

## Application Note

# Low-extractable Millex Smplicity® filters are ideal for preparing samples for sensitive HPLC detectors

The Smplicity® filtration system was developed to filter up to 8 samples directly into standard HPLC vials using vacuum-based filtration. The samples can then be used in downstream HPLC analysis. Given the high sensitivity of high performance liquid chromatography (HPLC) and ultra-high pressure liquid chromatography (UHPLC) analyses, it is critical to ensure that the filtered sample is not contaminated with extractables.

This application note reports the results of tests for extractables from Millex Smplicity® LCR (0.45 µm hydrophilic PTFE) and LG (0.20 µm hydrophilic PTFE) filters using three commonly used HPLC detectors. The detectors used were an ultraviolet/visible (UV-Vis) spectrophotometer, a mass spectrometer and a fluorescence detector.

## Materials and Methods

**Smplicity® Filtration System.** 0.45 µm and 0.2 µm Millex Smplicity® hydrophilic PTFE filters (Cat. Nos. SAMPLG001 and SAMPLCR01) were used with the Smplicity® filtration system (Cat. No. SAMPSYSGR (green) or SAMPSYSBL (blue)).

**Extraction.** Four or eight Millex Smplicity® filters were set on the Smplicity® filtration system and 1 mL of appropriate solvent (Milli-Q® water, acetonitrile (Cat. No. A955-4), methanol (Cat. No. A466-4), tetrahydrofuran (Cat. No. T425-1) or acetone (Cat. No. A949-4)) was added to the filter funnel. Sample was filtered and collected in standard (12 X 32 mm) HPLC vials. This extract was collected and labeled as the 1st mL extract. An additional 1 mL of solvent was then added to the same filters and extracts were collected in another set of clean

standard HPLC vials. This extract was labeled as the 2nd mL extract. The extracts were analyzed by HPLC separation followed by the various detectors.

**HPLC.** Samples were injected (100 µL per injection) and resolved on a Waters µBondapak® C18 column, 3.9 mm x 300 mm, 10 µm, 125 Å. Mobile phase A was Milli-Q® water, and mobile phase B was acetonitrile. Flow rate was 2 mL/min. HPLC gradient profile is shown in Table 1.

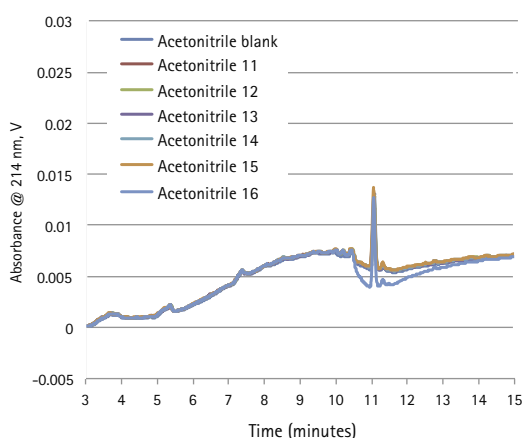
Time, min	% A	% B
0	100	0
10	0	100
13	0	100
16	100	0
20	100	0

Table 1.  
HPLC gradient profile.

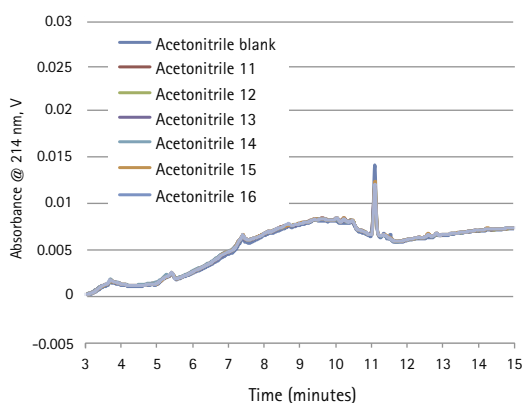
**Table 2.**  
Mass spectrometry detector  
settings

Q3 Scan	100–1500 m/z in 5 seconds
Source	Turbo ion spray
Curtain gas	20
Ion spray voltage	5000
Temp	OFF
Ion Source gas 1	20
Ion Source gas 2	0
Interface heater	ON
De-clustering potential	20
Focusing potential	200
Entrance Potential	10.0

**Figure 1.**  
HPLC-UV extractables pro-  
file for Millex Smplicity®  
0.20 µm Hydrophilic PTFE  
filters (acetonitrile as ex-  
traction solvent). The various  
traces represent extracts  
from replicate filter units.



**Figure 2.**  
HPLC-UV extractables pro-  
file for Millex Smplicity®  
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**UV Detection.** UV radiation-absorbing extractables were detected using a Waters 2489 UV Detector, Model # 87E, with three detection wavelengths: 214 nm, 254 nm and 280 nm.

**Fluorescence Detection.** Fluorescent extractables were detected using a Waters 2475 Fluorescence Detector, Model # 475. Excitation wavelength was 490 nm; emission wavelength was 525 nm.

**MS Detection.** Mass spectrometry of extracted samples was performed using a AB SCIEX API 2000™ Triple Quad MS/MS Turbo Spray source, using ESI + conditions. Detailed settings are listed in Table 2.

## Results and Discussion

**UV Extractables Analysis.** The Smplicity® system is validated for sample preparation for HPLC. Therefore, Millex Smplicity® 0.45 µm and 0.20 µm PTFE filters are certified for HPLC cleanliness using standard HPLC-UV analysis. The HPLC certification ensures users that the filter will not introduce any extractable impurities into the sample. Representative LC-UV chromatograms show that no detectable extractables were leached from Millex Smplicity® 0.45 µm and 0.20 µm PTFE filters using acetonitrile as an extraction solvent (Figures 1 and 2). Analysis of acetonitrile extracts using multiple replicate filter units yielded superimposable traces, showing that the results were highly reproducible.

The peak shown at ~ 11 min. is present in blank acetonitrile as well, and hence it is not considered an extractable peak originating from the Millex Smplicity® filters. Of the 3 wavelengths tested, typically the lowest wavelength (214 nm) shows the most absorbance. Similar UV extractables profiles were seen when using Milli-Q® water or methanol as extraction solvents.

**LC-MS Extractables Analysis.** LC-MS has not only become an extremely common analysis technique in pharmaceutical and biotechnology laboratories, but it is also being used in various other industries. Mass spectrometry offers highly sensitive and specific analysis of very low concentration analytes present in the sample. This means that any extractables that show up in the sample will affect the analysis and impact quantitation of analytes.

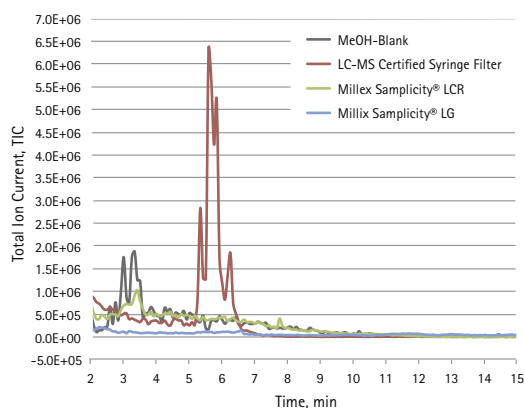
Determining MS extractables can be very challenging, because the conditions under which extractables are measured greatly affect the calculated level of extractables. In this study, we only used the ESI + ionization mode, and therefore may have missed some extractables which are only ionizable under ESI – / APCI ionization mode or are completely non-ionizable. We also compared the LC-MS extractables results with those of a commercially available MS-certified syringe filter (non-Merck Millipore) that is reported to have very low levels of extractables.

With water and acetonitrile as extraction solvents, all the three filters showed very similar levels of low extractables; however, with methanol as extraction solvent, the LC-MS certified syringe filter showed a much higher level of extractables, which can affect the downstream LC-MS analysis. Table 3 shows the highest observed total ion current for a given solvent and filter type; values were representative of 2-4 replicate filter units tested. Figure 3 shows the average LC-MS methanol extractables profile for each of the filters tested as well as methanol blank.

**HPLC-Fluorescence Detection.** Next to UV detection, fluorescence detection is the second most frequently used detection method used in HPLC analysis. Fluorescence detectors are very selective and sensitive, enabling detection and quantitation of analytes at levels close to few ng/mL with very low background noise. What this means from a sample preparation perspective is that the filter used for preparing samples prior to HPLC analysis should introduce minimal extractable impurities as detected by a fluorescence detector. Similar to the challenges with LC-MS, fluorescence intensity varies based on the excitation and emission wavelengths. In this study, we decided to choose the excitation and emission wavelengths that correspond to fluorescence properties of fluorescein. The emission efficiency of this fluorochrome is very high, facilitating highly sensitive detection.

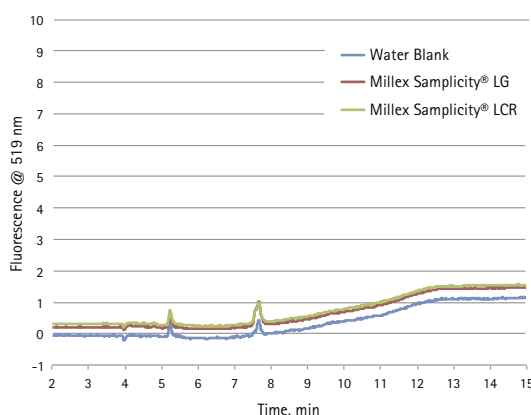
Five different solvents were selected for extractables analysis using HPLC-fluorescence. Both Millex Smplicity® 0.45 µm and 0.20 µm PTFE filters exhibited very low levels of extractables when using fluorescence detection following HPLC analysis (Figures 4 and 5).

	Water	Acetonitrile	Methanol
LC-MS-certified syringe filter	2x10 <sup>6</sup>	2 x10 <sup>5</sup>	2.5 x10 <sup>7</sup>
Millex Smplicity® 0.45 µm PTFE	3 x10 <sup>6</sup>	3 x10 <sup>5</sup>	1 x10 <sup>6</sup>
Millex Smplicity® 0.20 µm PTFE	2.5 x10 <sup>6</sup>	1 x10 <sup>5</sup>	5 x10 <sup>5</sup>

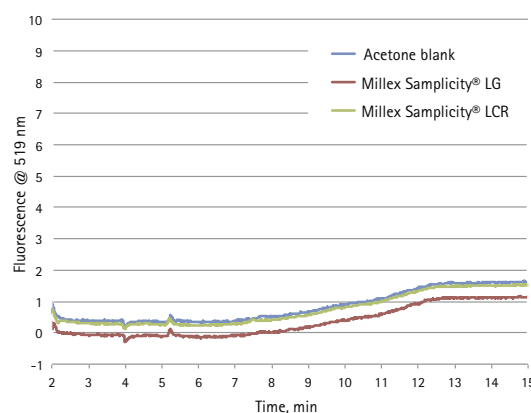


**Table 3.** Maximum observed total ion current based on LC-MS extractables profile. The most extractables were seen with LC-MS-certified syringe filters (highlighted box).

**Figure 3.** Average total ion current for methanol extractables showing very high level of extractables from LC-MS certified syringe filters. In comparison, both the Millex Smplicity® 0.45 µm and 0.20 µm PTFE show very low levels of LC-MS extractables, providing cleaner samples for downstream analysis.



**Figure 4.** Fluorescence intensity with water as extraction solvent.



**Figure 5.** Fluorescence intensity using acetone as extraction solvent.

Additional fluorescence data were also generated using methanol, acetonitrile and THF as extraction solvents, and these solvents also showed very low levels of extractables as observed by HPLC-fluorescence detection.

## Conclusion

As HPLC and its downstream detectors become more and more sensitive, a greater onus falls on the sample preparation devices to provide clean extracts for HPLC. Any device that comes in contact with an HPLC sample has a potential to introduce extractable impurities into the sample.

The Smplicity® filtration system was specifically developed for filtration of samples directly into a HPLC vial, thereby avoiding filtration into tubes and transfers into vials, both of which have potential for introducing extractable impurities into the sample. Therefore, as long as the Millex Smplicity® filters themselves do not introduce any extractables, the sample should remain free of extractable impurities.

In this study, we have shown extractables profiles of Millex Smplicity® 0.45 µm and 0.20 µm PTFE filters using three different commonly used HPLC detectors. In case of UV and fluorescence detectors, both the Millex Smplicity® 0.45 µm and 0.20 µm PTFE filters provided very clean extracts without addition of extractable impurities to the samples. When using MS as the detection method, the Millex Smplicity® filters actually provided cleaner extracts than those prepared using a non-Merck Millipore LC-MS-certified syringe filter. Clearly, Smplicity® systems with Millex Smplicity® filters provide an ideal solution for preparing samples for ultrasensitive chromatographic analyses.

## Ordering Information

Description	Qty/Pk	Catalogue No.
Smplicity® Filtration System, Green	1	SAMPSYSGR
Smplicity® Filtration System, Blue	1	SAMPSYSBL
Millex Smplicity® Filters, hydrophilic PTFE, 0.45 µm	96	SAMPLCR01
Millex Smplicity® Filters, hydrophilic PTFE, 0.45 µm	384	SAMPLCR04
Millex Smplicity® Filters, hydrophilic PTFE, 0.20 µm	96	SAMPLG001
Millex Smplicity® Filters, hydrophilic PTFE, 0.20 µm	384	SAMPLG004
Millex Smplicity® Filters, hydrophilic PVDF, 0.45 µm	96	SAMPHV001
Millex Smplicity® Filters, hydrophilic PVDF, 0.45 µm	384	SAMPHV004

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