Data Sheet

Mass Spectrometry: Effects of Extractables from Syringe Filters

Liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) are being increasingly used in analysis of samples, not only in the biotechnology and pharmaceutical industries, but also more generally in the analysis, identification and quantitation of complex samples containing low levels of analytes. The advantages of LC-MS or GC-MS over other analytical techniques include high sensitivity and specificity for analyte detection and quantitation.

Key requirements for samples prior to MS analysis are:

- Particle-free samples for LC-MS analysis
- Minimum interference from impurities leached from sample preparation devices
- High sensitivity and low limits of detection and quantitation (LLOD and LLOQ)

Though a number of syringe filters are certified for use in high performance LC (HPLC), most of those filters are certified using HPLC coupled to detection of ultraviolet (UV) absorbance. Though this method provides information about the levels of UV-absorbing extractables coming from a filter, it is still difficult to understand how the same filters would perform when using a mass spectrometry detector.

This data sheet presents the results of evaluation of a number of syringe filters for MS-detectable extractables using different solvents.

Evaluation Criteria:

Lot-to-lot reproducibility of extractables level

This parameter reflects the consistency with which filters are manufactured. Since there are very few MS-certified filters, this parameter helps select the right filter for MS applications and prevents variations in levels of extractables when different lots of syringe filters are used.

Intensity of signal contribution from extractables: Total Ion Current (TIC)

This parameter helps users understand the interference the extractables will have with downstream analysis. Comparing TIC under consistent experimental conditions makes it easy to compare extractable signals from different membranes and different filter vendors.

Type of extractables: low molecular weight, discrete peaks vs. polymeric peaks

Any type of extractables can confound downstream analysis, but the discrete peaks from low molecular weight extractables are typically less problematic than peaks from polymeric extractables, which always show peaks separated by a common mass difference ranging over a wide M/Z range. Polymeric extractables are also difficult to remove, even after extensive cleaning of the mass spectrometer.

Experimental Protocol

1. Rinse 10 mL syringe 2-3 times with the solvent from which extractable testing is to be carried out
2. Fill rinsed 10 mL syringe with the solvent
3. Attach the syringe filter
4. Filter 1 mL through the syringe filter and collect the filtrate in a low extractable vial
5. Filter 2nd mL through the same syringe filter and collect the filtrate in a second low extractable vial
6. Repeat steps 3-5 for four more filters and pool all the 1st mL and 2nd mL filtrates
7. Measure extractables using MS under infusion conditions
8. Solvent blanks (unfiltered) were also analyzed under the same infusion conditions

Solvents used for extraction studies:

- Milli-Q® water
- Methanol
- Acetonitrile
- 0.1% Formic acid in water
- 0.1% Trifluoroacetic acid in water
- 0.1% Ammonia in water
- 5% Isopropanol (IPA) in water
- 5% Tetrahydrofuran (THF) in water

MS conditions: AB-SciEX® API 2000 TQQ

- Ionization Mode: Electrospray positive ion
- Mol. Wt. Range: 100 – 1000 M / Z
- Curtain Gas: 25
- Ion spray voltage: 5000 V
- Temperature: 300 °C
- Flow Rate: 20 µL / min
- Run Time: 5 min

Total Signal: Average of 15 min run

Name	Membrane and Pore size	Pore size, µm	Filter size	Number of Lots Tested
Millex® LCR	Hydrophilic PTFE	0.45 µm	25 mm	3
Competitor P	Hydrophilic Polypropylene	0.45 µm	25 mm	2
Competitor W	Hydrophilic Polypropylene	0.45 µm	25 mm	2
Competitor P	Nylon	0.45 µm	25 mm	2
Competitor W	Nylon	0.45 µm	25 mm	2

Table 1. List of syringe filters evaluated for this protocol.

Results and Discussion

0.45 µm Syringe Filters:

Lot-to-lot reproducibility: The mass spectra shown in Figures 1 and 2 for extracts from 5% IPA in water clearly show the difference between two different vendors. For Millex® LCR filters, all the three lots of filters tested had very similar levels of extractables between the lots (mass intensities < 8 x 10^4 for all the three lots), whereas for the nylon syringe filters from Competitor W, the two lots tested had signal intensities ranging from 8 x 10^5 to 1.4 x 10^6. Such variations are likely to cause difficulties in downstream analyses.

Table 2 summarizes the reproducibility study conducted for five different filter types with eight different solvent types. Three different colors were used to qualitatively represent lot-to-lot variability. Green indicated least variability, whereas red indicated the most variability of mass spectral signal intensity. Millex® LCR filters as well as syringe filters with polypropylene membrane (Competitor W) showed the most reproducible mass spectral intensities for all the eight solvents tested. On the other hand, both the nylon syringe filters (Competitor P and W) showed high variability of signal across different lots as well as different solvents.

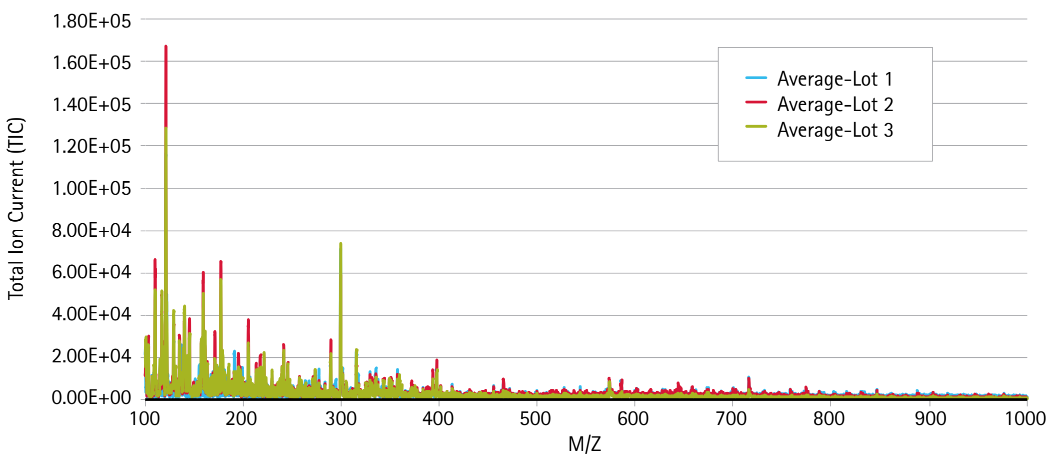

Figure 1.
Mass spectral intensity for extracts from 5% Isopropyl Alcohol in water. Data from three different lots of syringe filters are plotted for Hydrophilic PTFE syringe filters (Millex® LCR).

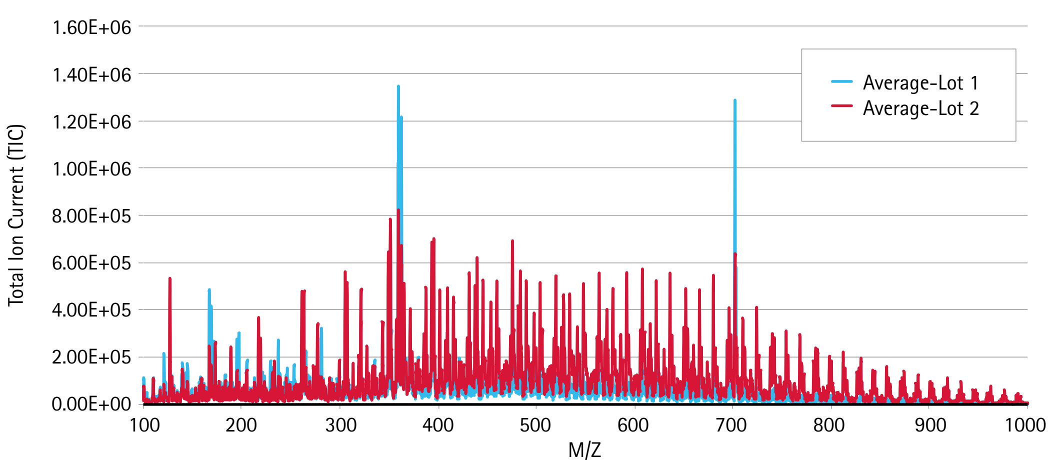

Figure 2.
Mass spectral intensity for extracts from 5% Isopropyl Alcohol in water. Data from two different lots of syringe filters are plotted for nylon syringe filters from Competitor W.

	Hydrophilic PTFE (Millex® LCR)	Polypropylene (Competitor P)	Polypropylene (Competitor W)	Nylon (Competitor P)	Nylon (Competitor W)
Lot-to-lot reproducibility					
Number of lots tested	3	2	2	2	2

Table 2.
Overall lot-to-lot reproducibility of mass spectral signal for five different types of syringe filters when tested using eight different solvents. Millex® LCR and polypropylene syringe filters from Competitor W show the most reproducible data for mass spectral signal intensity.

Signal intensity: The background signal coming from the filter contributes to overall baseline noise in the mass spectrum for the sample. With complex samples and low levels of analytes to quantitate, any increase in background signal negatively impacts LOQ and makes quantitation challenging. Figures 3 and 4 show representative mass spectra for extractables from 1% formic acid in water.

Millex® LCR filters (Figure 3) showed a highest peak intensity of about 8 x 10^5 for extractable masses, whereas polypropylene syringe filters from Competitor W showed extractable levels about 10 times higher (8 x 10^6). Such high signal intensity, which can be comparable to the signal from the analyte of interest, makes quantitation of analytes very challenging.

Figure 3.

Mass spectral intensity for extracts from 5% Isopropyl Alcohol in water. Data from three different lots of syringe filters are plotted for Hydrophilic PTFE syringe filters (Millex® LCR).

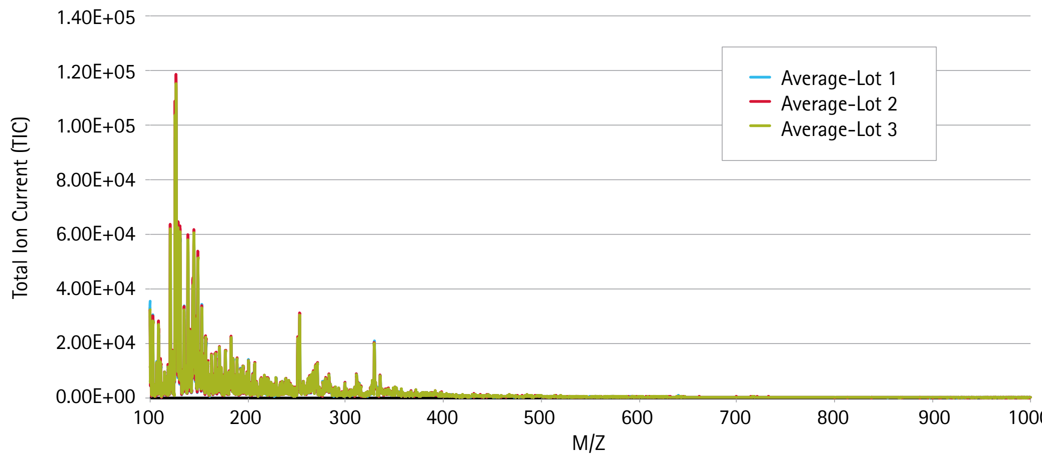

Figure 4.

Mass spectral intensity for extracts from 1% Formic acid in water. Data from two different lots of syringe filters are plotted for Polypropylene syringe filters (Competitor W). The highest signal intensity from extractables is about 8 x 10^6.

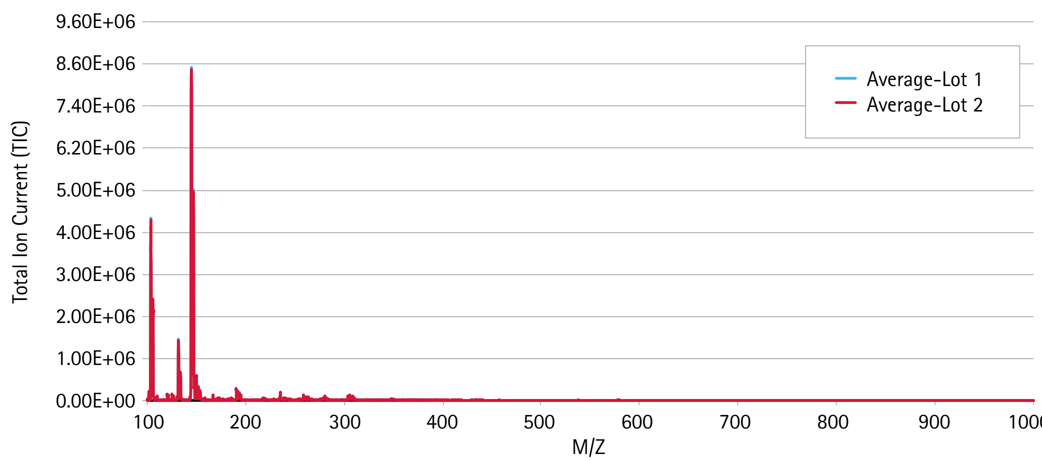

Table 3 compares signal intensities across eight different solvents and five different filters. Millex® LCR filters with hydrophilic PTFE membrane again showed the lowest level of background signal contribution. On the other hand, polypropylene syringe filters from competitor P and both the nylon syringe filters from Competitors P and W showed very high levels of extractables signal.

	Hydrophilic PTFE (Millex® LCR)	Polypropylene (Competitor P)	Polypropylene (Competitor W)	Nylon (Competitor P)	Nylon (Competitor W)
Signal intensity					
Number of lots tested	3	2	2	2	2

Table 3.

Overall mass spectral signal intensity for five different types of syringe filters when tested using eight different solvents. Millex® LCR filters show the lowest level of signal intensity (and therefore background noise). On the other hand, Polypropylene syringe filters from Competitor P as well as nylon syringe filters from Competitor P and W all show very high levels of extractables, impacting background signal.

Type of extractables: The ideal situation in any analysis is to have zero extractables from the syringe filter, but since this is rarely the case, it is important to understand the type of extractables a syringe filter introduces into the sample. Any surface that comes in contact with the sample has a potential to introduce extractables into the sample; syringe filters are therefore not the only source of extractables. Examining the averaged mass spectra revealed whether the extractable ions were discrete molecular entities or polymeric impurities (Figure 5 and 6). As can be clearly seen, the hydrophilic PTFE-based Millex® LCR syringe filters showed few discrete molecular ions as main impurities, whereas the polypropylene membrane-containing syringe filters from Competitor P showed high levels of polymeric extractables. As typically seen in mass spectra of polymers, these syringe filters showed multiple extractable peaks separated by a common mass difference (typical repeat unit difference). As these peaks spread over the molecular weight range of 300 – 800, the presence of these peaks makes it difficult to perform downstream analysis, especially of analytes in that molecular weight range. Table 4 shows the summary results from all the eight different solvents and five different filters. Only the Millex® LCR filters (containing hydrophilic PTFE) showed few extractable peaks at low intensity; all the other syringe filters showed polymeric extractable impurities.

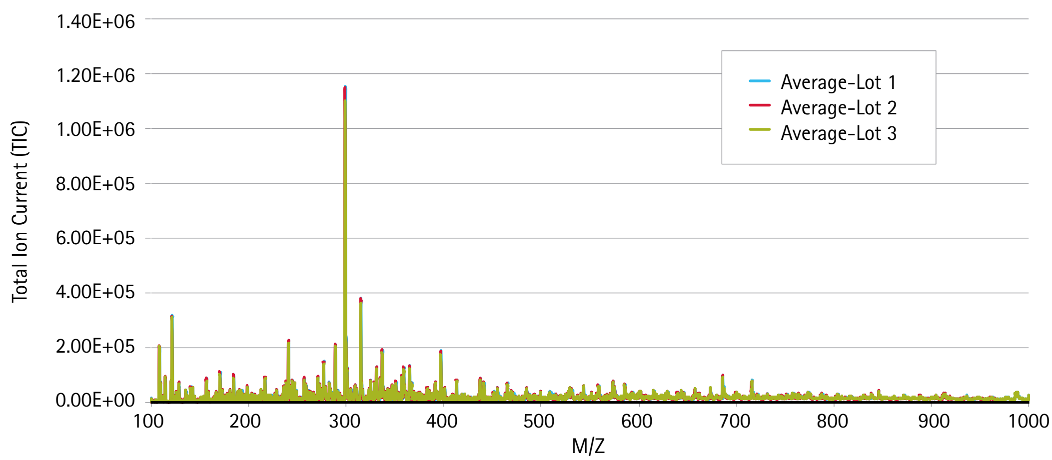

Figure 5.

Mass spectral intensity for extracts from Methanol. Data from three different lots of Millex® LCR syringe filters are plotted. Data show that there are discrete MS peaks as extractables coming from this syringe filter, the most intense peaks at 299 and 315 M / Z. Overall signal intensity is still fairly low.

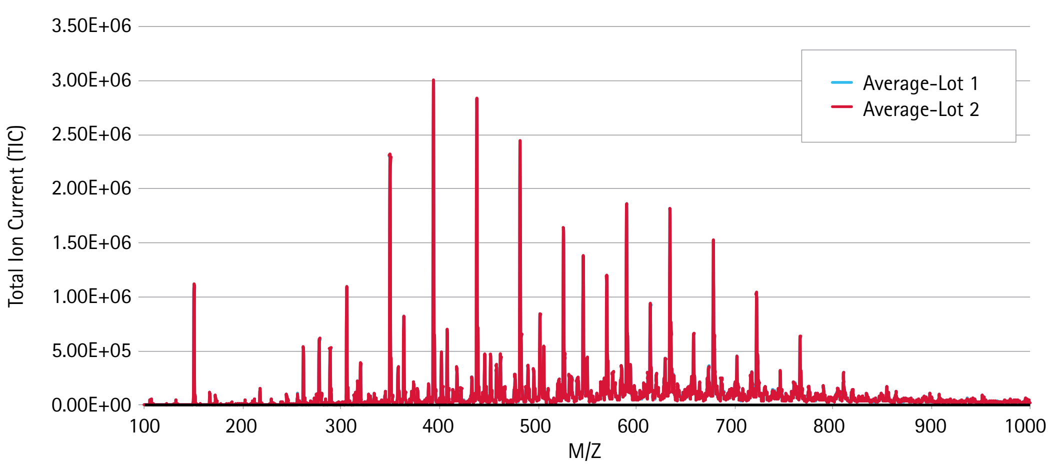

Figure 6.

Mass spectral intensity for extracts from Methanol. Data from two different lots of syringe filters containing polypropylene membrane (Competitor P) are plotted. Data show that there are a number of polymeric peaks coming from extractables from this filter. These peaks can be seen at 305, 349, 393, 437, 481, 525 all the way upto 722 M /Z. These peaks are separated by 44 M /Z, appearing to be a repeat unit difference characteristic of a polymer.

	Hydrophilic PTFE (Millex® LCR)	Polypropylene (Competitor P)	Polypropylene (Competitor W)	Nylon (Competitor P)	Nylon (Competitor W)
Nature of Extractables (MW Range)	Low MW range discrete peaks	Polymeric	Polymeric – Variable	Polymeric – Variable	Polymeric - Variable
No. of Lots Tested	3	2	2	2	2

Table 4.

Nature of extractables for five different types of syringe filters when tested using eight different solvents. Millex® LCR filters only show a few discrete peaks with low signal intensity (and therefore background noise). On the other hand, both the polypropylene and nylon syringe filters from Competitors P and W show polymeric extractables spanning from M / Z of 300 to 800.

	Hydrophilic PTFE (Millex® LCR)	Polypropylene (Competitor P)	Polypropylene (Competitor W)	Nylon (Competitor P)	Nylon (Competitor W)
Lot-to-lot reproducibility					
Signal Intensity					
Nature of extractables (MW range)	Low MW range discrete peaks	Polymeric	Polymeric – Variable	Polymeric – Variable	Polymeric - Variable
No. of Lots Tested	3	2	2	2	2

Table 5.
Overall summary of extractables for five different types of syringe filters.

Summary

Based on all these studies, Millex® LCR syringe filters showed the lowest level of extractables when compared with other suppliers' syringe filters, which contained polypropylene and nylon membranes. Table 5 shows the overall summary for all of these filters.

Description	Cat. No.
Millex®-LCR Filter, 13 mm, Hydrophilic PTFE, 0.45 µm, 100/pk	SLCR013NL
Millex®-LCR Filter, 13 mm, Hydrophilic PTFE, 0.45 µm, 1000/pk	SLCR013NK
Millex®-LCR Filter, 25 mm, Hydrophilic PTFE, 0.45 µm, 250/pk	SLCR025NB
Millex®-LCR Filter, 25 mm, Hydrophilic PTFE, 0.45 µm, 1000/pk	SLCR025NK

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