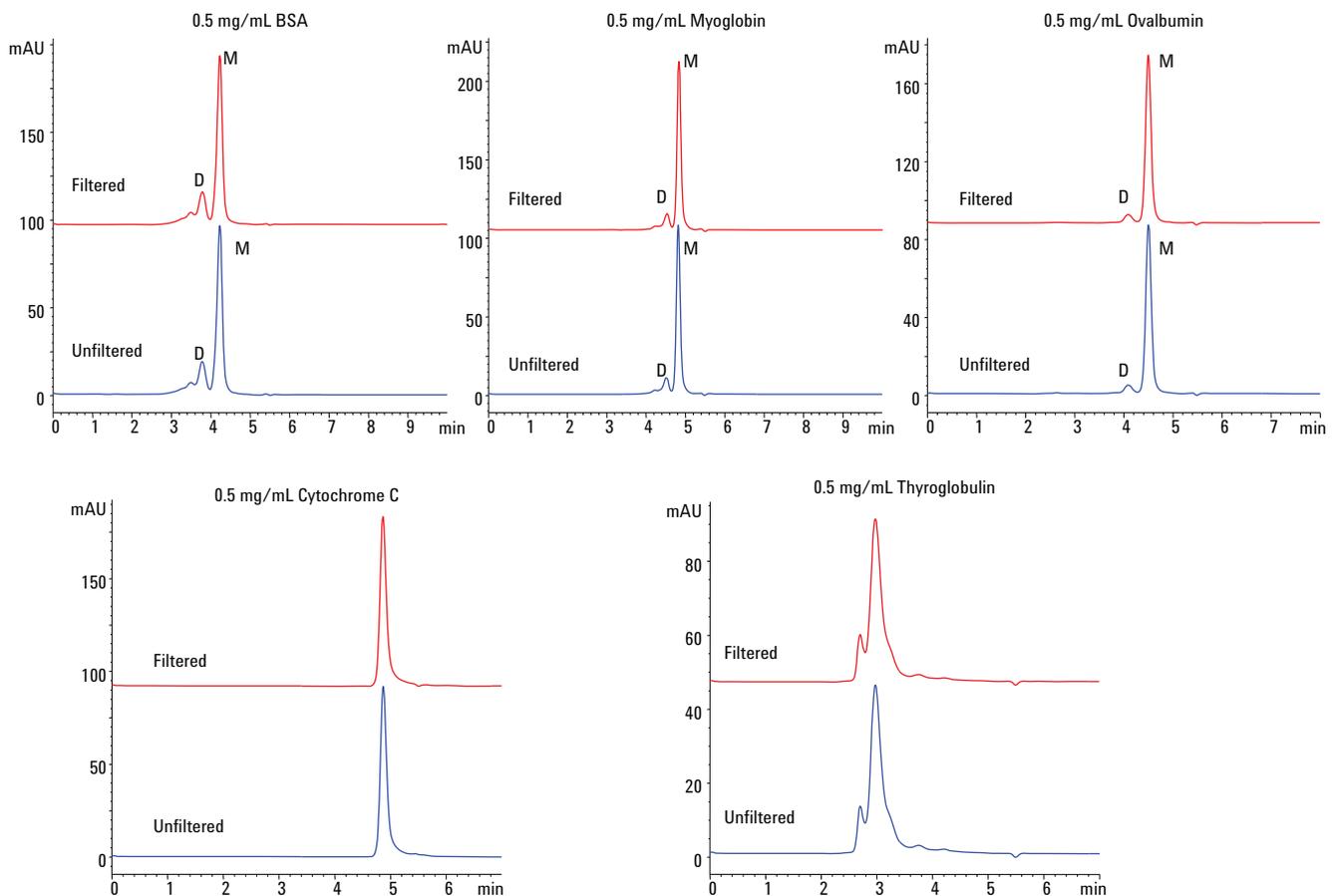


Figure 1. HPLC/UV chromatograms of common proteins and comparison of unfiltered sample to filtered sample using an Agilent Captiva Premium PES syringe filter, 0.2 μm , 15 mm. D: Dimer, M: Monomer.

Captiva Premium PES syringe filter versus PVDF syringe filters

PVDF membranes have been claimed to feature low protein binding, and thus they have been widely used for filtering biological samples targeted to protein and peptide analysis. Four types of PVDF syringe filters from different suppliers were thus selected for comparison. First, all 5 proteins were tested at 0.5 mg/mL to check their suitability for different proteins, and the results are shown in Figure 2. Clearly, the Captiva Premium PES syringe filter provided superior recoveries (> 97%) over other PVDF syringe filters for all proteins, and for dimers and monomers, as long as they could be separated chromatographically. This result was not true for all of the PVDF syringe filters. In particular, poor syringe filters caused significant loss of myoglobin and cytochrome C.

Subsequently, myoglobin was selected to investigate filtration recovery consistency over different levels. Four levels of myoglobin solution, 0.1, 0.2, 0.5, and 1 mg/mL, were prepared and used for evaluation. Figure 3 shows the stacked filtration recovery values of each myoglobin concentration for different syringe filters. The average recovery and RSD is shown at the top of each column. For the Captiva Premium PES syringe filter, excellent and consistent recoveries were obtained through low to high levels for dimer and monomer. All of the other PVDF syringe filters gave very poor recoveries at low levels (0.1 and 0.2 mg/mL), especially for dimer. The results demonstrate that Captiva Premium PES filters can firmly support the analysis of low level protein samples with minimal loss during filtration.

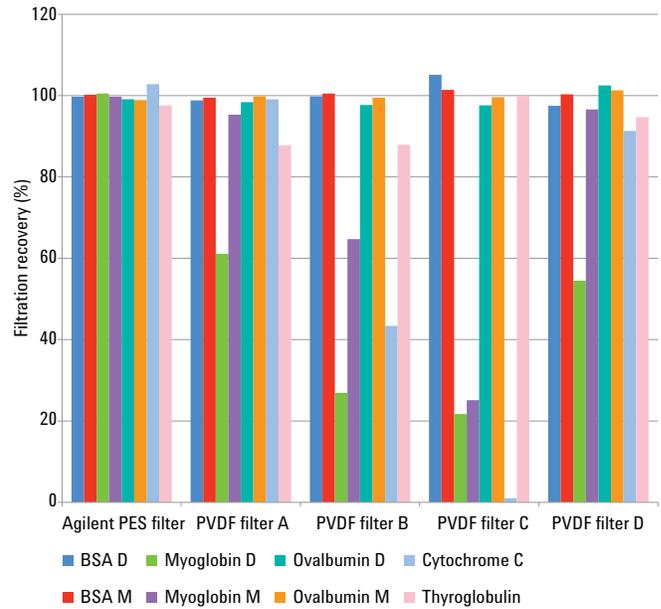


Figure 2. Filtration recovery comparison of the Agilent Captiva Premium PES syringe filter with PVDF syringe filters for different proteins at 0.5 mg/mL. D: Dimer, M: Monomer.

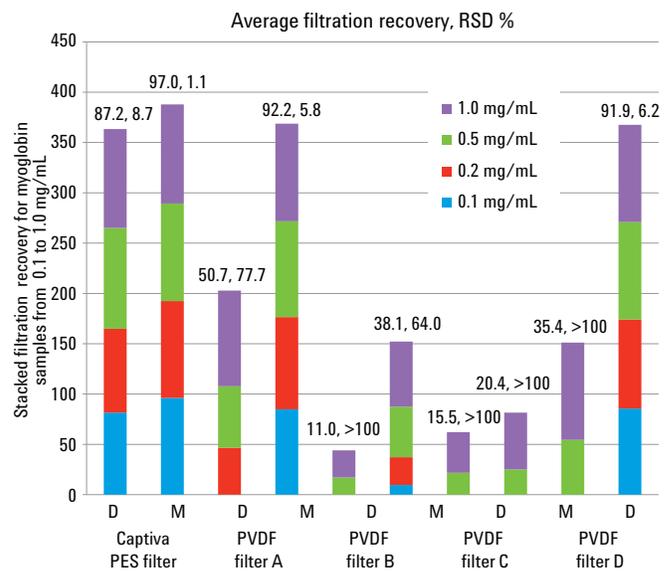


Figure 3. Filtration recovery comparison of the Agilent Captiva Premium PES syringe filter with PVDF syringe filters for myoglobin samples at different concentrations. D: Dimer, M: Monomer.

Captiva Premium PES versus other PES syringe filters

The previous comparison demonstrated the better suitability of Captiva Premium PES syringe filters for protein and peptide filtration than PVDF syringe filters. Do PES syringe filters from different suppliers always perform similarly? To answer this question, 3 non-Agilent PES syringe filters were compared with the Captiva Premium PES syringe filter. Similar tests were performed as above, and the results are shown in Figures 4 and 5.

The results show that PES syringe filters from different suppliers can perform very differently. Again, Captiva Premium PES syringe filters stand out in providing excellent and consistent filtration recovery for different varieties of proteins and for different concentrations of protein. Other PES syringe filters usually caused analyte loss for 'sticky' proteins, especially at low concentrations.

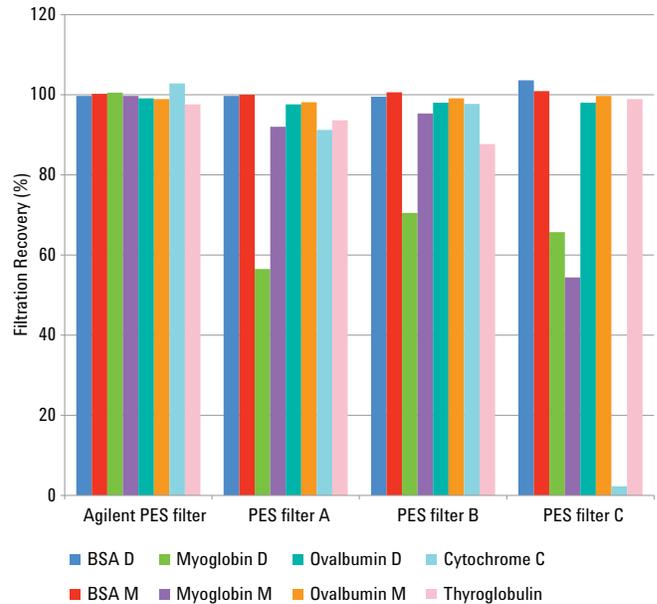


Figure 4. Filtration recovery comparison of the Agilent Captiva Premium PES syringe filter with non-Agilent PES syringe filters for different proteins at 0.5 mg/mL. D: Dimer, M: Monomer.

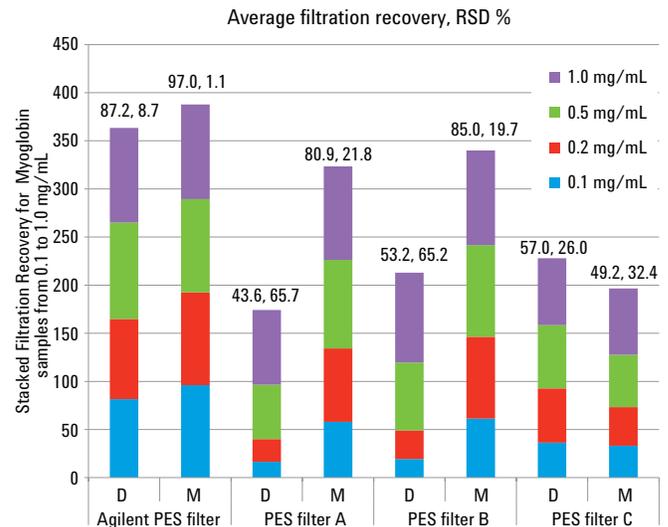


Figure 5. Filtration recovery comparison of the Agilent Captiva Premium PES syringe filter with non-Agilent PES syringe filters for myoglobin at different concentrations. D: Dimer, M: Monomer.

Chemical cleanliness

PES membranes have limited compatibility with organic solvents, and so PES syringe filters are more suitable for aqueous based or low percentage organic sample filtration. For protein and peptide analysis, samples are usually aqueous based or contain less than 30% methanol. Therefore, a 30% MeOH solution was used for the PES syringe filter chemical cleanliness evaluation and comparison. An LC/MS single quadrupole system under positive and negative mode was used to screen potential extractables from filtrates. Because the extractables were more evident in positive mode, and negative chromatograms were basically clean, the discussion focuses on a comparison of positive chromatograms.

Figure 6 shows the filtrate positive chromatograms for Captiva Premium PES and other non-Agilent PES syringe filters. Captiva Premium PES syringe filters were clearly the cleanest in comparison to non-Agilent PES syringe filters and provided identical chromatograms to the unfiltered sample. Non-Agilent PES syringe filters showed low to high interference peaks in the filtrate chromatograms, indicating that they introduced impurities into the samples and thus caused contamination.

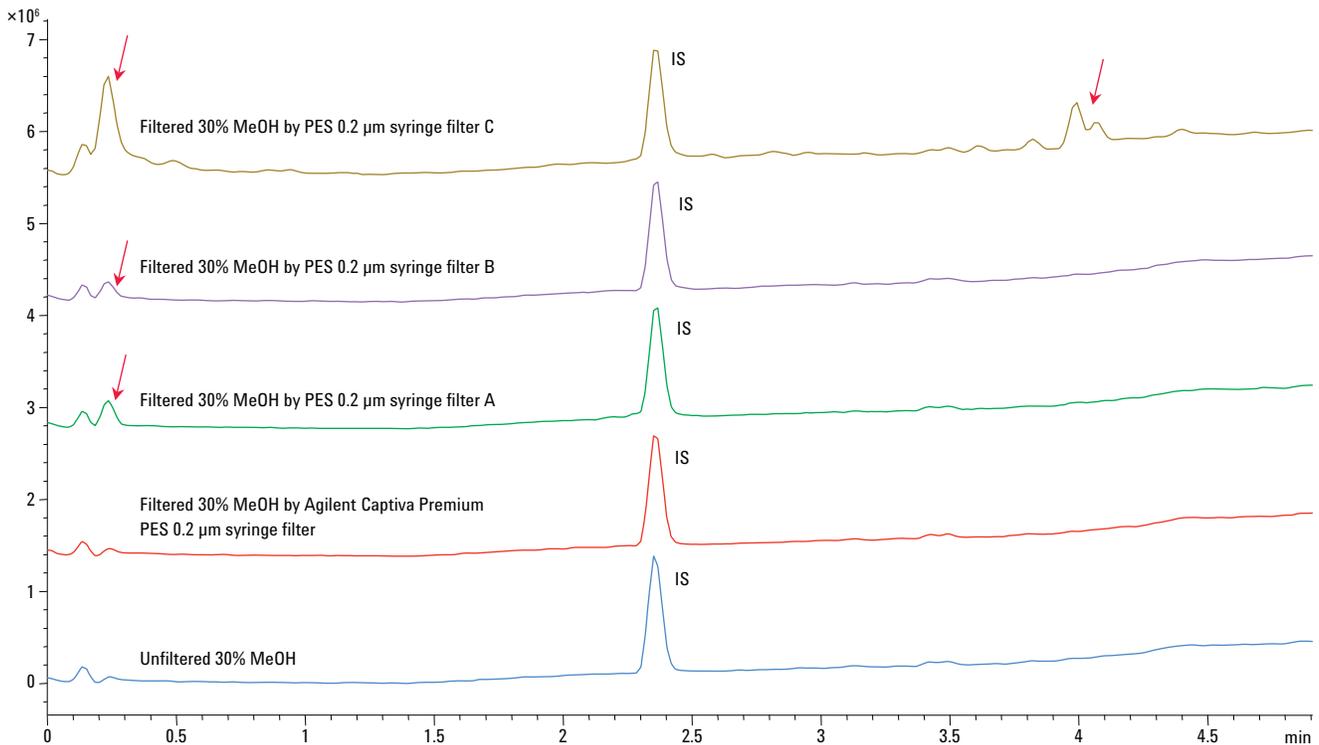


Figure 6. Filter cleanliness comparison of the Agilent Captiva Premium PES syringe filter with non-Agilent PES syringe filters using LC/MS under positive mode.

Figure 7 shows the filtrate positive chromatograms for Captiva Premium PES syringe filters and other PVDF syringe filters comparison.

In general, PVDF syringe filters cause contamination to samples during filtration. For the common UV/Vis detector used in protein analysis, extractables may not be observable due to limited detector sensitivity and selectivity, but they can still cause various uncertainties for accurate and precise protein analysis. Therefore, it is critical to use the cleanest filter to prevent the introduction of any contamination during sample preparation. Captiva Premium PES syringe filters were clearly demonstrated as the best option.

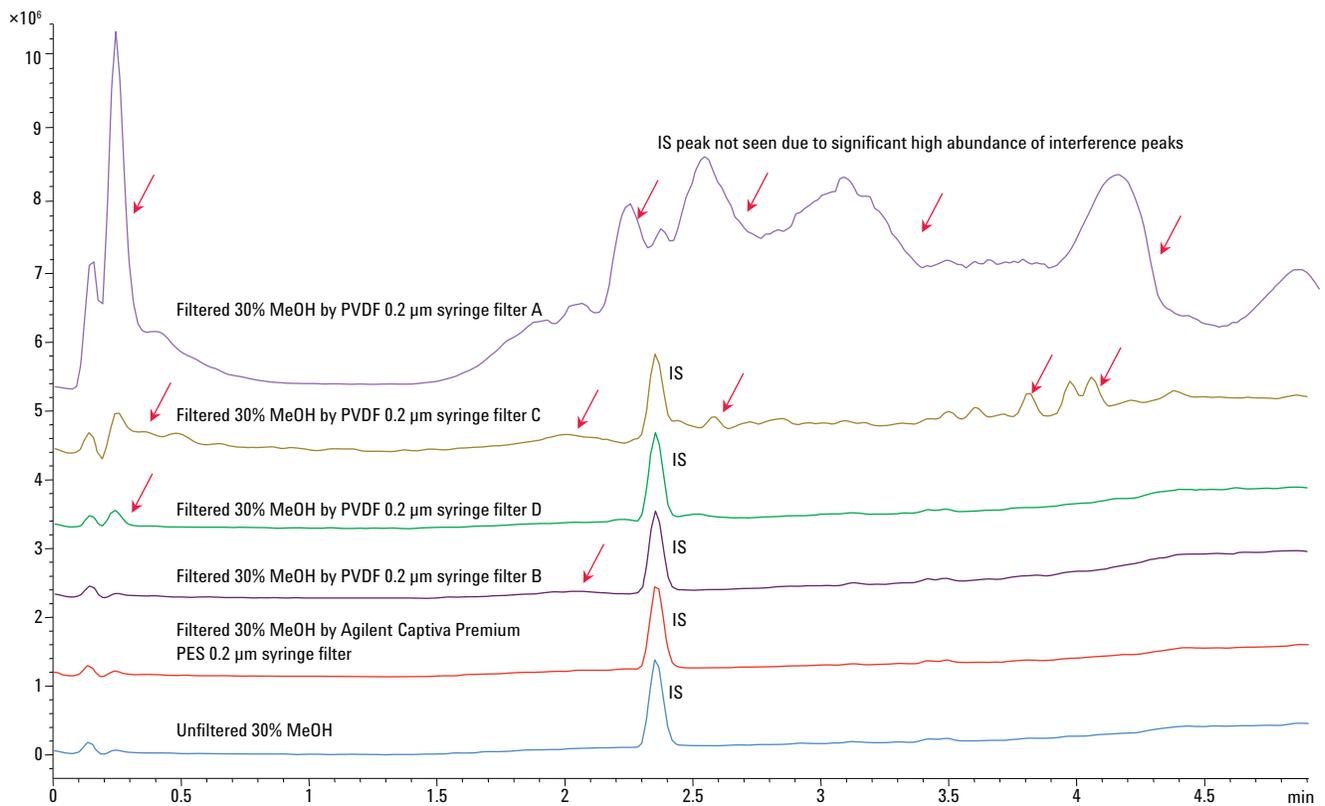


Figure 7. Filter cleanliness comparison of the Agilent Captiva Premium PES syringe filter with non-Agilent PVDF syringe filters using LC/MS under positive mode.

Conclusions

Agilent Captiva Premium PES syringe filters were evaluated and compared with other PES and PVDF syringe filters for filtration recovery, targeted to protein analysis and chemical cleanliness. For protein analysis, Captiva Premium PES syringe filters provided excellent and consistent filtration recovery for different proteins at low to high concentrations. Compared to other PES and PVDF syringe filters, Captiva Premium PES syringe filters clearly featured extremely low protein binding, thus significantly preventing protein analyte loss during filtration. In addition, they were also cleaner than other syringe filters, and therefore, did not cause chemical contamination during filtration. Captiva Premium PES syringe filters are the best option for the filtration of biological samples.

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