

July 23, 2020

Keywords or phrases:

Leachables, membrane filter discs, eluent filtration, HPLC, HRMS

Do Filter Membranes Release Leachable Compounds into the Mobile Phase Following Vacuum Filtration?

Klaus Schöne^{1*}, Stephanie Schweizer¹, Tanja Verena Maier^{2*}, Roberto Menzel²

1. Sartorius Lab Instruments GmbH & Co.KG, Otto-Brenner-Str. 20, 37079 Göttingen, Germany

2. Sartorius Stedim Biotech GmbH, August-Spindler-Str. 11, 37079 Göttingen, Germany

* Correspondence

E-Mail: tanja.maier@sartorius.com, klaus.schoene@sartorius.com

Abstract

Vacuum filtration of eluents is a common practice to protect chromatographic columns from particles. Leachables, from components that a solvent comes in contact with, can cause interference during chromatographic analysis. Sartorius membrane filter discs were therefore tested to determine if leachable compounds are detected in the eluents following filtration of the solvents through these filters. The solvents - pure water, a phosphate buffer, and pure acetonitrile were used to simulate typical eluents to be filtered. The most frequently used membrane types, regenerated cellulose (RC) and polyamide (PA), with a pore size of 0.2 µm were tested as filter materials. While the RC filter showed no difference to the reference, we found small peaks for the PA filter using HPLC-UV/VIS and HRMS. The observation was supported by measurement of the total organic carbon (TOC) of the water extracts. Overall, the amount of leachables is very low. Therefore, it can be concluded that for most applications the filter discs made of both RC and PA are well-suited for the filtration of eluents.

Introduction

With the development of chromatographic methods based on densely packed columns, as used in HPLC or UHPLC, the chromatographic performance - the separation of analytes - significantly improved compared to packed columns and is now an indispensable part of routine analysis. However, the high packing density of the stationary phase using 5 µm or smaller silica particles cause the problem that the columns are extremely sensitive to particulate impurities. Clogging the stationary phase reduces the quality of the results and can lead to down time of the analytical system generating costs. This risk is minimized by using various partially combined techniques such as inlet filters, guard columns, as well as syringe filters for sample filtration or membrane discs for buffer filtration. In this note we want to highlight the latter technique where the aqueous and/or organic mobile phase is filtered by vacuum using a filter holder and an inserted membrane disc with a pore size of 0.2 µm. This type of eluent filtration is particularly widespread in cost-efficient routine analysis. It removes particulate impurities, such as precipitates or dust particles, from the respective solvent and at the same time many users see an advantage in degassing the solvent simultaneously by the applied vacuum (Ref. 1, 2).

Nevertheless, this type of filtration involves contact between the respective eluent and a membrane. There is a risk that compounds can leach from the membrane and produce undesired interference peaks or increase the background noise and even create an "out-of-specification" situation.

To investigate the influence of vacuum membrane filtration on the release of compounds into the respective eluent, two typical membrane filter materials, regenerated cellulose and polyamide (Nylon), were selected. Commonly used solvents such as pure water, acetonitrile and NaH₂PO₄ buffer were filtered with both filter materials. The released compounds into the filtrates were analyzed by HPLC-UV/VIS, TOC and high-resolution mass spectrometry (HRMS) and then compared with the respective reference.

Methods

Two different Sartorius membrane types - regenerated cellulose (RC, 18407, lot 1704923) and polyamide (25007, lot 1852193) - with a pore size of 0.2 µm were used. The performance of selected membranes used for the filtration of eluents is tested by filtering different model solutions through membranes using a glass filter holder (All-Glass Vacuum Filter Holder, 16309). The filtered solutions were tested as eluents using HPLC-UV/VIS (pure water and NaH₂PO₄ buffer) and LC-HRMS (Acetonitrile) in direct infusion mode to generate an eluent-specific mass chromatogram. Acetonitrile (p.a. Honeywell or LC grade) was purchased from Sigma-Aldrich. Pure water (RO water) was used as eluent and to prepare a 10 mm NaH₂PO₄ buffer solution for HPLC-UV/VIS analyses.

In addition, the TOC of pure water filtered through the RC or the polyamide membrane was analyzed.

TOC analyses were performed on a Shimadzu TOC-V CSH (Autosampler: ASI-V). In total, 350-400 mL of pure water (TOC < 50 ppb) was filtered through the filter holder equipped with the respective membrane. Additionally, solutions were passed through the filter holder without a filtration membrane. These controls are referred to as the blank.

System used for high resolution mass spectrometry (HRMS) analyses was a Xevo G2-XS ESI-QToF mass analyzer from Waters. MS parameters: mass range m/z 50-1500; source temperature 120°C; cone gas flow 50 L/h, desolvation gas temperature 550°C and flow 950 L/h; collision mode: ramp from 15 to 40 eV, analysis run in ESI negative and ESI positive ionization mode. Software Data processing was UNIFI™ v1.8.2.

The HPLC-UV/Vis measurements were carried out with an Agilent 1200 Infinity equipped with a VWD detector. An artificial test sample of pure water was injected. Gradient separation was performed on a Nucleosil C18 column (5 µm x 250 mm x 4.6 mm, 100 Å) with a Nucleosil 100-5 C18 guard column (5 µm x 3 mm, 100 Å) in front. Solvents as eluents for the test were A) pure acetonitrile and B) pure water or NaH₂PO₄ buffer filtered through the membrane disc holder. For HPLC-UV/VIS analyses acetonitrile was not filtered. Conditions were as follows: flow 1 mL/min, column temperature 40°C, injection volume 20 µL and detection wavelength 220 nm. The gradient used is shown in Table 1.

Table 1: Gradient Parameters for HPLC-UV/VIS

HPLC-UVV/Vis Method at 220 nm	
0 - 10 min	isocratic 10/90 acetonitrile/aqueous
10 - 35 min	gradient 40/60 acetonitrile/aqueous
35 - 70 min	isocratic 90/10 acetonitrile/aqueous

Results and Discussion

Three different analytical methods were used to detect and analyze compounds that potentially leach from the membrane filter disc. The value provides a first estimate of the organic contamination in the sample. HPLC-UV/VIS analysis is used to investigate the influence of different membranes on eluent filtration under realistic conditions in routine analysis. In addition, high-resolution mass spectrometry (HRMS), a high sensitivity detection method, was applied to generate an eluent-specific mass chromatogram and to be able to identify possible “contaminants” from their mass signals.

The TOC values of the reference as well as both eluent extracts were at the same range below ≤ 200 ppb (ng/mL) which indicates a very low level of organic contamination. It was slightly higher for the filtrate from the PA membrane indicating a slightly higher organic content in these samples.

Table 2: Gradient Parameters for HPLC-UV/VIS

Sample	TOC [ng/mL]
Reference	125
0.2 μm Regenerated Cellulose	150
0.2 μm Polyamide	200

The results of the HPLC-UV/Vis analyses of the model eluents are depicted as chromatograms in Figure 1 for the NaH_2PO_4 buffer (top) and for the pure water eluent (bottom).

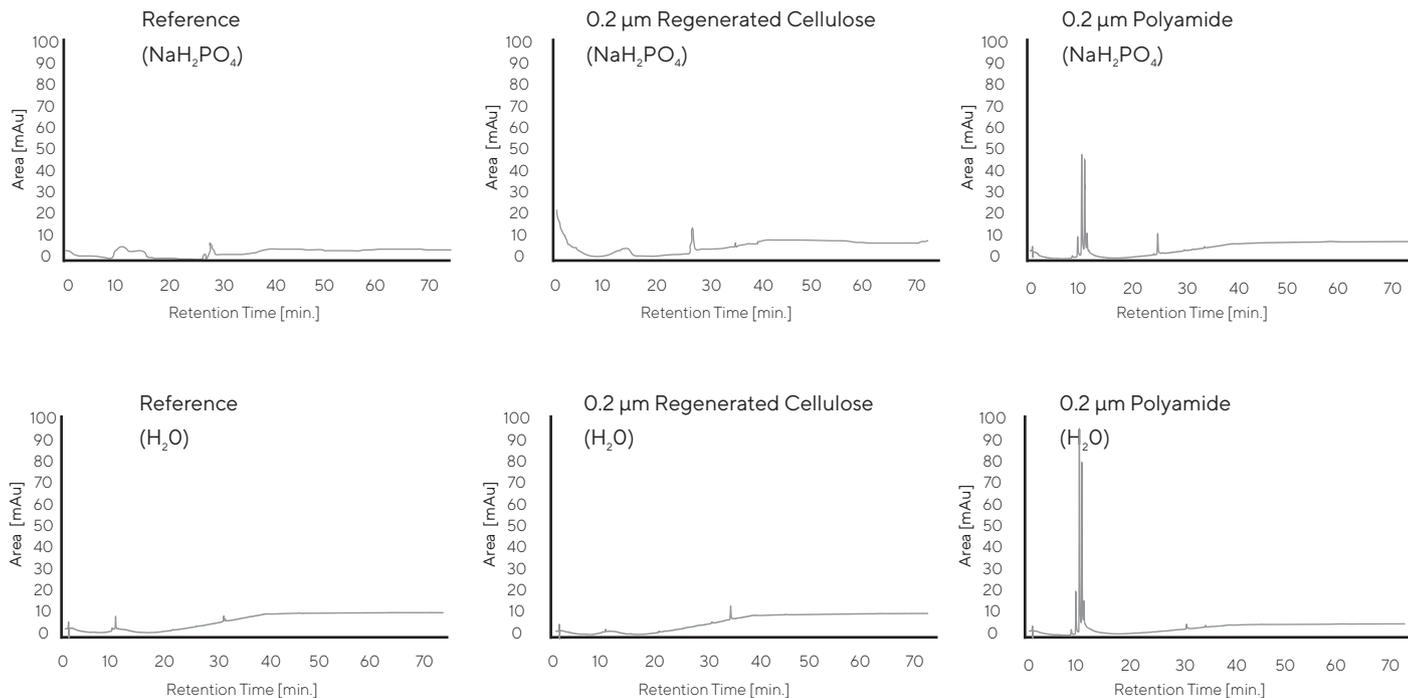


Figure 1: Top: HPLC-UV/VIS chromatogram of the NaH_2PO_4 buffer samples filtered through the glass filter holder w/o membrane (left), the 0.2 μm Regenerated Cellulose (middle) and 0.2 μm Polyamide membrane (right). Bottom: HPLC-UV/VIS chromatogram of the water samples filtered through the glass filter holder w/o membrane (left), the 0.2 μm Regenerated Cellulose (middle) and 0.2 μm Polyamide membrane (right).

In general, the chromatograms revealed a very low chromatographic background and low level of contaminants. It was observed, that the NaH_2PO_4 buffered eluent filtered through the polyamide membrane showed some peaks at the beginning of the gradient separation, indicating the presence of organic compounds, which were identified as polyamide oligomers. This result is quite plausible and confirms the slightly higher TOC values of the water samples of this membrane. In general, both membrane filters are well suited for eluent filtration. However, it is suggested to use the polyamide membrane in HPLC-applications with a gradient starting with a higher organic solvent composition. This reduces a potential pre-concentration of these polyamide oligomers minimizing the UV signals in the beginning of the chromatogram. This should solve a possible overlap of eluent-related and analyte signals.

Using high-resolution mass spectrometry the effect of acetonitrile on the two membranes was tested since it represents a critical organic solvent with strong extraction propensities. Testing the NaH_2PO_4 buffer solutions was not possible because of the background signals from the buffer itself. The resulted mass spectra of the filtered acetonitrile are depicted in Figure 2.

In general, there were only minor differences between all mass spectra of the eluents and references. The spectra for the two membrane filtrates are equivalent and as low as the reference in the ESI negative ionization mode. This corresponds to common solvent background signal.

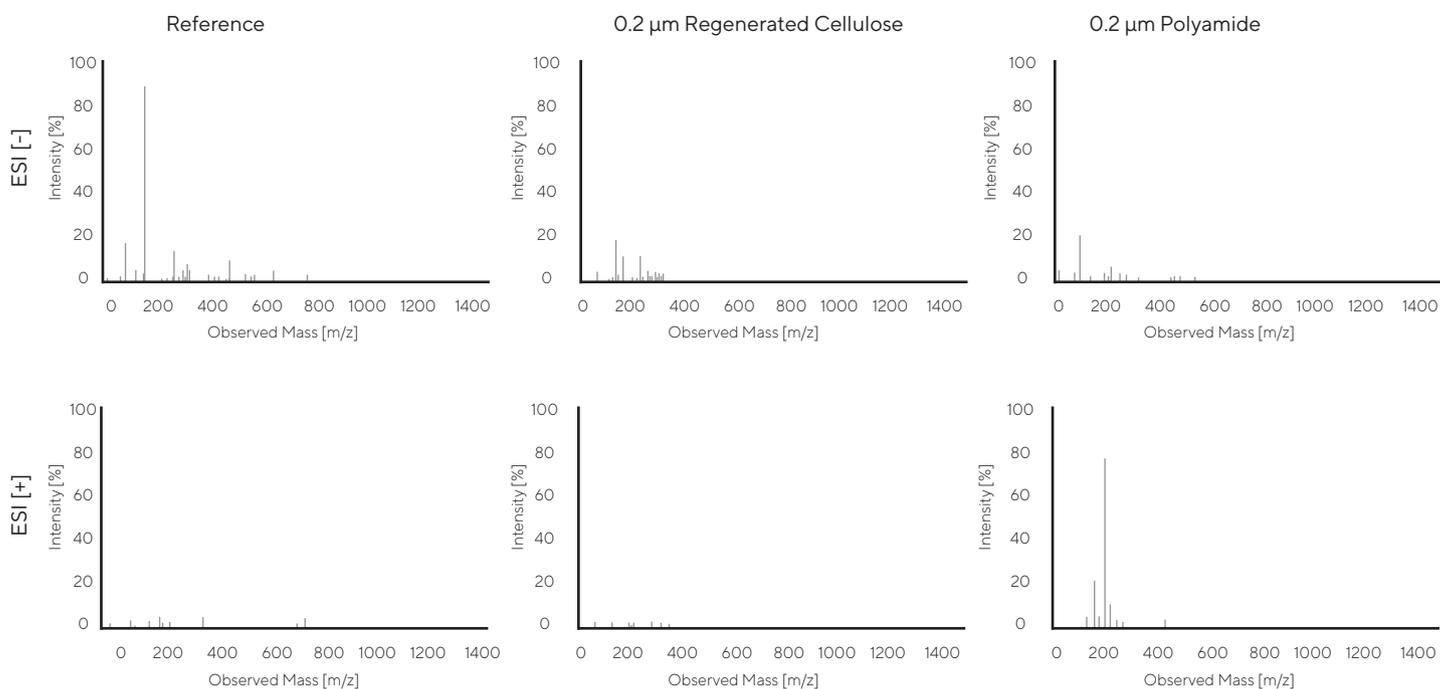


Figure 2: Comparison of two eluents filtered through two types of membranes using high-resolution mass spectrometry. Direct infusion high-resolution mass spectra of the reference sample (right), 0.2 μm regenerated cellulose sample and 0.2 μm polyamide sample (left) in ESI [-] (top) and ESI [+] (bottom) ionization mode. Mass spectra were scaled to $2.5 \cdot 10^6$ (ESI [-]) and $1.5 \cdot 10^7$ (ESI [pos]), mass range is m/z 50 – m/z 1500.

The mass spectra of the acetonitrile filtrates obtained in ESI positive mode showed some small additional mass signals for the PA membrane which might be assigned to polyamide oligomers. This is in alignment with the TOC and HPLC-UV/VIS results of this filter disc material. However, it should be noted that the mass signals were recorded from the filtered solution itself. In routine chromatographic separation, the influence of these additional mass signals will be low since they are eluted in the early fraction and can be separated from analyte signal by mass spectrometry.

Summary and Conclusion

We assessed the likelihood of two filter materials, RC and PA, releasing organic compounds following the filtration of solvents/ eluents that are routinely used in liquid chromatography. No additional peaks were present in HPLC-UV/VIS analysis using pure water and phosphate buffer filtrated eluents using regenerated cellulose filter discs. Small early eluting peaks, probably due to polyamide oligomers, were detected for polyamide filters disc in HPLC-UV/VIS but also in mass screening of the eluent in LC-HRMS. The TOC results confirmed that regenerated cellulose filters released no measurable organic content into the filtrate of pure water. Polyamide filters might release some small level of organics which should be considered in their application and can be reduced by adjusting the gradient, for example.

Based on the results it can be concluded that both regenerated cellulose and polyamide filter membranes are well-suited for the filtration of aqueous or organic mobile phases in liquid chromatographic applications. Regenerated cellulose filters are the first choice in eluent filtration due to an exceptionally low release of organic compounds and an excellent compatibility with most solvents. Polyamide filter discs are an ideal alternative and can be used for eluents with high or low pH. The release of polyamide oligomers is known and should be considered HPLC-UV/VIS method development.

References

1. Terri Thompson, Tips for Preparing and Using Buffers with Your Chromatography System, March 14, 2016, <https://www.biocompare.com/Bench-Tips/183975-Tips-for-Preparing-and-Using-Buffers-with-Your-Chromatography-System/>
2. Michael W. Dong, Modern HPLC for Practicing Scientists, 2006, p. 115f, ISBN-13: 978-0-471-72789-8

Abbreviations

RC	Regenerated cellulose
PA	Polyamide (Nylon)
(U)HPLC	(Ultra) high performance liquid chromatography
UV/VIS	Ultraviolet-visible spectrophotometry
HRMS	High-resolution mass spectrometry
ESI	Electrospray ionization
TOC	Total organic carbon

Sales and Service Contacts

For further contacts, visit
www.sartorius.com

Germany

Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone 1.631.254.4249