Filtration Approaches to Sample Preparation and Cleanup

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Today's Agenda

How does filtration work – a brief overview

Why filter – benefits of filtration, including applications

Introduction to Agilent filtration products

Selecting the right filtration product

Questions and wrap up

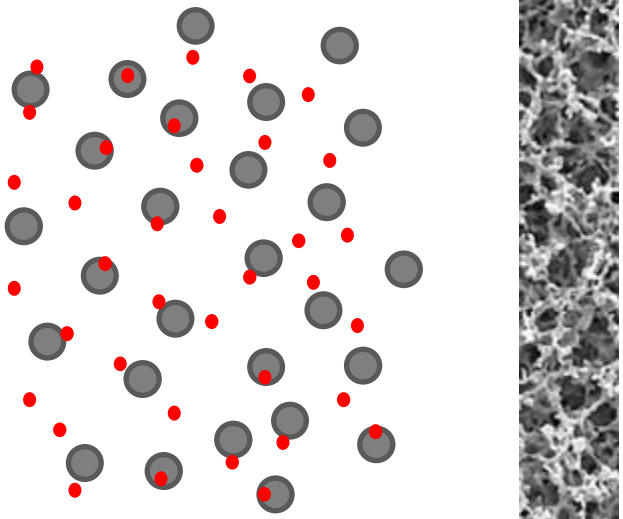
MECHANISMS OF FILTRATION: A BRIEF OVERVIEW

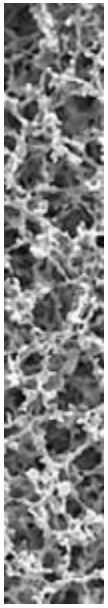
Filtration

Filtration – How does it work?

Filtration: separating substances based on particle size

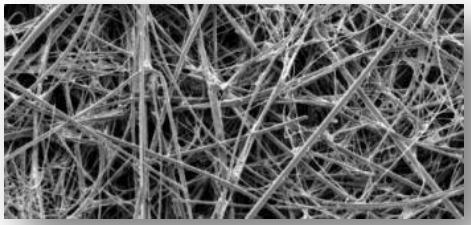
- Solid particles in liquids (suspension)
- Insoluble droplets in liquids (emulsion)





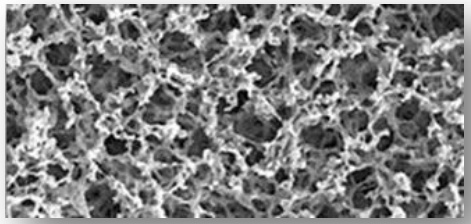
Microfiltration

Two types of membranes



1. Depth filters: for heavily particle-laden samples, ideal for pre-filtration

Glass Fiber



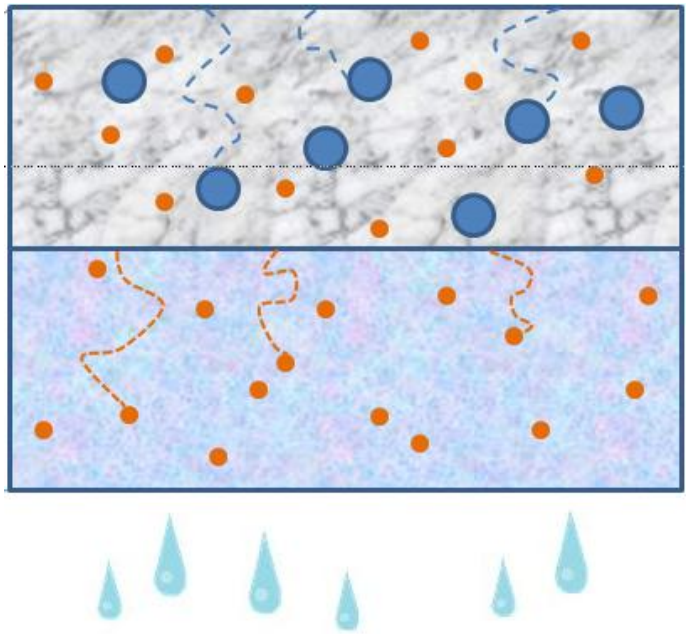
2. Microporous filters: high safety against breakthrough, for complete removal of particles

CA : Cellulose Acetate	RC : Regenerated Cellulose
PES : Polyethersulfone	PP: Polypropylene
PTFE : Polytetraflourethylene	Nylon

Dual-Depth Filter

Captiva Dual-Depth Filtration

1) First layer is a larger-porosity membrane. It traps larger particles, which travel in non-linear path, thereby preventing clogging and creating more surface area in which to capture the particulates
2) The second, smaller-porosity membrane, traps smaller particles resulting in all unwanted particles being removed from sample

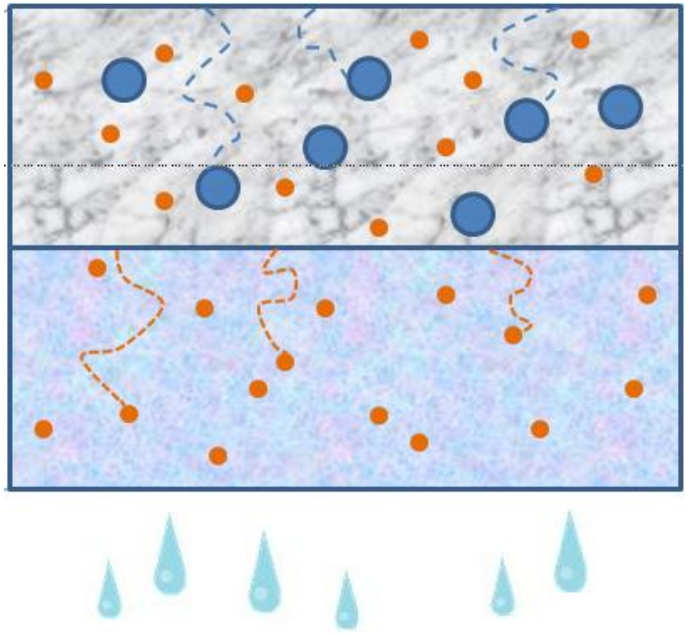


(Close-up of filter layers)

Mechanical Combined with Functional Filtration

Captiva ND Lipids Filtration

- Combines a depth-filter for mechanical filtration with a functional component designed to remove lipids from samples



Dual-depth filter schematic showing the differences in sizes between the two layers. A proprietary product offers a combination of mechanical and functional filtration for effective lipid removal.

WHY FILTRATION?

Filtration

Filtration and Other Sample Preparation Techniques: An Overview

	More Specific ← Instrument Separation and Detection Specificity ← *Less Specific*								
	Less Specific → *Sample Preparation Specificity* → *More Specific*								
Sample Prep Technique / Interference Removed	**Dilute & Shoot**	**Filtration**	**Liquid/Liquid Extractions**	**Supported Liquid Extractions (SLE)**	**Dried Matrix Spotting**	**Precipitation filtration**	**QuEChERS**	**Precipitation-Lipid Removal 'Hybrid' Filtration**	**Solid Phase Extraction**
Lipids	No	No	No	Some	No	No	Yes	Yes	Yes
Oligomeric Surfactants	No	No	No	No	No	No	No	Yes	Yes
Particulates	No	Yes	No	Some	No	Yes	Yes	Yes	Yes
Pigments	No	No	No	Some	No	No	Yes	No	Yes
Polar Organic Acids	No	No	Yes	Yes	No	No	Yes	No	
Proteins	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Salts	No	No	Yes	Yes	No	No	No	No	Yes

Filtration is suggested with any LC or GC method of sample preparation

Why Filter?

Benefits of Filtration

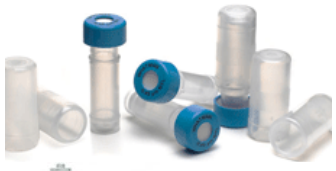
Optimal Instrument Performance	Less System Downtime	Extend Column Lifetime	Greater Sample Integrity
			

Achieve lower detection limits

Agilent recommends filtering prior to chromatographic analysis to remove particulates from the sample

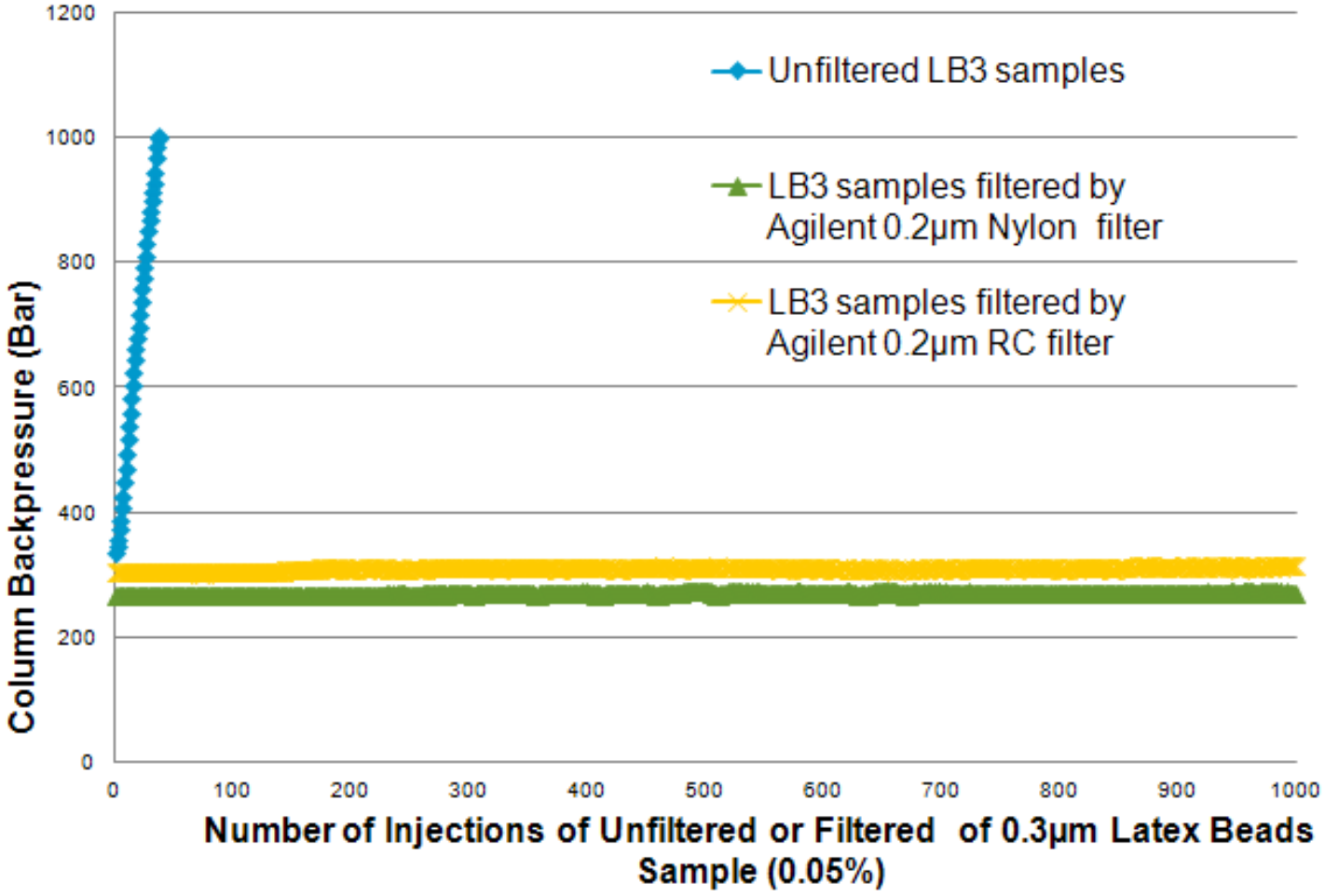
Why Filter the Sample?

Extreme Performance Requires Better Sample “Hygiene”

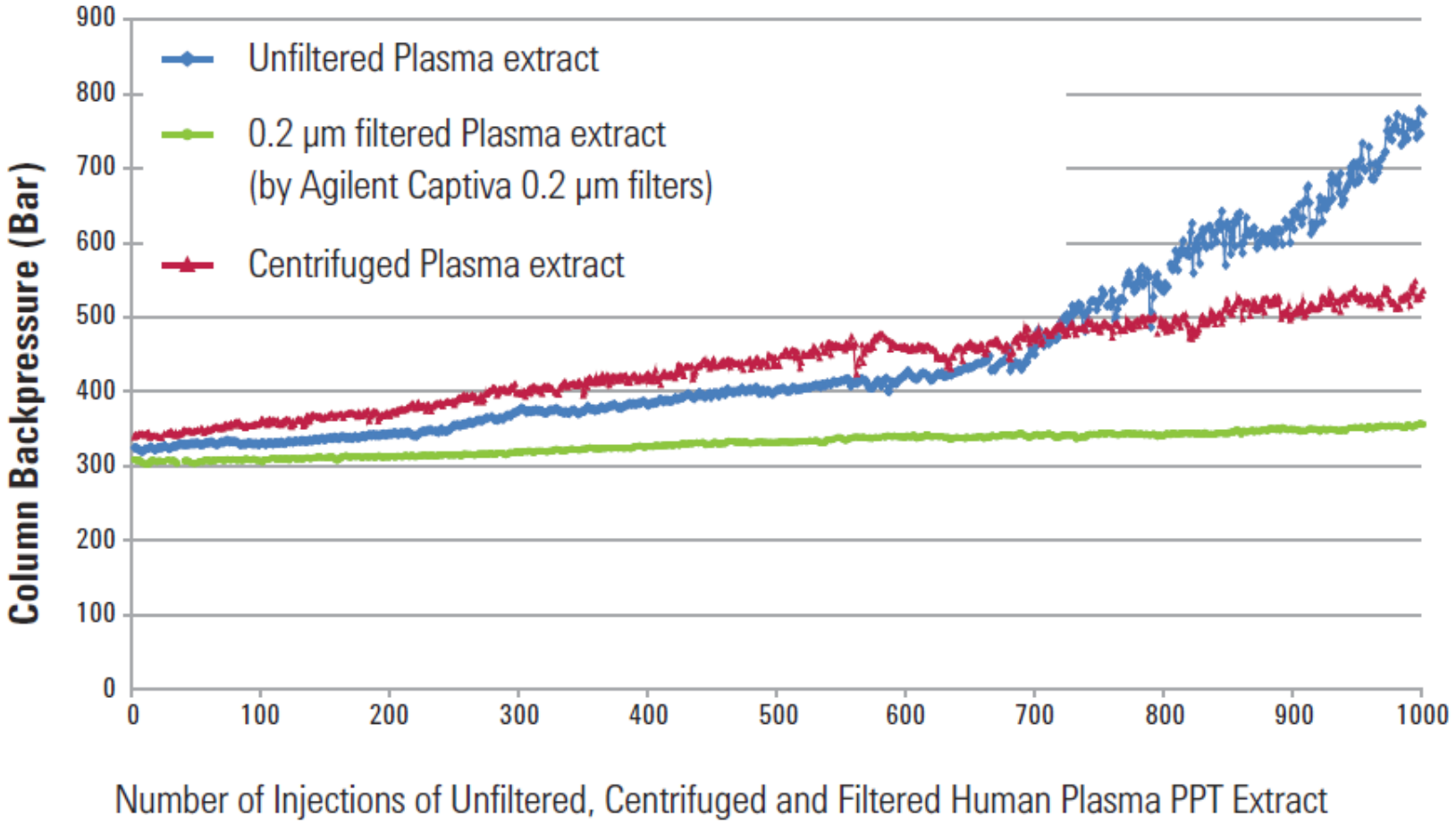


- Prevents blocking of capillaries, frits, and the column inlet (especially important for UHPLC)
- Results in less wear and tear on the critical moving parts of injection valves
- Results in less downtime of the instrument for repairs
- Produces improved analytical results by removing potentially interfering contamination
- “Functionalized” filtration can improve results over mechanical filtration by removing chemical interferences in addition to particulates

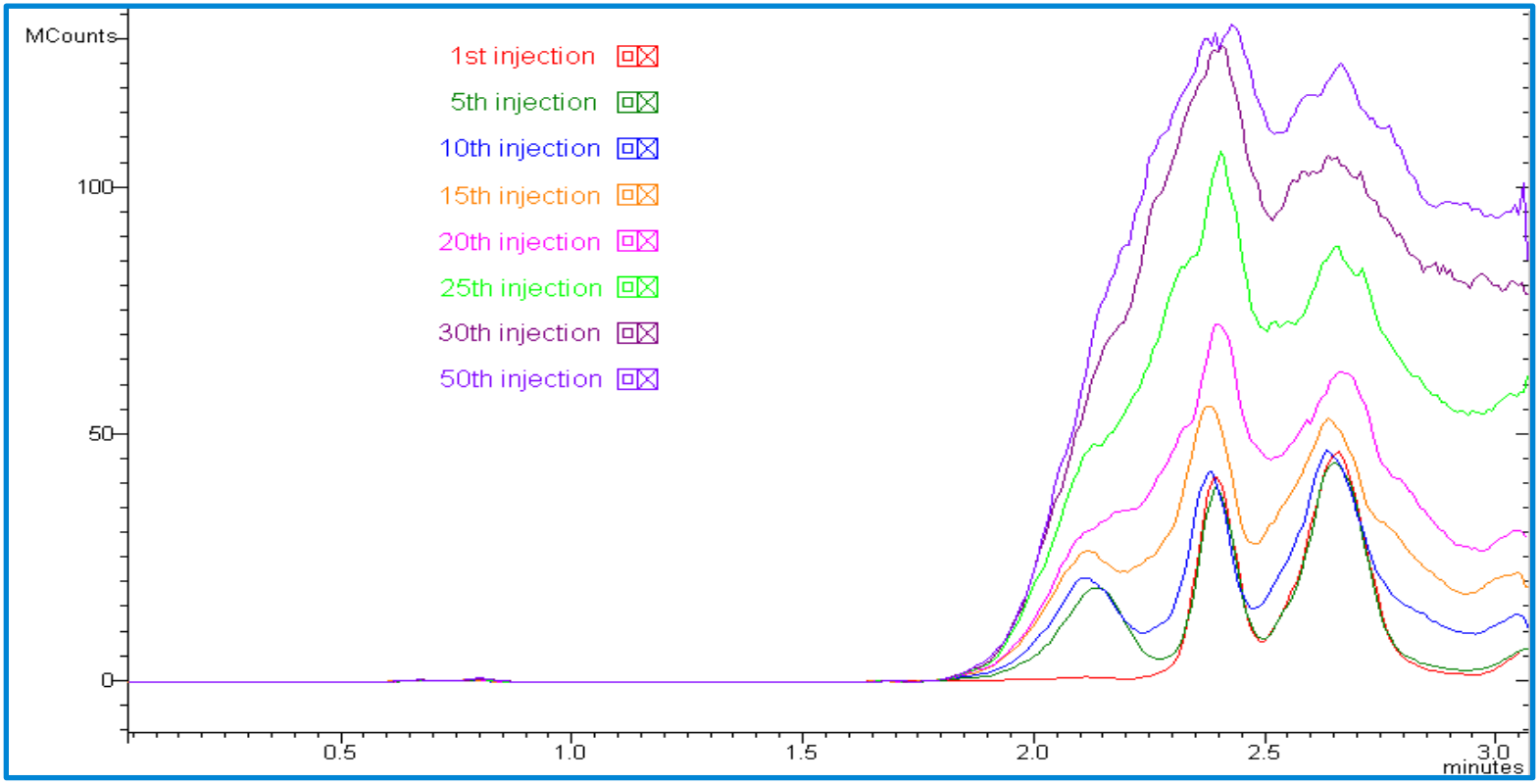
Syringe Filter Benefit: Improved sub-2-micron LC Column Lifetime – Standardized Sample



Syringe Filter Benefits: Improved sub-2 micron LC Column Lifetime – With Human Plasma Extract



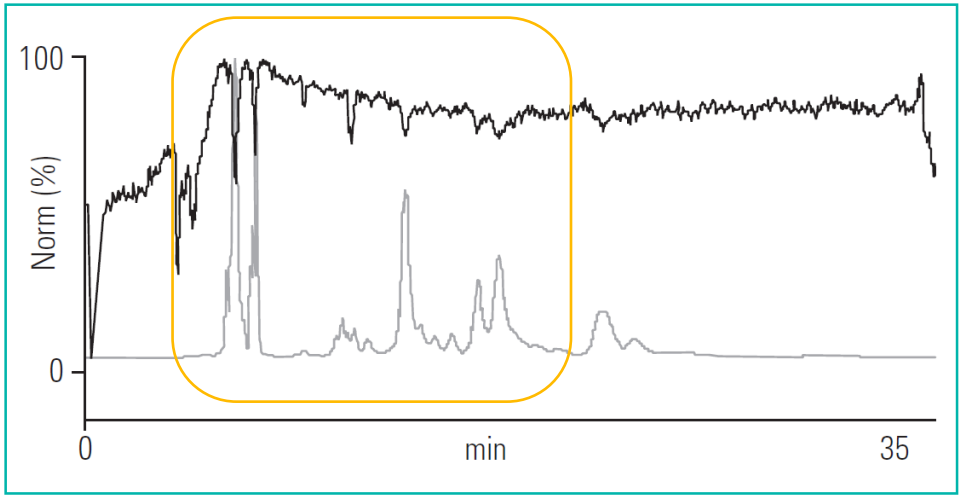
The Problem: Lipid Build-up and Ion Suppression in LC-MS Applications



If lipids are present in the sample and the gradient does not flush out lipids, they will build up as the injection number increases.

More lipids → ion-suppression

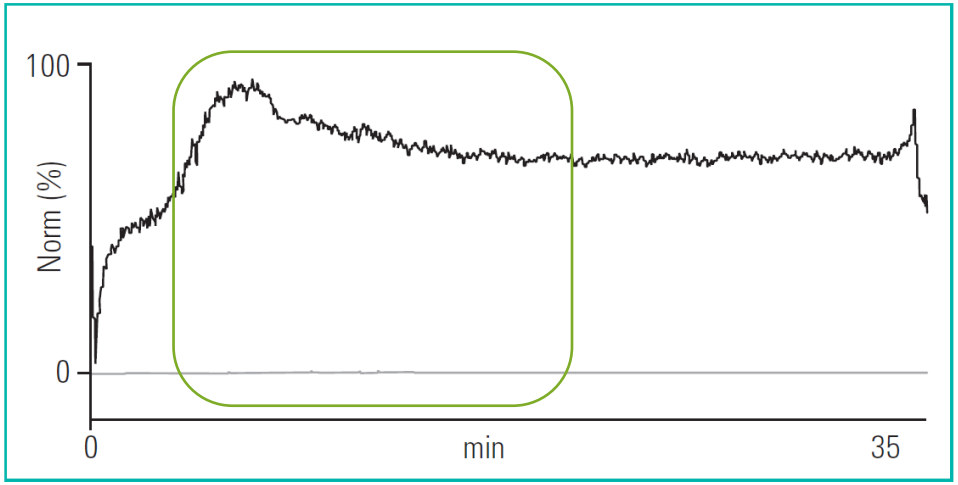
Functional and Mechanical Filtration Benefit: Reduced Ion Suppression by Removing Lipids



Post-column infusion of albuterol (top trace)

Plasma sample showing elution of phospholipids (bottom trace)

- Ion suppression events correspond to elution of phospholipids



Post-column infusion of albuterol (top trace)

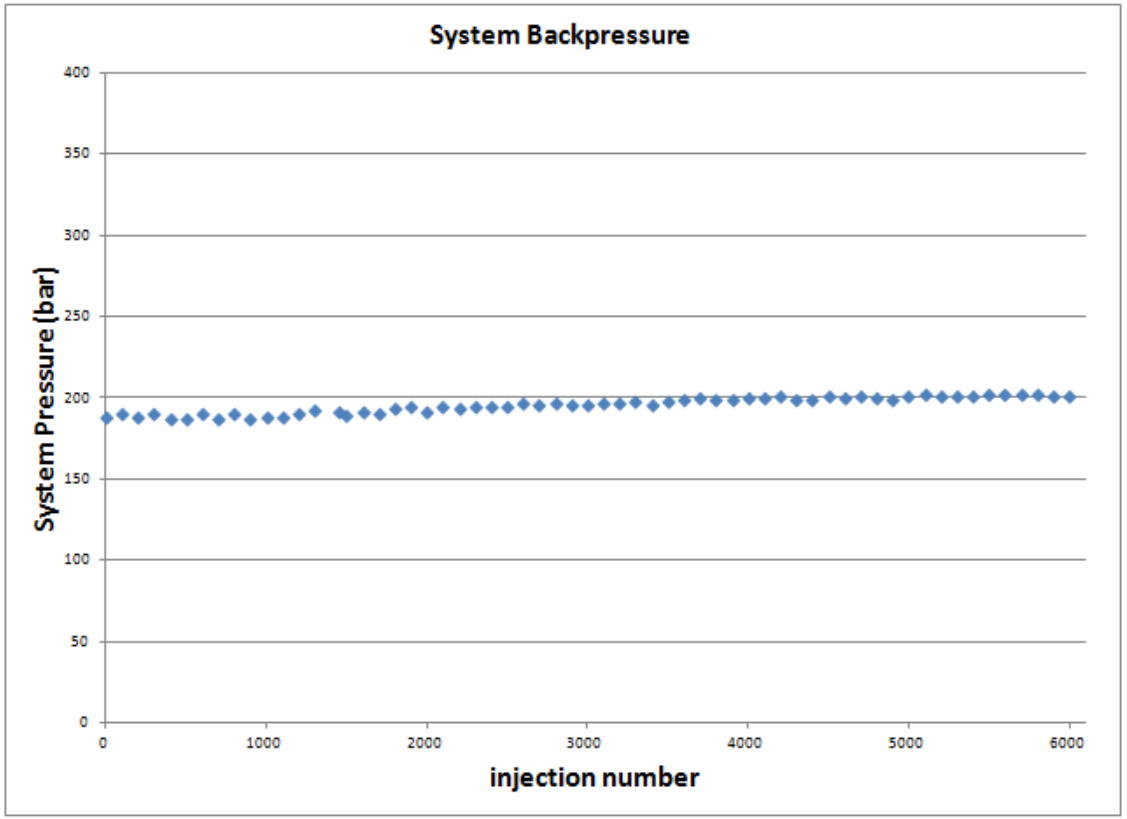
Sample prepared with Captiva ND Lipids to remove proteins, lipids, particulates (bottom trace)

- Ion suppression events are nearly absent – better results

Functional and Mechanical Filtration Benefit: Reducing Backpressure Increases Over Time

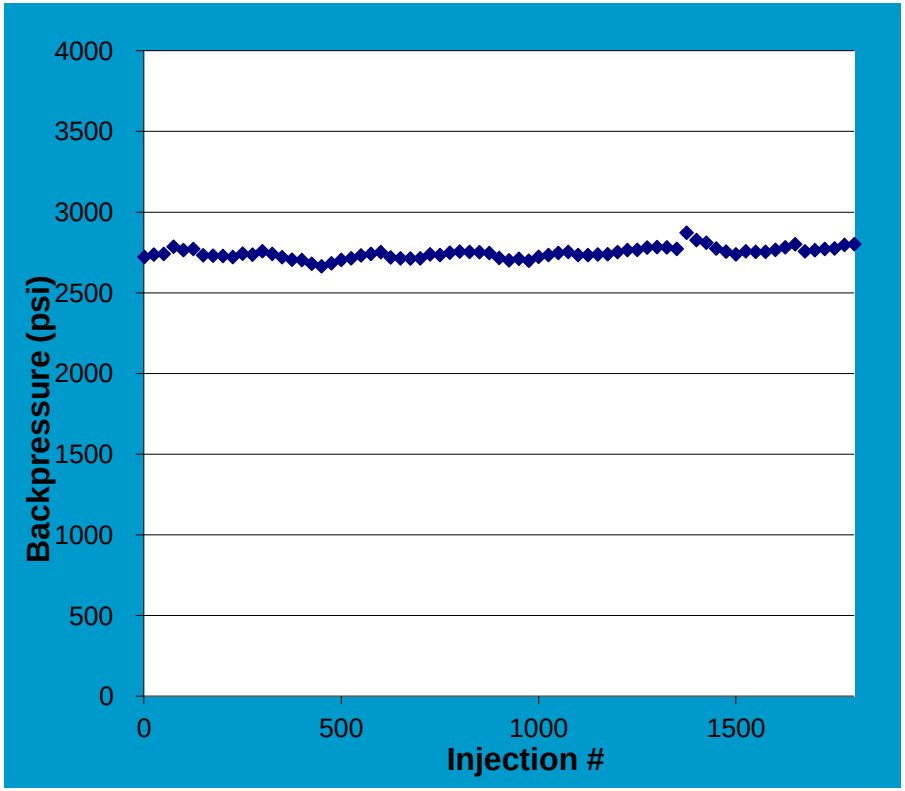
Conditions:
- 6,000 continuous injections
- Human plasma
- UHPLC instrument and column

Results:
Filtration with Captiva ND Lipids led to minimal system backpressure build-up (~7% for entire 6,000 injections)



Backpressure Build-up Comparison

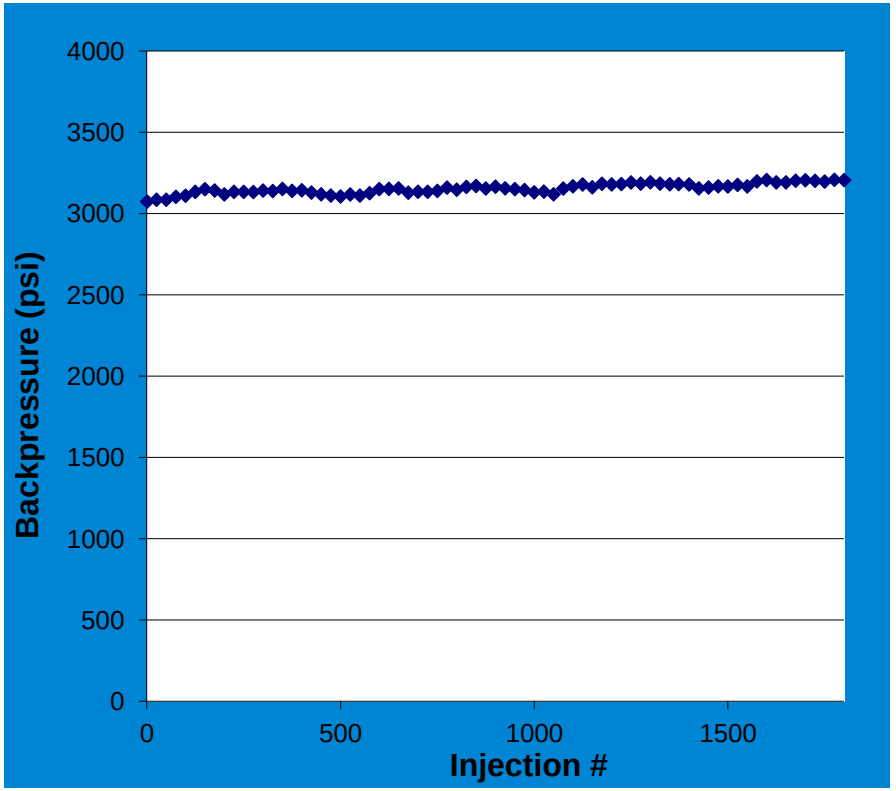
Captiva ND Lipids



Backpressure increase during 1800 injections was 60 psi.

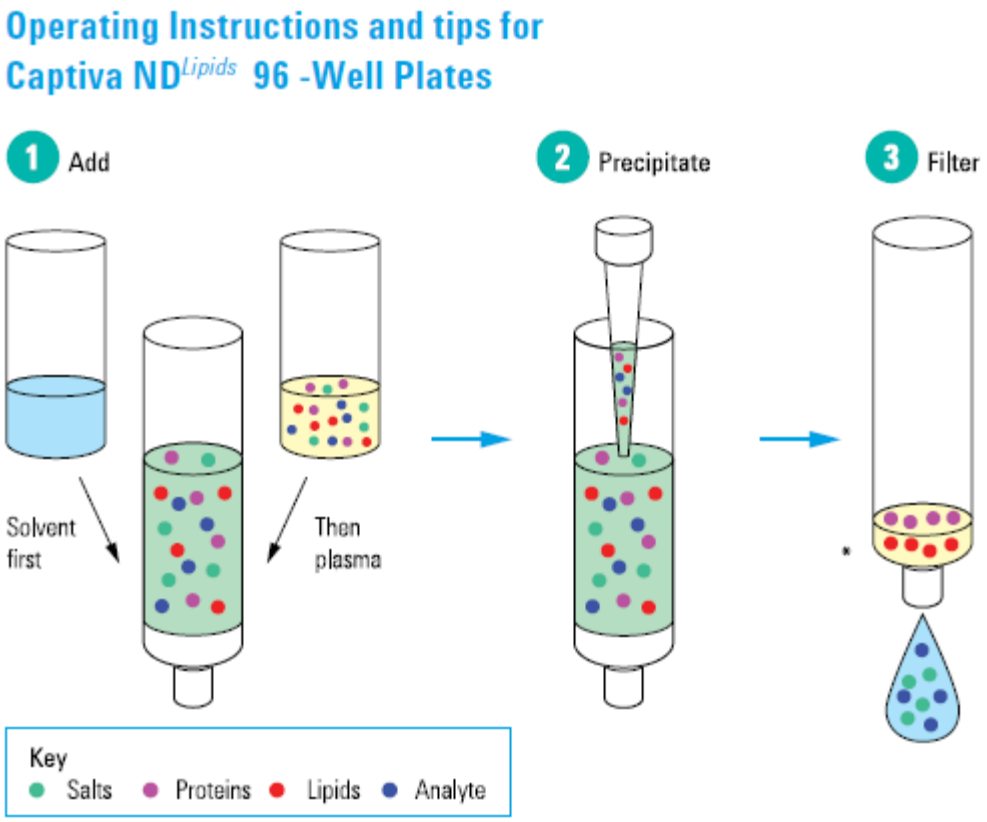
→ **53% less backpressure increase than PPT-centrifugation**

PPT followed by centrifugation

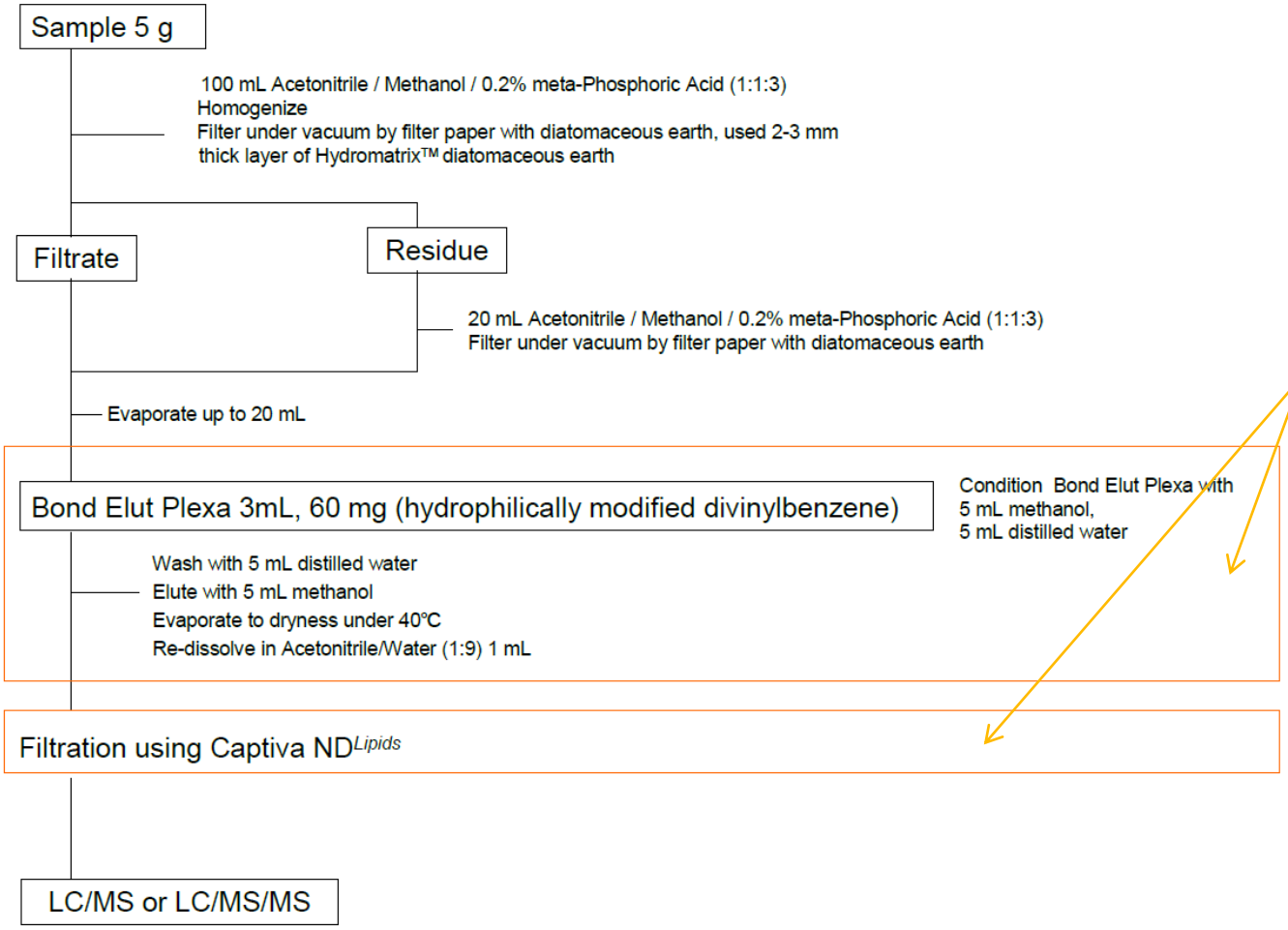


- Starting backpressure is higher than Captiva ND Lipids
- Backpressure increase during 1800 injections was 92 psi.

Simple Methodology - Captiva ND Lipids

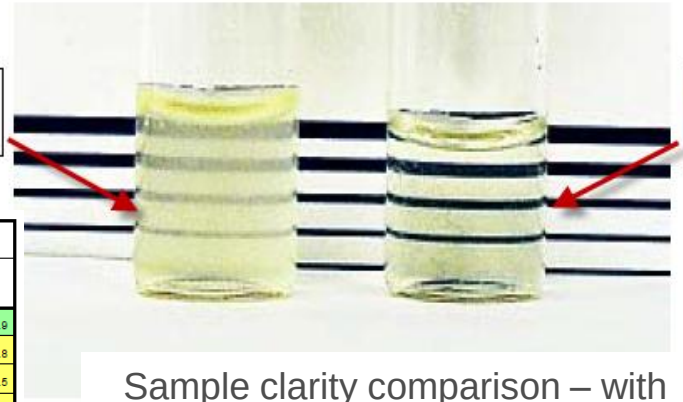


Filtration Benefit: Pair Filtration with Other Sample Preparation Approaches to Improve Results



5 g of animal samples analyzed for veterinary drugs using a multi-step approach to sample cleanup incorporated classical SPE with functional/mechanical filtration (Captiva ND Lipids) for improved results

Filtration Plus SPE – Improved Results and Cleaner Samples



Sample clarity comparison – with and without Captiva ND Lipid filtration

		Milk		Pork		Fish (Yellowtail)		Fish (baked Eel)	
Veterinary Drug	Retention Time	Without Captiva ND Lipids	Filtered by Captiva ND Lipids	Without Captiva ND Lipids	Filtered by Captiva ND Lipids	Without Captiva ND Lipids	Filtered by Captiva ND Lipids	Without Captiva ND Lipids	Filtered by Captiva ND Lipids
Sulfadiazine	7.21	77.9	97.5	109.5	105.8	64.2	103.3	62.1	81.9
Sulfathiazole	8.88	63.5	92.6	90.9	127.9	30.0	127.3	14.9	33.8
Sulfapyridine	9.53	67.9	74.3	75.8	94.9	42.1	96.0	38.2	60.5
Sulfamerazine	9.60	76.5	89.0	91.9	103.3	46.0	99.3	37.1	62.3
Sulfachlorpyridazine	9.70	57.6	74.5	65.1	93.9	28.2	90.8	23.4	40.9
Sulfamethoxazole	9.74	70.0	85.4	83.6	94.8	34.6	87.0	25.6	53.7
Sulfamethoxypyridazine	10.32	66.0	85.1	68.4	86.5	23.7	80.0	22.3	36.8
Thiamphenicol	10.55	38.1	88.4	154.5	117.1	72.3	135.1	32.7	101.2
Sulfadoxine	10.66	71.7	85.3	79.9	97.6	34.3	87.1	32.0	48.4
Sulfadimidine	11.20	66.4	89.3	81.1	96.8	31.3	88.1	26.8	50.5
Sulfamonomethoxine	11.24	68.0	79.1	72.8	90.8	28.0	92.8	21.8	41.6
5-Propylsulfonyl-1 H-benzimidazole-2-amine	12.73	48.6	65.7	50.9	66.3	18.9	70.8	13.1	18.2
Sulfaquinoxaline	12.83	69.9	82.2	24.8	61.4	8.7	49.5	9.2	16.8
Levamisole	12.87	54.1	87.5	82.7	108.1	37.8	104.4	34.0	66.5
Sulfadimethoxine	12.99	58.4	69.2	48.1	78.7	22.5	66.2	20.9	33.3
Trimethoprim	13.02	61.0	85.9	86.4	99.3	38.5	95.1	39.3	63.1
Ormetoprim	13.70	56.2	82.6	75.0	88.9	36.1	90.3	27.1	54.2
Sulfanitran	16.10	56.3	72.6	38.5	81.9	20.9	66.5	28.2	65.7
Thiabendazole	16.19	37.7	56.5	39.5	55.3	17.5	53.4	12.1	21.1
Ethopabate	16.48	55.3	76.4	49.0	78.6	28.9	70.9	17.8	38.0
alpha-Trenbolone	19.91	17.8	33.2	11.3	23.8	6.0	17.8	9.0	19.2
beta-Trenbolone	20.39	30.6	52.2	19.5	34.7	10.0	28.6	9.6	21.2
Zeranol	20.60	31.5	61.0	22.8	50.1	14.3	38.7	21.2	43.9
Melengestrol Acetate	23.00	35.6	86.4	24.4	90.5	4.7	55.3	15.2	62.6
Average Improvement		Milk	21.5%	Pork	20.0%	Yellowtail	49.8%	Eel	22.6%

Recovery as % response versus neat standard in 10% Acetonitrile (10ppb level), n=6.

Key: 0 - 30% | 30 – 70% | 70 – 120% | > 120%

Adding the functionalized filtration step improves recoveries dramatically

Filtration Benefits – Summary

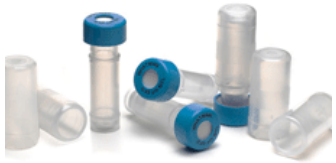
- Filtration produces improved analytical results by removing potentially interfering contamination
- Functional filtration can improve results over mechanical filtration by removing chemical interferences in addition to particulates
- Protecting the instrument and maximizing analytical performance and uptime is straightforward with both mechanical and functional filtration approaches

FILTRATION OPTIONS

Filtration

Agilent Captiva Filtration Products:

Setting a New Standard in Filtration



Captiva Premium Syringe Filters

- Certified to be free of UV detectable extractables on HPLC
  - PES & Glass Fiber also certified for LC/MS
- Color-coded boxes for easy identification
- Comprehensive portfolio to meet your application needs

Premium Syringe Filters						
Membrane	Diameter / Pore size					
	4 mm		15 mm		25 mm (* 28 mm)	
	0.2 µm	0.45 µm	0.2 µm	0.45 µm	0.2 µm	0.45 µm
PTFE	♦	♦	♦	♦	♦	♦
Nylon			♦	♦	♦	♦
PES	♦	♦	♦	♦	♦	♦
Regenerated Cellulose	♦	♦	♦	♦	♦	♦
Cellulose Acetate					♦	♦
Glass Microfiber			♦		♦*	
Depth filters: Glass / PTFE			♦	♦	♦	♦*
Depth filters: Glass / Nylon			♦	♦	♦	♦

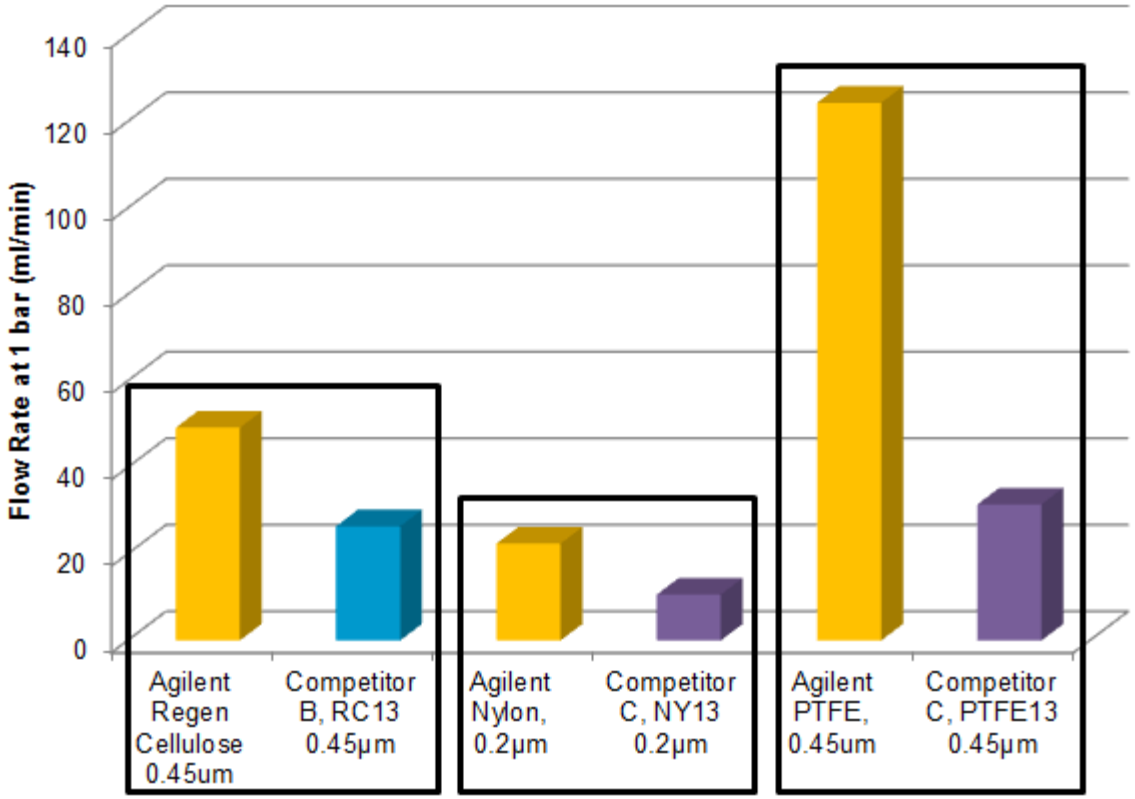
Cellulose Acetate | Nylon | Glass Fiber/ PTFE | Glass Fiber | PES | PTFE | Regenerated Cellulose | Glass Fiber/ Nylon

Captiva Premium Syringe Filters: Advantages

Feature	Advantages	Benefits
Unique Syringe Filter construction	Cleanliness; certified to be free of UV-detectable extractables	• Minimizes introduction of contamination by the filter • Reduce need for retesting • Accuracy
	Fastest flow rates	• Productivity / throughput
	High loading capacity	• Productivity
	Minimal analyte adsorption	• Maximum target analyte recovery • Accuracy • Reproducibility
Ultrasonic weld and reinforced membrane	Withstands high pressure and force	• Rugged • Reliable

Captiva Premium Syringe Filters: Fastest Flow Rates

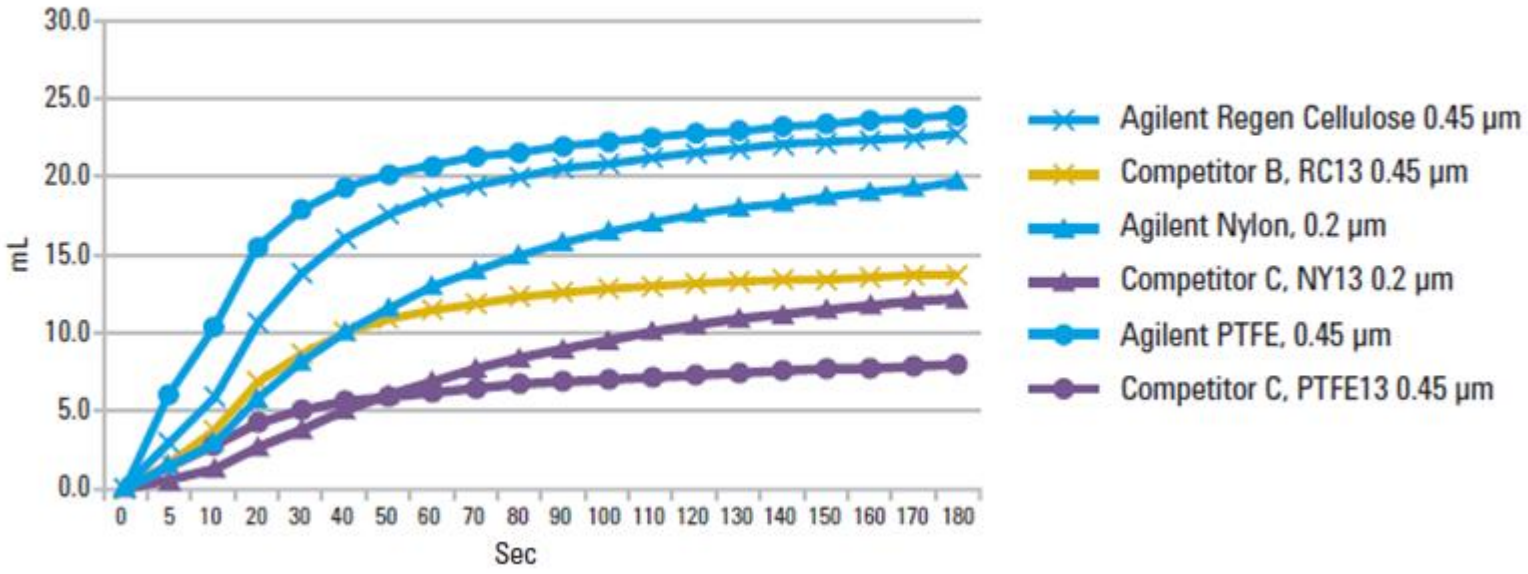
Agilent **RC**, **Nylon** and **PTFE** filters compared to competitors



Captiva Premium Syringe Filters: High Loading Capacity

- ✓ Due to nature of membrane, there is less clogging by particulates
- ✓ Assures more sample will be filtered

Capacity (volume) of 15 mm syringe filters over time (with Particulate-Laden Samples)

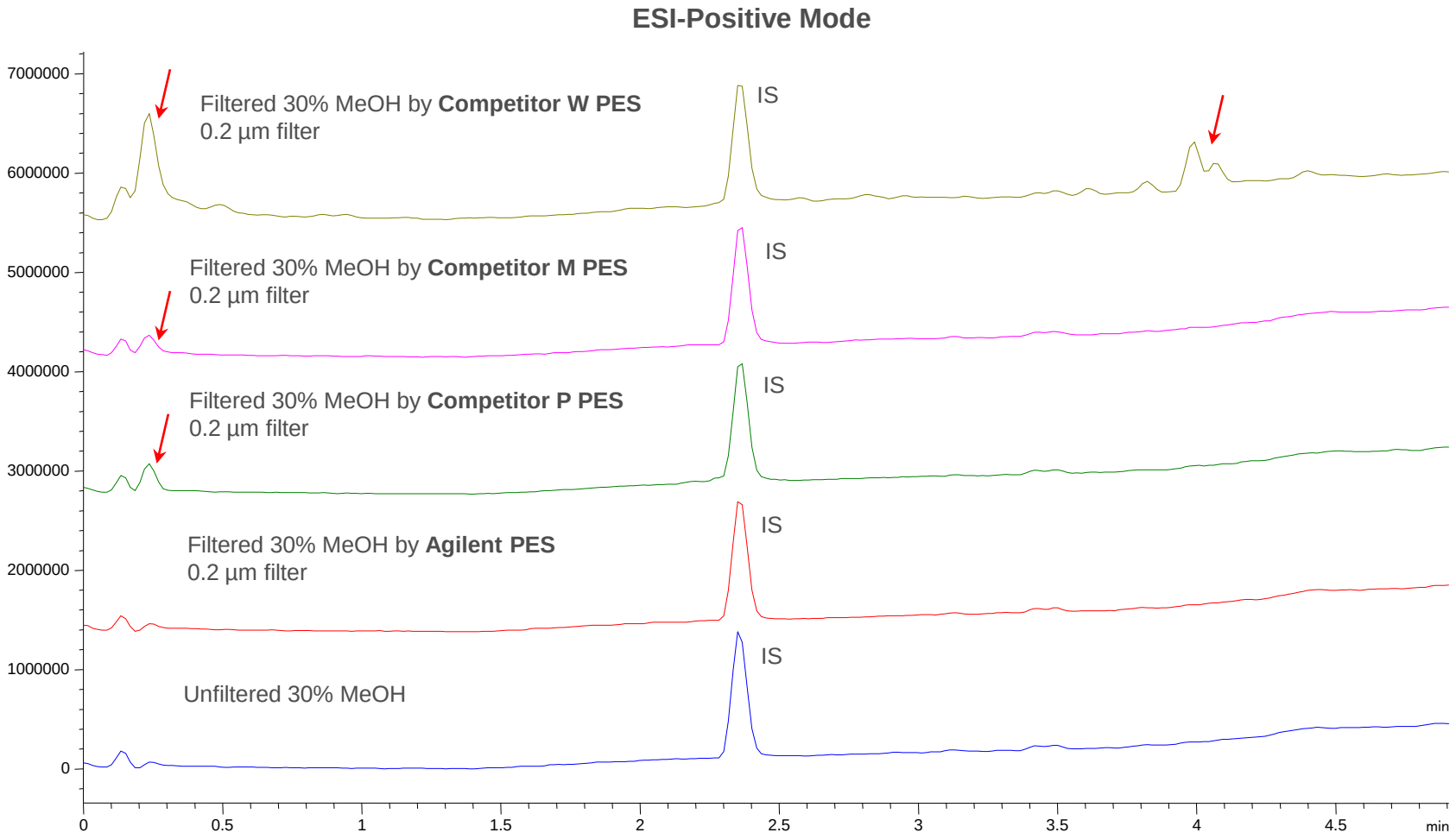


Captiva Premium Syringe Filters: Performance

Agilent Captiva Syringe Filters provide consistent and higher than 90% filtration efficiency on particulate removal

	Agilent premium 0.2µm syringe filters						Agilent premium 0.45µm syringe filters					
	Nylon	PTFE	RC	PES	GF/NY	GF/PTFE	Nylon	PTFE	PES	CA	GF/NY	GF/PTFE
1	96.0	92.3	89.8	92.1	99	99.4	95.2	97	93.6	92.4	96.8	98.4
2	95.9	91.4	90.6	91.4	99	98.9	93.2	96.5	93.6	95.0	97.1	98.8
3	94.5	93.3	90.3	89.5	99.2	99.0	95.5	97.5	93.5	96.3	96.4	97.7
4	96.6	92.3	91.7	99.0	99.6	98.6	95.4	96.6	88.5	97.2	99.3	98.8
5	95.4	91.2	92.4	96.3	98.8	98.8	94.9	96.0	88.2	96	99.0	99.7
6	95.6	91.1	90.8	99.9	99.3	98.5	95.3	95.7	92.3	95.6	100	96.8
7	99.9	91.1	98.2	99.0	99.4	99.4	99.5	95.2	94.9	96.7	98.2	97.6
8	99.8	91.2	99.0	97.8	95.0	99.0	98.0	97.8	89.4	93.8	98.9	98.5
9	99.7	90.9	96.4	95.2	95.9	99.9	97.7	94.9	87.3	92.5	100.2	98.0
10	99.2	91.3	95.7	96.1	94.7	99.6	99.7	94.8	87.5	92.8	100.5	101.3
Average Eff (%)	97.3	91.6	93.5	95.6	98.0	99.1	96.4	96.2	90.9	94.8	98.6	98.6
RSD (%)	2.2	0.8	3.7	3.7	2.0	0.5	2.2	1.1	3.3	1.9	1.5	1.3

Cleanliness of Agilent Captiva PES Filters



Additional Tools to Help You Select the Right Syringe Filter

Membrane compatibility: online, in each box, in brochu

Chemical Compatibility

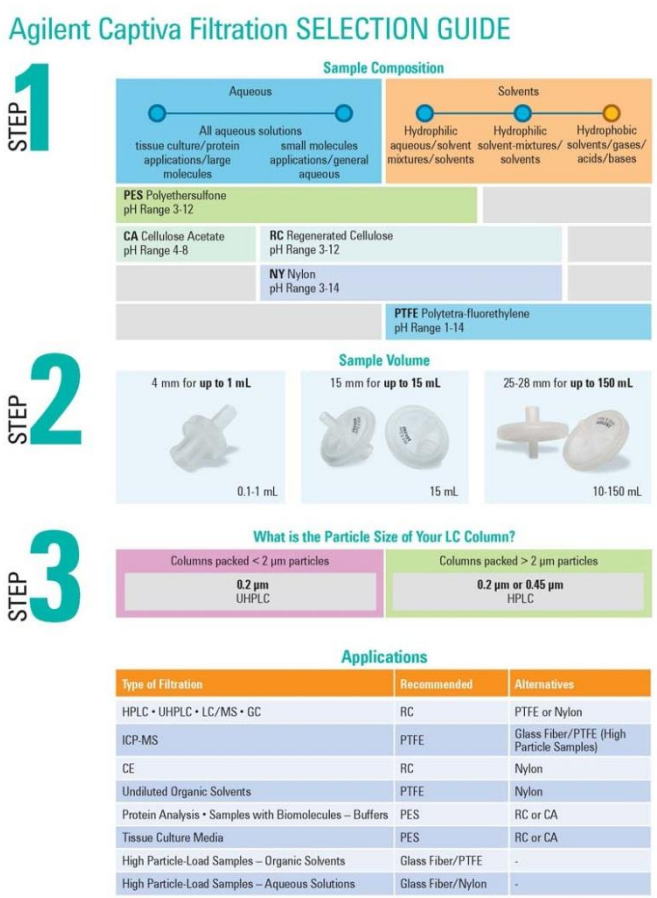
LEGEND	
Compatible	••
Limited compatibility	•
Not compatible	–
Not analyzed	N/A

Contact time: 24 hours at 20 °C.
Chemical compatibilities can be influenced by various factors. Therefore, we recommend that you confirm compatibility with the liquid you want to filter by performing a trial filtration run before you start your actual filtration.

FILTER	Polypropylene membrane	Polyethersulfone membrane	Cellulose Acetate membrane*	Polytetrafluoroethylene membrane	Regenerated Cellulose membrane	Nylon membrane	Glass Fiber membrane*	Housing Methacrylate Butadiene Styrene	Housing Polypropylene
Housing	PP	PES	CA	PTFE	RC	Nylon	GF	MBS	PP
Solvents									
Acetone	••	–	–	••	••	••	••	–	••
Acetonitrile	•	–	–	••	••	N/A	••	–	••
Benzene	–	–	•	••	••	••	••	–	••
Benzyl alcohol	••	–	–	••	••	••	••	–	•
n-Butyl acetate	N/A	–	–	••	••	••	••	–	••
n-Butanol	••	•	•	••	••	••	••	••	••
Carbon tetrachloride	•	–	–	••	••	••	••	–	–
Chloroform	•	–	–	••	••	••	••	–	••
Cyclohexane	••	–	•	••	••	••	••	•	•
Diethylacetamide	••	–	–	••	••	••	••	–	••
Diethyl ether	•	–	•	••	••	••	••	–	••
Dimethyl formamide	••	–	–	••	•	•	••	–	•
Dimethylsulfoxide	••	–	–	••	••	••	••	–	••
Dioxane	•	–	–	••	••	••	••	–	••
Ethanol, 98%	••	••	•	••	••	••	••	–	•
Ethyl acetate	•	–	–	••	••	••	••	–	•
Ethylene glycol	••	••	•	••	••	••	••	••	••
Formamide	N/A	••	–	••	•	••	••	••	••
Gasoline	•	•	•	••	••	••	••	••	••
Glycerin	••	••	•	••	••	••	••	•	•
n-Heptane	–	••	•	••	••	••	••	•	••
n-Hexane	–	••	•	••	••	••	••	•	•
Isopropanol	••	••	•	••	••	••	••	–	••
Isopropyl acetate	N/A	–	–	••	••	••	••	–	••
Methanol, 30%	••	••	N/A	••	••	••	••	••	••
Methanol, 98%	••	•	–	••	••	••	••	••	•
Methyl acetate	•	–	–	••	••	••	••	–	•
Methylene chloride	•	–	–	••	••	••	••	–	••
Methyl ethyl ketone	•	–	–	••	••	••	••	–	•
Methyl isobutyl ketone	•	–	–	••	••	••	••	–	•

*CA and GF membranes in MBS housing for 28 mm size

Syringe Filter Selection Guide: Online, in brochure



Captiva, Captiva ND, and Captiva ND Lipids Plate and Cartridge Formats

In addition to syringe filters, Captiva filtration products also include 96-well plates and 3 mL cartridge formats, to support a wide variety of filtration needs.

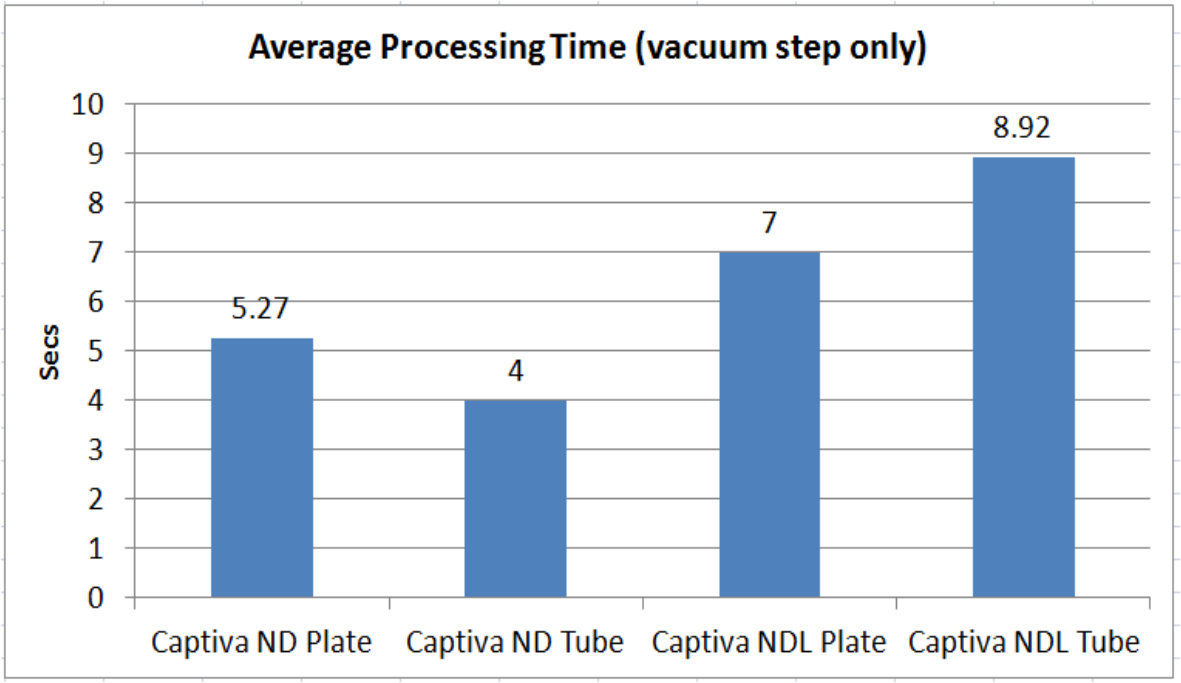
- Plate formats suitable for automation platforms, small sample volumes, and high throughput
- 3 mL cartridges support larger sample volumes, automation (by vacuum/positive pressure manifolds), and lower throughput
- Available in Captiva filtration and in the “ND” – Non-Drip – configurations. Non Drip configuration allows for protein precipitation inside the cartridge/well. No dripping until vacuum/positive pressure applied
- “Functional” filtration with Captiva ND lipids supports lipid removal to provide cleaner samples

Captiva Average Processing Time:

Plates vs Cartridges

Conditions:
- 300 µL ACN + 100 µL plasma
- Vacuum step only
- ~15 in Hg

Results:
Average processing time of the Captiva products show good correlation, and supports the benefit of fast flow.



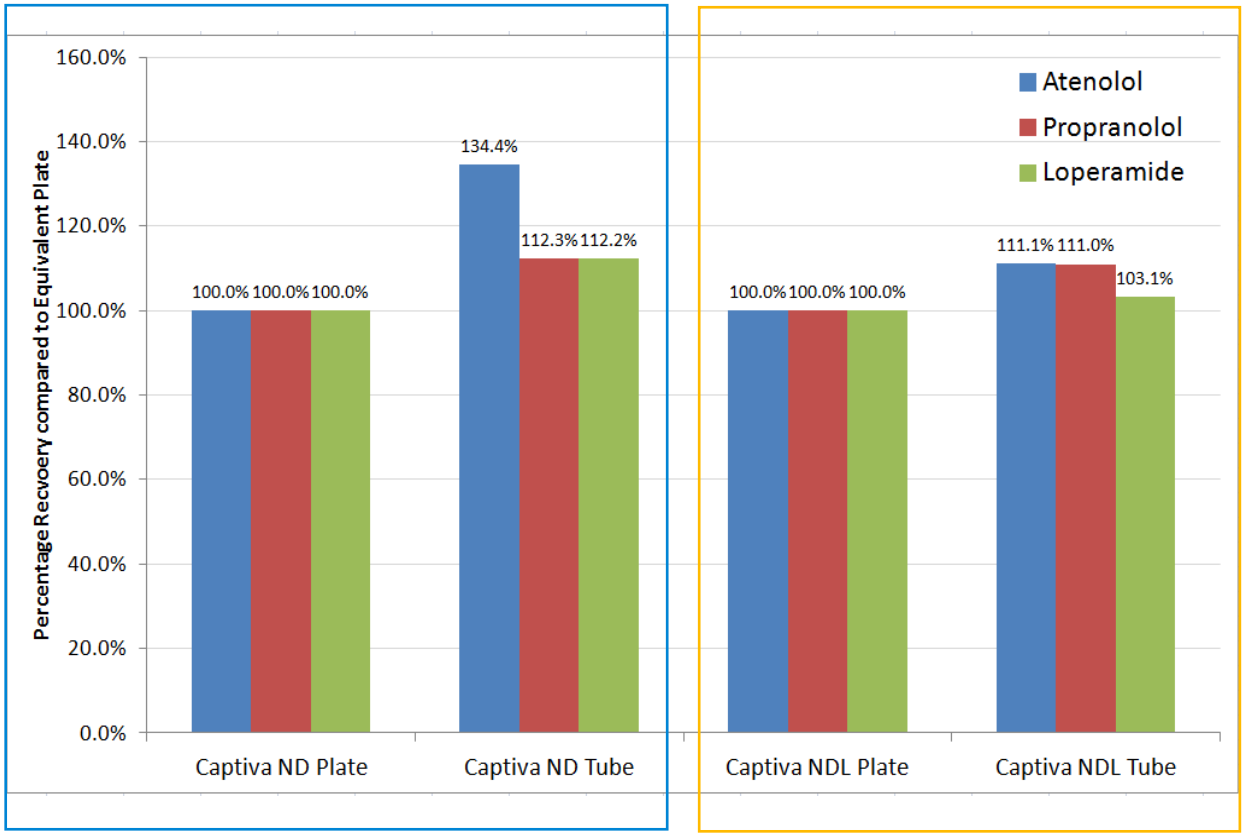
Captiva ND and ND Lipids - Recoveries

Conditions:

- Captiva Plates: 100 µL plasma
- Captiva Cartridges: 500 µL plasma (contains 4X more lipid stripping sorbent than plate)
- Drugs: Atenolol, Propanolol, Loperamide

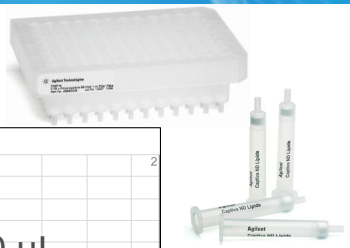
Results:

Cartridges showed comparable recovery of three drugs compared to plates. This demonstrates correlation



Captiva ND Lipids

Phospholipid-stripping performance



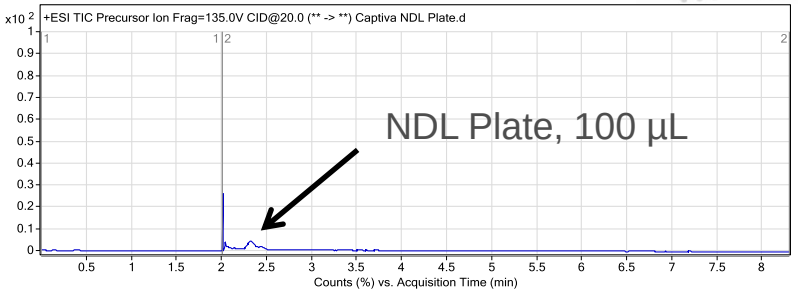
Conditions:

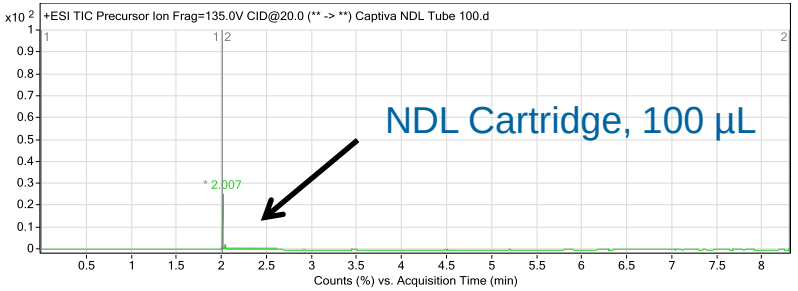
- Captiva NDL vs off-line protein precipitation
- Sample = plasma
- Tube tested with both 100 µL (same sample size as plate) and 500 µL (ideal for 3 mL tube)
- Instrument = Agilent 1260 LC module with a 6460 QQQ LC/MS/MS

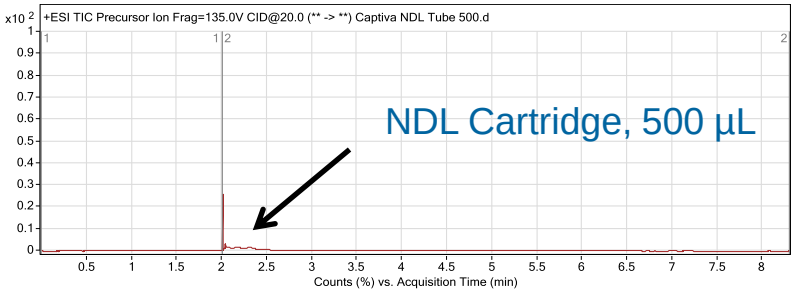
Results:

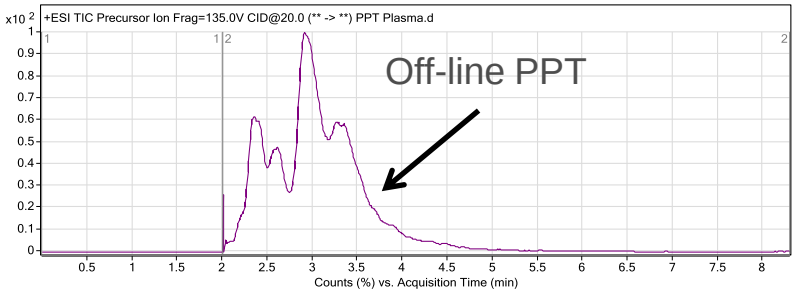
Compared to off-line PPT (no lipid stripping), we can see the effectiveness of the Captiva NDL products, and how completely they remove ion-suppressing phospholipids

Technique	% phospholipid remaining in sample
PPT	100%
NDL plate	**1.24%**
NDL cartridge 100 µL	**0.33%**
NDL cartridge 500 µL	**1.04%**







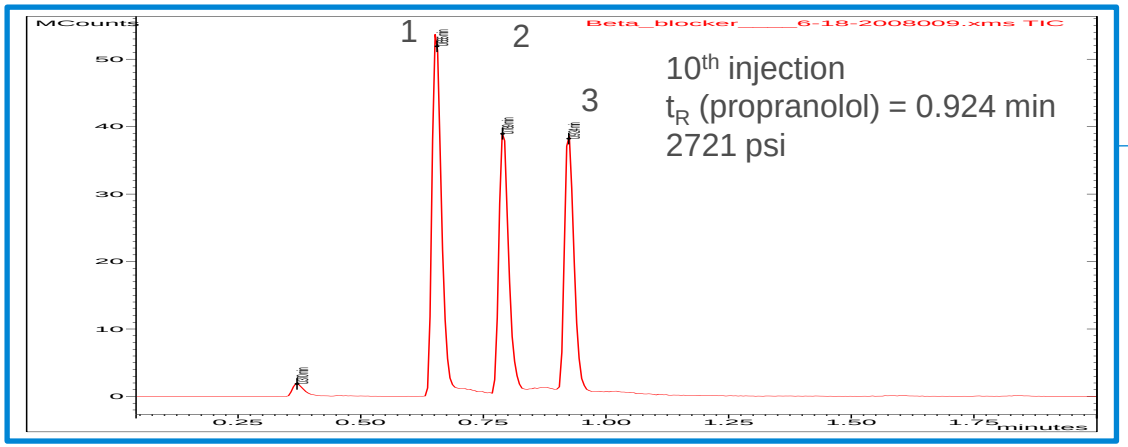


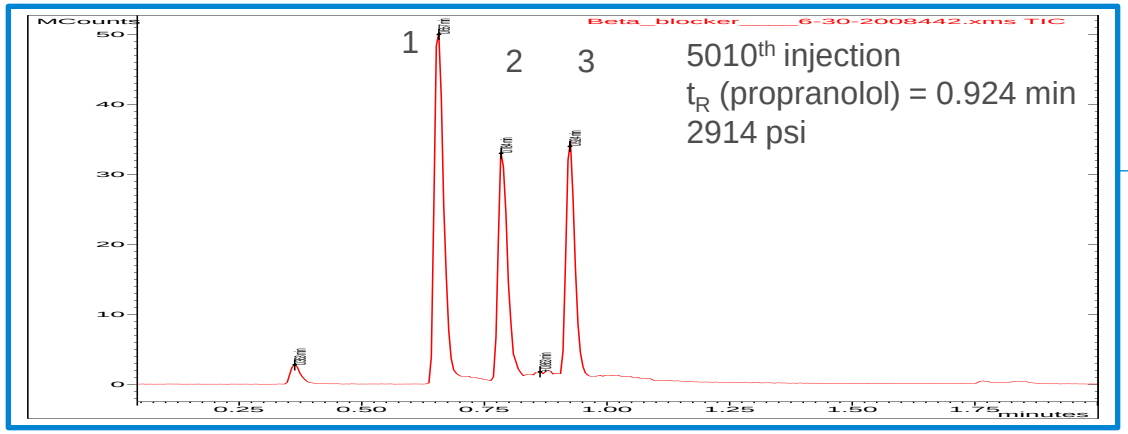
Sample Preparation Time Comparison PPT (centrifugation) vs. Captiva ND Lipids

Captiva ND Lipids	Time (min)
Add 0.6 mL of MeOH and 0.2 mL of plasma sample to Captiva ND 96-well plate.	5
Mix each well with a pipette 5 times and apply vacuum for filtration.	
Directly transfer injection plate for analysis.	0
Total time required for sample preparation	**5**

This time comparison is based on the preparation of 96 samples.

Captiva ND Lipids Application: Reproducibility





LC/MS chromatograms over 5000 injections show no retention time shift.

1. Atenolol
2. Pindolol
3. Propranolol

Captiva Accessories

- Captiva Disposable Syringes (5, 10, 20 mL)

- CaptiVac vacuum collar, Captiva deep-well collection plate, and pierceable plate covers

- Vac Elut 20 and 12 port vacuum manifolds

- Vac Elut SPS 24 vacuum manifold

Captiva plates and Cartridges: Advantages

Feature	Advantages	Benefits
Non-Drip Filtration Capabilities	In-cartridge or in-plate protein precipitation	• Automation • Reproducible recoveries • No dripping without vacuum or positive pressure
Dual-depth Filter	Non-linear path with two layers	• High capacity for particulates • More residence time for mechanical filtration and adsorption to occur for cleaner samples
Non-Drip Lipids Capability	Phospholipid removal	• Increased cleanup of samples through removal of lipids and phospholipids • Longer instrument uptime • Minimized ion suppression in LC-MSMS due to phospholipid interference

FILTRATION RECOMMENDATIONS & SUMMARY

Filtration

Filtration and Other Sample Preparation Techniques

	More Specific ← **Instrument Separation and Detection Specificity** ← *Less Specific*								
	Less Specific → ***Sample Preparation Specificity*** → *More Specific*								
Sample Prep Technique / **Interference Removed**	**Dilute & Shoot**	**Filtration**	**Liquid/Liquid Extractions**	**Supported Liquid Extractions (SLE)**	**Dried Matrix Spotting**	**Precipitation**	**QuEChERS**	**Lipid Removal 'Hybrid' Filtration**	**Solid Phase Extraction**
Lipids	No	No	No	Some	No	No	Yes	Yes	Yes
Oligomeric Surfactants	No	No	No	No	No	No	No	Yes	Yes
Particulates	No	Yes	No	Some	No	Yes	Yes	Yes	Yes
Pigments	No	No	No	Some	No	No	Yes	No	Yes
Polar Organic Acids	No	No	Yes	Yes	No	No	Yes	No	
Proteins	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Salts	No	No	Yes	Yes	No	No	No	No	Yes
Suggested Agilent Product	Agilent Autosampler Vials	Captiva Syringe Filters		Chem Elut	Bond Elut DMS	Captiva ND	Bond Elut QuEChERS	Captiva ND LIPIDS	Bond Elut Silica and Polymeric SPE

Agilent Captiva Filtration Products are recommended for use with any LC or LC-MS method

Captiva Filtration Product Recommendation

	Filtration Needs/Situation						
Agilent Captiva Product Format	**Low Throughput**	**Vacuum processing**	**In-well protein precipitation**	**Particulate filtration only**	**Automation Friendly**	**Lipid removal**	**Larger sample size**
Single cartridge	X	X		X	X		X
Single ND cartridge	X	X	X	can be done	X		X
Single ND Lipids cartridge	X	X	X		X	X	X
96-well plate		X		X	X		
96-well ND plate		X	X	can be done	X		
96-well ND Lipids plate		X	X		X	X	
Syringe filter	X	can be done		X	can be done		X

Conclusions and Summary

- Filtration is a straightforward means of preparing samples when minimal cleanup is needed
- Mechanical filtration to remove particulates maximizes analytical uptime
- Functionalized filtration can further enhance standard sample preparation methods
- Combining filtration with other sample preparation processes can improve result quality and maximize analytical uptime
- Agilent offers a wide range of filtration products to suit your needs, from syringe filters through specialized filtration products

Additional Resources and Application Support

Reference Materials and Guides:

Agilent Captiva Plates and Cartridges Guide:

http://www.chem.agilent.com/Library/flyers/Public/5990-9061EN.pdf

Agilent Captiva Syringe Filter Interactive Selection Tool:

www.agilent.com/chem/SelectFilters

Agilent Captiva Syringe Filter Selection guide:

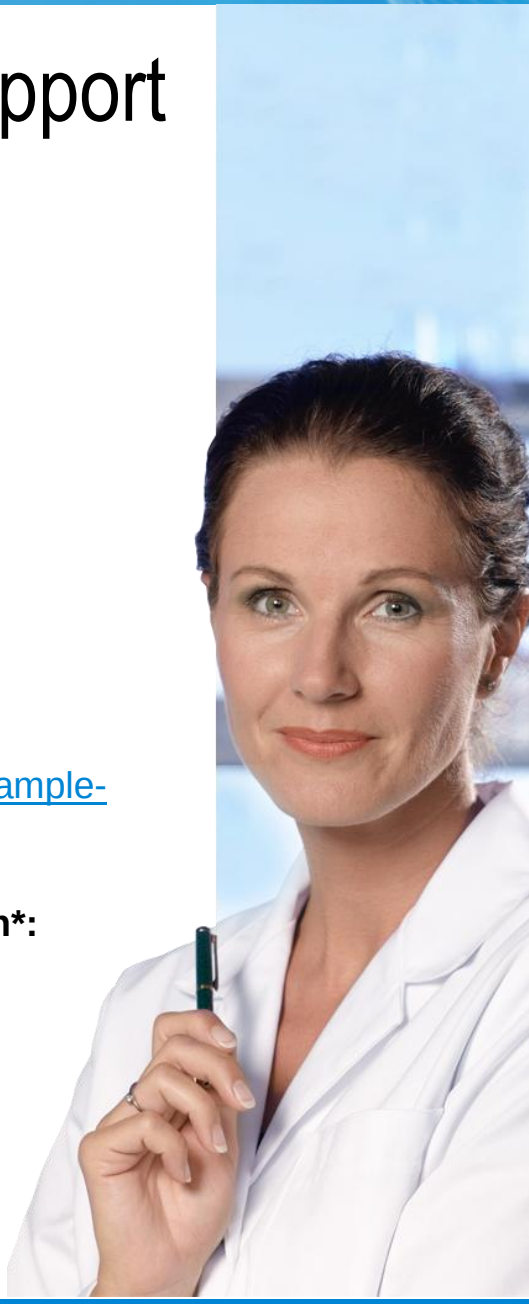
www.chem.agilent.com/en-US/products-services/Columns-Sample-Preparation/Sample-Preparation/Filtration/Syringe-Filters/Pages/Selectionguide.aspx

Agilent Sample Preparation Products Technical Support Contact Information*:

Phone: 800-227-9770, Options 3, 3, 3

Email: spp-support@agilent.com

* *North America*



Acknowledgements

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Questions?