Successful Starts in LC-MS/MS

Critical considerations when choosing a syringe filter for HPLC, UHPLC, and LC-MS/MS analysis

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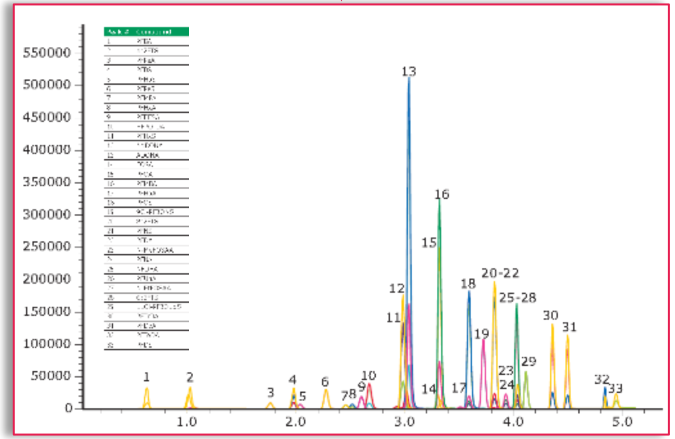
LC-MS/MS Analytical Methods
Three Components for Success

HPLC Columns + **Mobile Phase Components** +

Sample preparation removes particles, contaminants and interferences from the sample, which brings it to a form amenable to downstream analysis.

- Analyte properties
- Impurities present
- Matrix components
- Choice of water



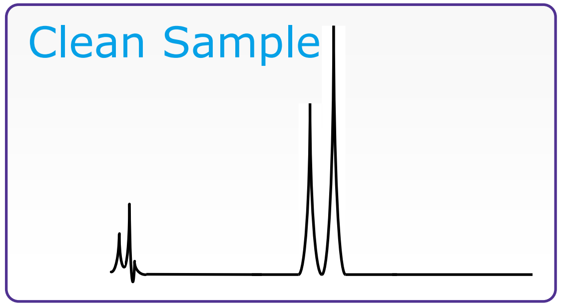
https://www.sigmaaldrich.com/US/en/campaigns/pfas-testing

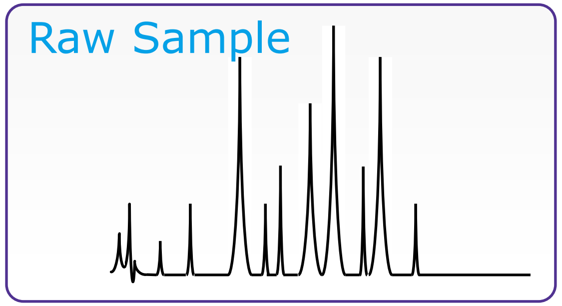
- For high-particulate matrices, SPE and filtration are common

Why Sample Preparation
Importance of sample preparation

Without sample preparation, instrument performance and data quality will not be optimal:

- Undissolved particles in a sample / mobile phase clog and reduce the life of the chromatography column.
- Sample matrix containing lots of impurities makes chromatograms challenging to interpret.
- Particles held up on the column can clog the column and/or leach contaminants into the eluent (and affect current and subsequent samples), thereby increasing background noise.

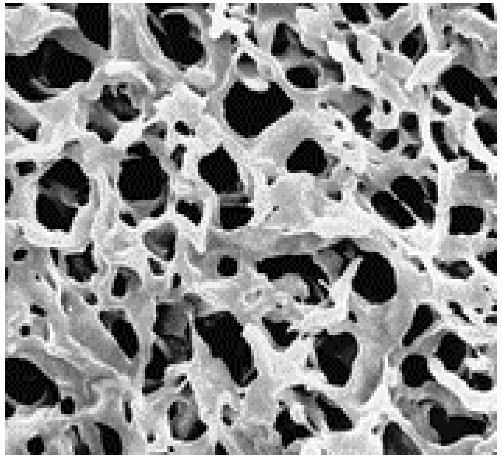




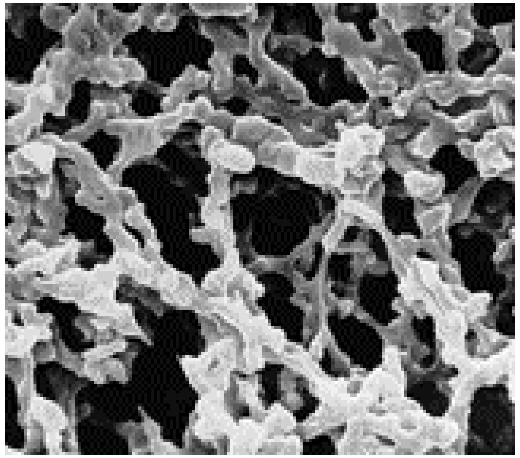
- Sample matrix impurities
- Undissolved particles
- Particle leaching

80%

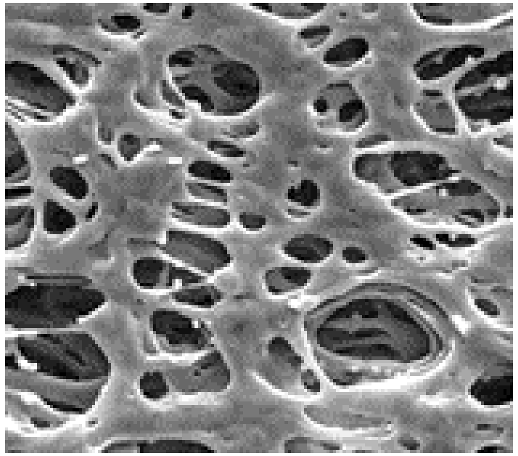
Which Membrane, and Why?
Membrane Morphology & Applications



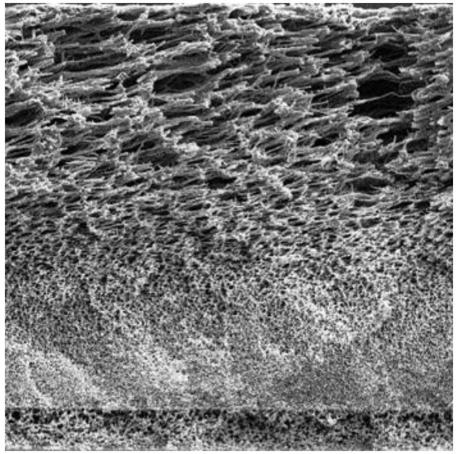
Polyvinylidine Fluoride (PVDF): *Low binding and fast flow for protein sample prep*



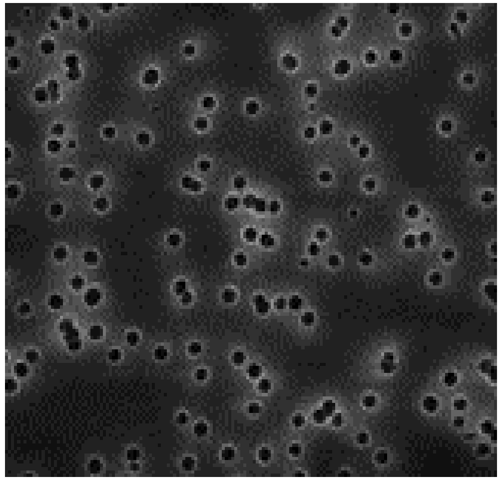
Mixed Cellulose Esters (MCE): *Biologically inert, versatile, smooth and uniform*



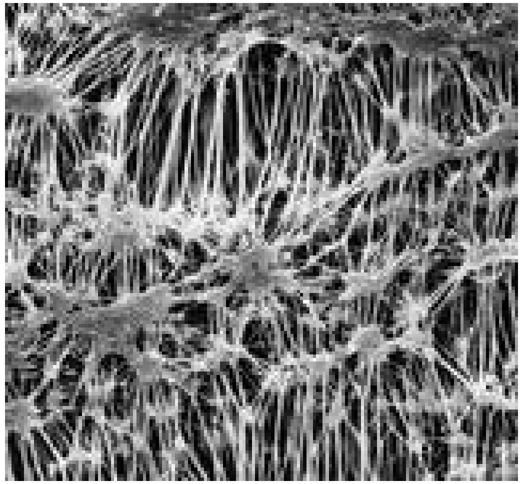
Polyethersulfone (PES): *Quick flow and high capacity, asymmetric for high-particulate water samples*



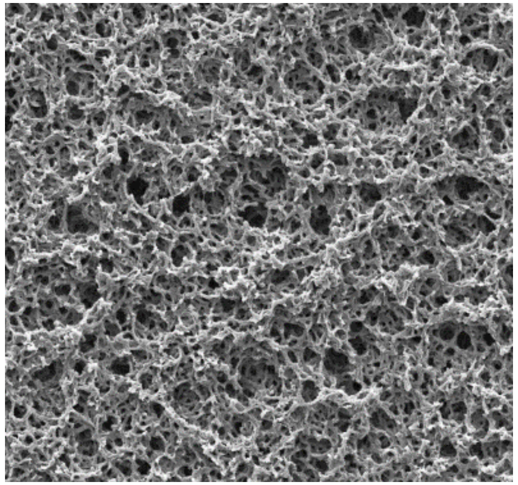
PES Cross-section



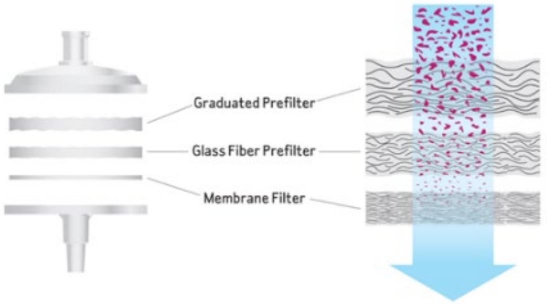
Polycarbonate (PC): *preferred for microscopy and cell-based applications*



Polytetrafluoroethylene (PTFE): *Low extractables and high chemical compatibility*



Nylon: *Broad compatibility and commonly used for HPLC*



Nylon with a Glass Fiber Prefilter (HPF): *One-step cleanup of large and small particulates without clogging*

Which Membrane, and Why?
Chemical Compatibility, Extractables and Retention

	Housing Type		Membrane Type			
	HDPE	**PP**	**Nylon**	**PES**	**GFF**	**PTFE***
AA	**E**	**ND**	**P**	**E**	**E**	**E**
ACN	**E**	**E**	**E**	**G**	**E**	**E**
MeOH	**E**	**E**	**G**	**G**	**E**	**E**
EtOH	**E**	**E**	**E**	**E**	**E**	**E**
3M NaOH	**E**	**E**	**P**	**P**	**ND**	**E**
NH_4OH	**E**	**E**	**P**	**P**	**E**	**E**
Na_2CO_3 Solution	**G**	**E**	**P**	**P**	**ND**	**E**
1N HCl	**E**	**E**	**G**	**E**	**E**	**E**
Brine	**E**	**E**	**E**	**E**	**ND**	**E**
SDS	**G**	**G**	**G**	**G**	**ND**	**E**

E Excellent **G** Good **P** Poor

Abbreviations: HDPE = high-density polyethylene; PP = polypropylene; PES = polyethersulfone; GFF = glass fiber filter; MCE = mixed cellulose ester; AA = acetic acid; ACN = acetonitrile; MeOH = methanol; EtOH = ethanol; SDS = sodium dodecyl sulfate; ND=not determined/testing recommended

*PTFE/PVDF not recommended for PFAS testing applications

Pore size is important to consider in consider with respect to your column. For U/HPLC columns with <3µm packing, use a 0.2µm, for sizes above this, a 0.45µm is sufficient.

Retention doesn't always depend on pore size, but also membrane manufacturing method, symmetry and chemical structure, and even lot-by-lot

	0.2/0.22µm Syringe Filters	0.45µm Syringe Filters
Material	**% Retention of 0.24µm PS Beads**	**% Retention of 0.5µm PS Beads**
Nylon	100.0±0.1	100.0±0.05
Nylon-HPF	54.2±27.3	--[a]
PES	69.4±28.1	99.5±0.79
PTFE (Vendor 1)	49.8±31.8	99.98±0.10
PP (Vendor 1)	25.3±0.9	100.0±0.06
RC (Vendor 2)	15.8±2.2	48.16±4.27

[a]Pore size not available.

Case study: PFAS analysis

Analytical Methods for PFAS Developed at a Rapid Pace
Regulatory Landscape

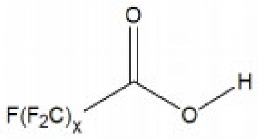
Method(s)	Update/ Revision	Matrix/Matrices	Sample Prep	Analytical Method
EPA 537.1	Jun. 2020	Drinking water	SPE	LC-MS/MS
EPA 533	Dec. 2019	Drinking water	SPE	LC-MS/MS
SW-846 Method 8327*	Jul. 2021	Non-potable groundwater, surface water, wastewater	SPE, **filtration**	LC-MS/MS
ASTM D7968-17a	Sep. 2017	Environmental solids	Solvent extraction, **filtration**	LC-MS/MS
ASTM D7979-19	Sep. 2021	Water matrix (no drinking water)	Solvent extraction, **filtration**	LC-MS/MS
ISO 21675	Oct. 2019	Drinking, natural and wastewater	SPE, **filtration** as needed	LC-MS/MS
FDA C-010.02	Dec. 2021	Foods	QuEChERS, SPE, **filtration**	LC-MS/MS
OTM-45	Jan. 2021	Air Emissions (stationary sources)	Sampling train: **filtration**, impingers	LC-MS/MS
EPA Draft 1633	Feb. 2022	Aqueous, soil, biosolids, sediment, tissue	SPE, **filtration**	LC-MS/MS
EPA Draft 1621*	Apr. 2022	Aqueous matrices	TSS, GAC column cleanup	CIC

- Almost exclusively LC-MS/MS based methods
- SPE & filtration are common sample preparation
- Increased focus on high-particulate matrices
- Additional watchouts for PFAS sample preparation

**screening method only*
Abbreviations: SPE = solid phase extraction; TSS = total suspended solids determination; GAC = granular activated carbon; CIC = combustion ion chromatography
Selected methods; does not include all drinking water and international methods

Do Nylon or PES Syringe Filters Contaminate Samples with PFAS?
Experimental

Overview of Modified EPA 537.1



- 250 mL water sample + surrogates
  - QC blank internal standard spike = 0.08 ppb

- Filtration with 0.22µm or 0.45µm Millex® syringe filter

SPE

- SDVB SPE cartridge extraction (Methanol)

Concentration

- Concentrate samples to 1 mL in 96:4% v/v Methanol:Water

LC-MS/MS

- LC-MS/MS, C18 column
- Analysis by internal standards

LC-MS/MS Conditions

Column:	C18, 100 x 2.1mm ID, 2.7 µm superficially porous particles
Mobile phase:	[A] DI Water, 0.1% (v/v) acetic acid; [B] Methanol (MeOH), 0.1% (v/v) acetic acid
Gradient:	See table below
Flow rate:	See gradient table
Detection:	MS/MS, ESI(-), details of MS/MS conditions can be requested from the author
Column temp:	50.0 °C
Injection volume:	3-5 µL autosampler injection
Sample :	SPE eluate concentrated to 1 mL methanol: water, 96:4% (v/v)

Gradient:

Time (min)	A %	B %	Flow (mL/min)
0-0.0	65%	35%	0.4
0-7.0	0%	100%	0.4
7.0-10.0	0%	100%	0.7
10.0-11.0	0%	100%	0.7
11.0-15.0	65%	35%	0.4

Data collected in collaboration with: SGS

(Orlando, FL)

Results

Category	Compound	Abbre-viation	RL (ppb)	MDL (ppb)	SLGP033 0.2µm PES			SLHP033 0.45µm PES			SLGN033 0.2µm Nylon			SLGNM25 0.2µm Nylon-HPF	
					Lot1	Lot2	Lot3	Lot1	Lot2	Lot3	Lot1	Lot2	Lot3	Lot1	Lot2
[1] Perfluoroalkylcarboxylic acids	Perfluorobutanoic acid	PFBA	0.0040	**0.0020**	**ND –** Not detected in filtrate						**ND –** Not detected in filtrate				
	Perfluoropentanoic acid	PFPeA	0.0020	**0.0010**											
	Perfluorohexanoic acid	**PFHxA**	0.0020	**0.0010**											
	Perfluoroheptanoic acid	**PFHpA**	0.0020	**0.0010**											
	Perfluorooctanoic acid	**PFOA**	0.0020	**0.0010**											
	Perfluorononanoic acid	**PFNA**	0.0020	**0.0010**											
	Perfluorodecanoic acid	**PFDA**	0.0020	**0.0010**											
	Perfluoroundecanoic acid	**PFUnDA**	0.0020	**0.0010**											
	Perfluorododecanoic acid	**PFDoDA**	0.0020	**0.0010**											
	Perfluorotridecanoic acid	**PFTrDA**	0.0020	**0.0010**											
	Perfluorotetradecanoic acid	**PFTeDA**	0.0020	**0.0010**											
[2] Perfluoroalkylsulfonic acids, Perfluorooctane sulfonamides, and Perfluoroctanesulfon-amidoacetic acids	Perfluorobutanesulfonic acid	**PFBS**	0.0020	**0.0010**	**ND –** Not detected in filtrate						**ND –** Not detected in filtrate				
	Perfluoropentanesulfonic acid	PFPeS	0.0020	**0.0010**											
	Perfluorohexanesulfonic acid	**PFHxS**	0.0020	**0.0010**											
	Perfluoroheptanesulfonic acid	PFHpS	0.0020	**0.0010**											
	Perfluorooctanesulfonic acid	**PFOS**	0.0020	**0.0010**											
	Perfluorononanesulfonic acid	PFNS	0.0020	**0.0010**											
	Perfluorodecanesulfonic acid	PFDS	0.0020	**0.0010**											
	PFOSA	PFOSA	0.0040	**0.0020**											
	N-MeFOSAA	**MeFOSAA**	0.0040	**0.0020**											
	N-EtFOSAA	**EtFOSAA**	0.0040	**0.0020**											
[3] Fluorotelomer sulfonates and Next Generation PFAS Analytes	4:2 Fluorotelomer sulfonate	8:2 FTS	0.0080	**0.0020**	**ND –** Not detected in filtrate						**ND –** Not detected in filtrate				
	6:2 Fluorotelomer sulfonate	6:2 FTS	0.0080	**0.0020**											
	8:2 Fluorotelomer sulfonate	8:2 FTS	0.0080	**0.0020**											
	HFPO-DA	**GenX**	0.0040	**0.0020**											
	ADONA	**ADONA**	0.0080	**0.0020**											
	9C1-PF3ONS (F-53B Major)	**--**	0.0080	**0.0020**											
	11C1-PF3OUdS (F-53B Minor)	**--**	0.0080	**0.0020**											

*Highlighted **teal** indicates requirement of EPA 537.1*

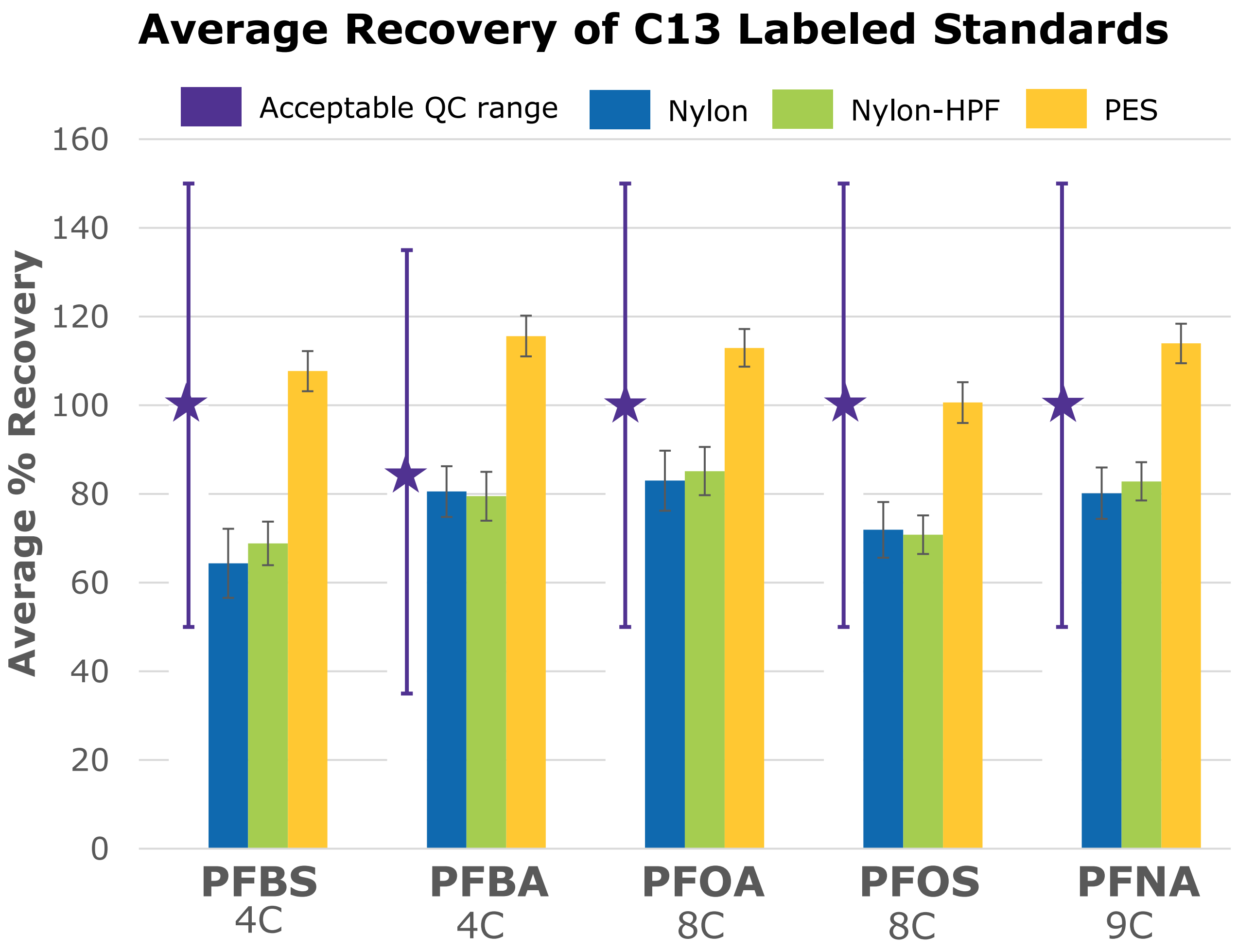
PFAS Analysis
The Known Challenges

Known Challenges

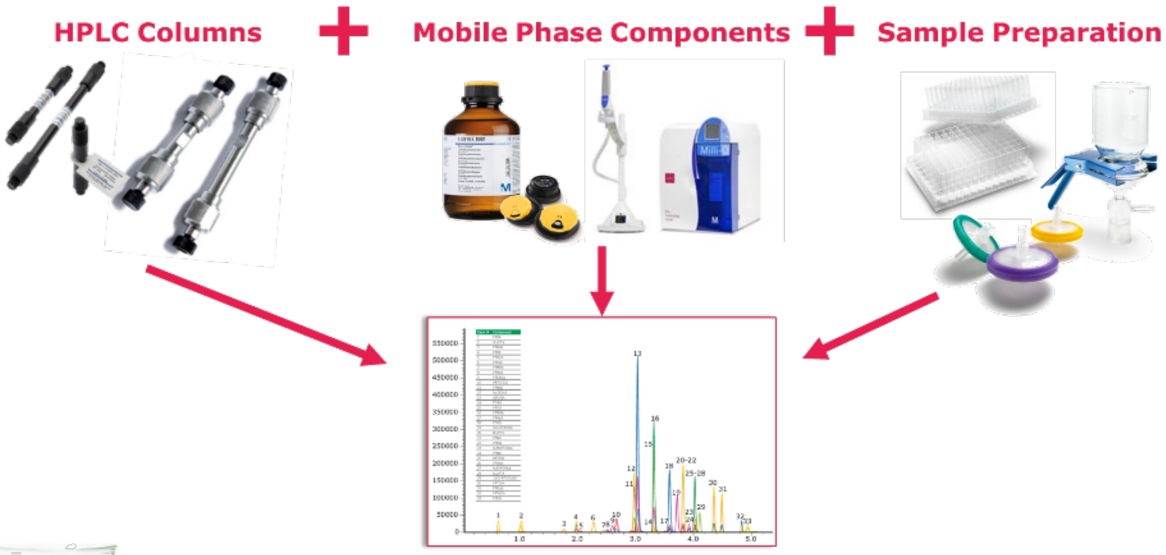
- Contamination
- Volatility
- Longer chain compounds
- Recovery and absorption
- Consumable vs. process

Solutions

- Know your membrane & vendor
- For nylon, a simple wash can reduce absorption from detectable to undetectable levels [1,2]
- Choose the proper membrane for targeted compounds



Successful Starts in LC-MS/MS
Summary



- Sample preparation is one of the critical components of successful LC-MS/MS analysis
- With higher particulate matrices, sample preparation needs increase (ex., filtration, SPE)
- **Choosing the right filter for sample preparation depends on many properties:**
  - Material, chemical compatibility, pore size & retention, prefiltration, sample volume
- For PFAS analysis specifically, always consider the known challenges when choosing a consumable for sample preparation:
  - **Risk of contamination**
  - **Risk of analyte loss**

We tested PES (0.2µm and 0.45µm), Nylon (0.2µm) and Nylon with a glass fiber prefilter (0.2µm) for PFAS extractables using a modified EPA 537.1 LC-MS/MS method

None of the filters demonstrated PFAS extractables, and are thus appropriate for prepping high particulate samples for PFAS analysis

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PREPARED FOR PFAS TESTING

- https://sigmaaldrich.com/pfas-testing
- http://sigmaaldrich.com/pfassamplefiltration

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