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HPLC sample preparation – a study using Whatman™ brand filtration devices

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Sample preparation methods for HPLC analysis have the potential to influence analytical results. Disposable devices can alter the sample in several ways. For example, they can release extractables into the sample, bind analytes nonspecifically, or retain liquid. This white paper presents experimental data for several of GE Healthcare's syringe and syringeless filters evaluated on these properties. All tested devices contained membranes composed of regenerated cellulose, which has broad compatibility for aqueous and organic solvents. The results support the use of the tested devices for typical HPLC sample preparation needs. Further, the data supports GE's recommendation that users consider standardizing on a membrane such as RC across multiple encapsulated formats. Standardization reduces the variation associated with sample preparation on multiple membranes and increases lab process consistency.

Introduction

Prior to analytical high-performance liquid chromatography (HPLC) applications, samples are prepared by a variety of methods. All of these methods have the potential to influence analytical results by altering the sample injected into the system. For example, disposable devices could release extractable compounds into the sample, remove analytes, or remove solvent.

Filtration is one way to remove insoluble particles from liquid samples prior to HPLC analysis. This step helps to protect the column and extends the lifetime of both the injector and the column. Because a fundamental goal of many laboratories engaged in analytical testing is to develop consistent processes that underpin consistent results, GE Healthcare suggests that standardizing on a single type of filtration membrane is a way to contribute to process rigor. This white paper presents data on the filtration performance of the regenerated cellulose (RC) membrane, which is broadly compatible across many solvent types (see Table 5). This property makes RC a viable candidate for such standardization.

Considerations for choosing a suitable filtration device

Selection of a filtration device with an appropriate pore size ensures effective particulate removal while minimizing sample loss. A lack of interaction with analytes (e.g., by binding) is another consideration relevant to analytical HPLC.

Ensuring compatibility between the materials in the filtration device and the solvent will minimize the levels of extractable compounds that might interfere with analyte detection. One option for addressing this issue across the various solvents used in analytical laboratories is to choose a membrane that is broadly compatible with both aqueous and organic solvents. Regenerated cellulose is a material that meets this criterion. See the *Characteristics of regenerated cellulose* section for more information.

This white paper presents experimental data on the described properties to inform the selection of devices for pre-HPLC filtration. Several of GE Healthcare's syringe and syringeless filters with RC membranes (0.2 µm and 0.45 µm pore sizes) were evaluated to address the range of sample types and sampling methods used in analytical laboratories.

Table 1. UV absorbances (mAU) of solvents filtered through SPARTAN™ syringe filters, which contain RC membranes. Values for two samples from one lot are shown

Filtration device	Water		Methanol		Acetonitrile	
	215 nm	254 nm	215 nm	254 nm	215 nm	254 nm
SPARTAN 30 mm, 0.2 µm	2.5, 3.0	3.6, 2.1	2.1, 6.4	1.9, 4.4	0.6, 10.3	0.4, 2.8
SPARTAN 30 mm, 0.45 µm	10.8, 8.1	0.6, 2.3	8.2, 3.3	1.2, 6.2	3.4, 5.6	0.9, 2.9

Table 2. UV absorbances (mAU) of solvents filtered through SPARTAN 13 mm diameter, 0.2 µm pore size syringe filters, which contain RC membranes. Values for three devices from three different lot numbers are shown

	Water		Methanol		Acetonitrile	
	215 nm	254 nm	215 nm	254 nm	215 nm	254 nm
Lot A	1.2	0.3	2.2	0.7	0.7	0.7
Lot B	1	0.3	2.2	1.2	0.5	0.4
Lot C	0.9	0.4	4.5	2	1.3	1.4
Average	1.03	0.33	2.97	1.30	0.83	0.83
Standard deviation (SD)	0.12	0.05	1.08	0.54	0.34	0.42
Coefficient of variation (CV)	0.12	0.14	0.37	0.41	0.41	0.50

Testing for extractables

Methods and results

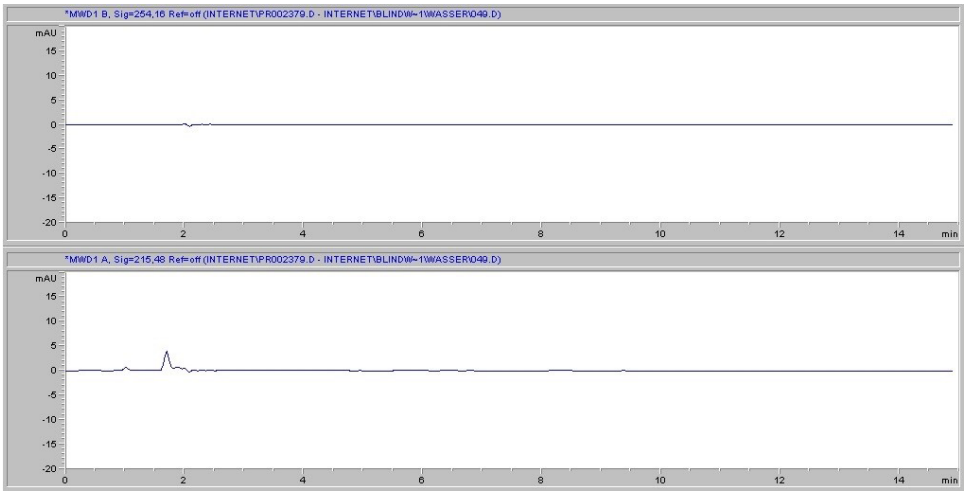
The aim of this evaluation was to establish extractable profiles for filtration devices with RC membranes when subjected to standard solvents used for HPLC analysis. These profiles will give an analytical testing lab confidence that their results accurately reflect the profile of the sample and that the contribution from the filter itself is minimal.

Three types of SPARTAN syringe filters were selected for evaluation – 30 mm diameter with 0.2 or 0.45 µm pore size, and 13 mm diameter with 0.2 µm pore size. Two devices from one lot were evaluated for each pore size of the 30 mm diameter filters. Three devices from three different lots were evaluated for the 13 mm diameter filters. Water, 100% methanol, and 100% acetonitrile were chosen as typical solvents. A 500 µL volume of each solvent was filtered separately through the individual filter devices. Each filtrate was collected and injected into a HPLC system. Analyses were performed by ultraviolet-visible (UV) spectrophotometry with measurements at 215 and 254 nm. Results are presented in Tables 1 and 2. Typical chromatograms for a SPARTAN syringe filter are shown in Figure 1.

Conclusion

The tested SPARTAN syringe filters demonstrated low levels of background extractables when challenged with standard solvents used in analytical HPLC. Low extractable levels were demonstrated through low peak heights at the wavelengths of interest and the lack of erroneous peaks in the chromatogram. Overall, these results indicate a high level of compatibility between the three solvents evaluated and the regenerated cellulose membrane. The data also suggests that SPARTAN devices will generate minimal interference with UV detection of biological analytes. In addition, where SPARTAN filters do contribute low levels of interference, that interference was demonstrated to be consistent, thus allowing a user to account for it in analysis.

(A) SPARTAN 30 mm, 0.2 µm – water



(B) SPARTAN 30 mm, 0.2 µm – methanol

(C) SPARTAN 30 mm, 0.2 µm – acetonitrile

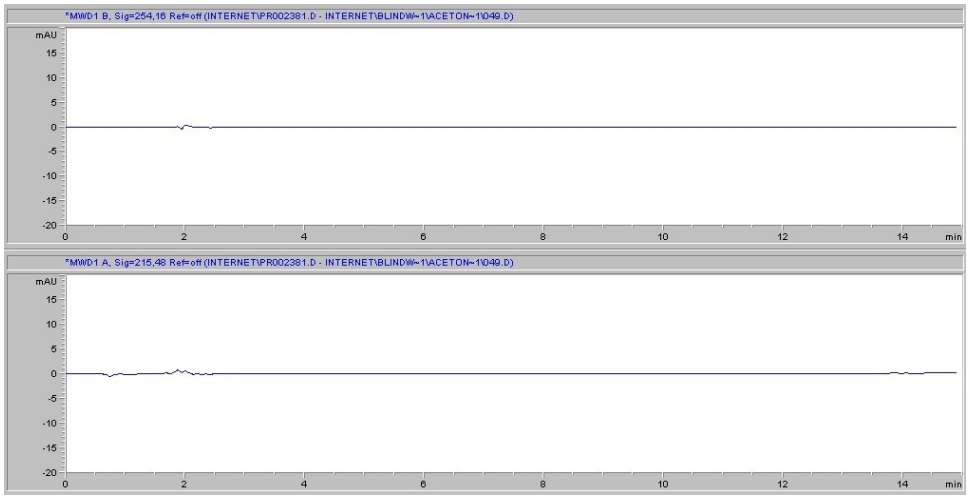


Fig 1. Representative chromatograms of solvents filtered through SPARTAN syringe filters and subjected to HPLC analysis. Top panel in each set of chromatograms shows results at 254 nm; bottom panel shows results at 215 nm.

Testing for nonspecific protein binding

Methods and results

The aim of this evaluation was to measure nonspecific protein binding of filtration devices containing RC membranes when challenged with a "typical" protein.

Four filtration devices with RC membranes were selected for evaluation – 13 mm diameter SPARTAN syringe filters with 0.2 and 0.45 µm pore sizes, 25 mm diameter Whatman GD/X™ syringe filters with 0.2 µm pore size, and Mini-UniPrep™ syringeless filters with 0.2 µm pore size. Bovine serum albumin (BSA) was selected as the typical protein. A 5 mg/mL stock was prepared in PBS buffer, pH 7.4, then prefiltered using a SPARTAN 30 mm, 0.2 µm syringe filter. The stock was diluted with PBS to prepare 0.5 mg/mL and 1 mg/mL solutions for evaluation.

For each type of fitration device, 1 × 2 mL of 0.5 or 1 mg/mL BSA was passed through the device in triplicate. Filtrates were collected into a 5 mL, 48-well UNIPLATE. For blanks, 1 × 2 mL PBS buffer was passed through each type of device in triplicate and collected in a UNIPLATE collection plate.

Triplicate aliquots (300 µL) of filtered and unfiltered sample solutions and filtered PBS blanks were transferred to a UV plate (Costar) and read at A_{280}. The amount of protein loss associated with filtration was then calculated. Results are presented in Table 3.

Table 3. Percent recovery of BSA after passing through filtration devices with RC membranes (N = 3)

Filtration device	Recovery at 1 mg/mL (%)	SD	Recovery at 0.5 mg/mL (%)	SD
SPARTAN 13 mm, 0.2 µm	98	0.6	97	0.3
SPARTAN 13 mm, 0.45 µm	99	0.7	99	0.9
GD/X 25 mm, 0.2 µm	86	1.9	78	2.2
Mini-UniPrep* 0.2 µm	95	0.9	94	2.5

SD = standard deviation, *Polypropylene housing

Conclusion

The tested devices, which all contained RC membranes, demonstrated low levels of nonspecific protein absorption when challenged with two concentrations of a model protein, BSA. These results suggest that these devices, including housings and membranes, will not substantially alter the concentration of protein analytes.

The higher nonspecific binding seen with the GD/X format is indicative of a multilayer filter designed for difficult-to-filter samples. Because full recovery is not expected with such samples, a user will need to weigh the acceptability of this loss against alternative methods of dealing with difficult-to-filter samples (e.g., using multiple syringe filters, which might also lead to sample loss).

Testing for hold-up volume

Methods and results

The aim of this evaluation was to assess the loss of sample resulting from the filtration process itself (i.e., the amount of sample retained in the device after filtration).

Three filtration devices with RC membranes were evaluated – 13 mm diameter SPARTAN syringe filters with 0.2 and 0.45 µm pore sizes and 25 mm GD/X syringe filters with 0.45 µm pore size. Deionized water was passed through each device. The volume of sample remaining in the device after air purge was determined by weighing a device before and after filtration. The lost (hold-up) volume was calculated. An air purge was then conducted on each device to remove as much of the remaining water as possible. The device was weighed again to give a final loss calculation. Results are presented in Table 4.

Table 4. Hold-up volumes of water in filtration devices containing RC membranes. Results before and after air purge

Filtration device	Hold-up volume (µL)		Final hold-up volume (µL)	
	Average	SD	Average	SD
SPARTAN 13 mm, 0.2 µm	135	36	9.3	0.9
SPARTAN 13 mm, 0.45 µm	135	31	9.2	1.1
GD/X 25 mm, 0.45 µm	1683	42	158	13

SD = standard deviation, N = 10

Conclusion

During filtration some loss of sample is expected due to the dead volume in the device itself. The data demonstrates low sample loss as a result of the hold-up volume, especially after an air purge. GD/X syringe filters have a comparatively high hold-up volume. Because full recovery is not expected with such samples, a user will need to weigh the acceptability of this loss against alternative methods of dealing with difficult-to-filter samples (e.g., using multiple syringe filters, which might also lead to sample loss).

For applications that do not require absolute quantitation of analyte or maximum yield, standard syringe filtration is appropriate. However, an air purge is recommended when it is important to maximize recovery.

Table 5. Solvent compatibility of regenerated cellulose

Solvent	Regenerated cellulose	Solvent	Regenerated cellulose	Solvent	Regenerated cellulose
Acetic acid	R	Cresol	R	Hydrofluoric acid	NR
Acetic acid, glacial	NR	Cyclohexane	R	Nitrobenzene*	R
Acetone	R	Cyclohexanone	R	Pentane	R
Acetonitrile	R	Diethylacetamide	R	Perchloroethylene	R
Ammonia, 6 N	LR	Dimethylformamide	LR	Phenol 0.5%	R
Amyl acetate	R	Dioxane	R	Pyridine	R
Amyl alcohol	R	DMSO	LR	Sodium hydroxide, 6 N	NR
Benzene*	R	Ethanol	R	Sulfuric acid, conc.	NR
Benzyl alcohol*	R	Ethers	R	Tetrahydrofuran	R
Boric acid	R	Ethyl acetate	R	Toluene*	R
Butyl alcohol	R	Ethylene glycol	R	Trichloroethane*	R
Butyl chloride*	R	Formaldehyde	LR	Trichloroethylene*	R
Carbon tetrachloride*	R	Freon	R	Water	R
Chloroform*	R	Formic acid	LR	Xylene*	R
Chlorobenzene*	R	Hexane	R		
Citric acid	R	Hydrochloric acid, conc.	NR		

R = resistant, LR = limited resistance
NR = not recommended
* = Short-term resistance of housing

Characteristics of regenerated cellulose

Regenerated cellulose (RC) provides broad compatibility for aqueous and organic solvents, including those commonly used in analytical HPLC (Table 5).

GE's regenerated cellulose is produced from pure cellulose without the addition of wetting agents. The starting polymer is cellulose acetate, which is solubilized and cast into a uniform, reproducible membrane. This membrane is then hydrolyzed to remove the acetate, which returns it to the fundamental cellulose structure. The robust formulation of the RC membrane produces a hydrophilic membrane with spontaneous wetting properties and compatibility with both organic and aqueous solutions. The membrane is mechanically stable with good wet strength. GE devices that include an RC membrane can be sterilized.

Summary

Several properties of filtration devices are important to consider when choosing a product suitable for HPLC sample preparation. A selection of GE's syringe and syringeless filters were evaluated against these properties. Devices with regenerated cellulose (RC) membranes were chosen based on the broad compatibility of RC with a wide range of aqueous and organic solvents. The tested devices provided low levels of UV-absorbing extractables after exposure to typical HPLC solvents. Overall, nonspecific protein absorption and sample retention in the device were determined to be low. The acceptability of the comparatively higher values for the device designed to handle difficult-to-filter samples will have to be weighed by a user against alternative methods of dealing with such samples (e.g., using multiple syringe filters, which might also lead to sample loss).

The data presented here supports the use of GE's syringe and syringeless filters containing RC membranes for typical HPLC sample preparation needs. Because of its broad solvent compatibility, regenerated cellulose is expected to be compatible with a wide range of solvents used to prepare samples in an analytical laboratory. GE also recommends that users consider standardizing on a membrane such as RC across multiple encapsulated formats. Standardization reduces the variation associated with sample preparation on multiple membranes and increases lab process consistency.

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