Tips for Developing Successful Solid Phase Extraction Methods

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October 4, 2022

Agilent InfinityLab

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Agenda

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1. Solid Phase Extraction (SPE) – Why and How?
2. Selecting the right SPE – How?
   - Analyte
   - Sample matrix
   - Sorbents and capacity
   - SPE formats
   - SPE processing
3. Developing SPE methods and troubleshooting
   - Nonpolar method
   - Ion exchange method
   - Mixed-mode method
4. What's New?
5. Summary

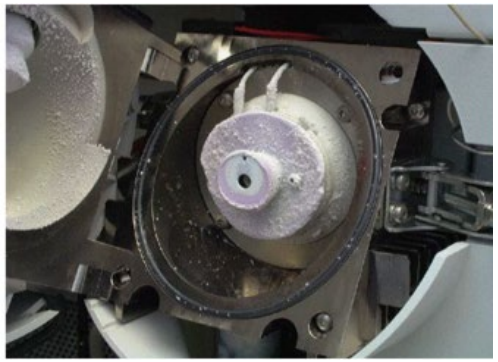
Solid Phase Extraction – Why and How?

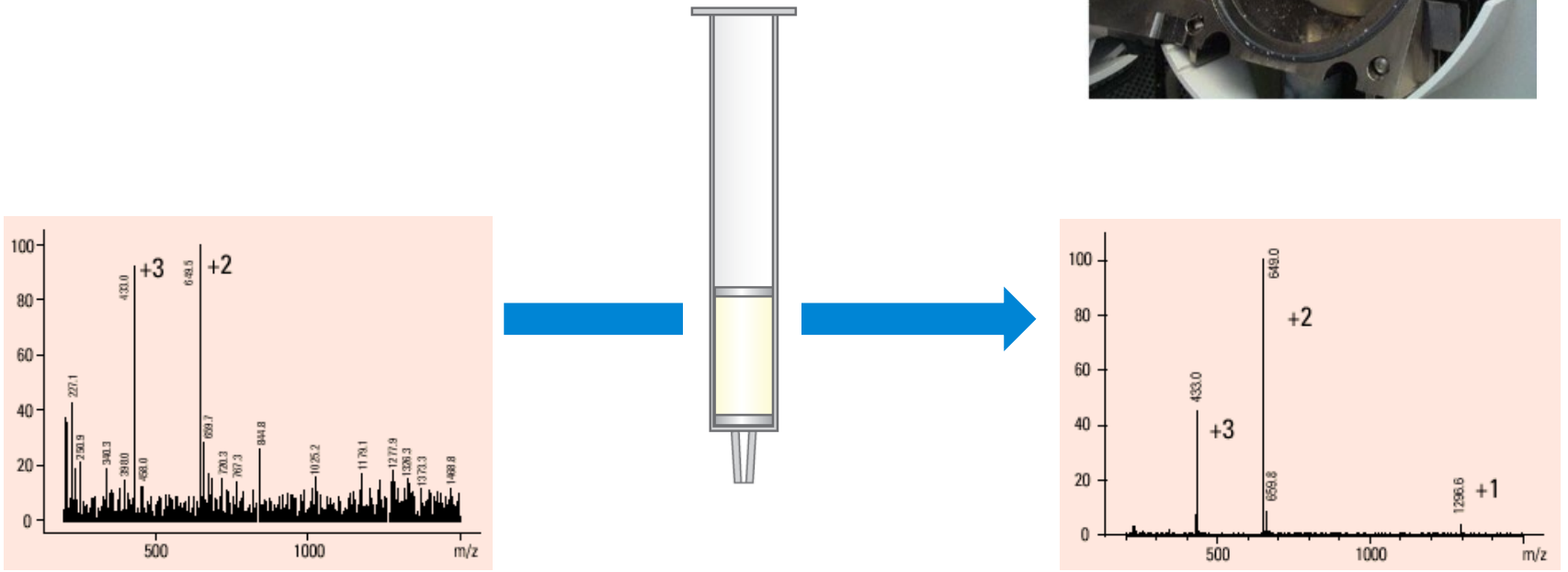
Offline Options for Sample Matrix Removal

	More specific ← **Instrument separation and detection specificity** ← *Less specific*					
	Less specific → ***Sample preparation specificity*** → *More specific*					
Sample Preparation Technique / **Interference Removed**	**Filtration**	**Supported Liquid Extractions (SLE)**	**Protein Precipitation and Filtration**	**QuEChERS**	**Protein Precipitation, Filtration and Lipid Removal**	**Solid Phase Extraction**
Lipids	No	No	No	Yes	Yes	Yes
Oligomeric Surfactants	No	No	No	No	Yes	Yes
Particulates	Yes	Some	Yes	Yes	Yes	Yes
Pigments	No	Some	No	Yes	No	Yes
Polar Organic Acids	No	Yes	No	Yes	No	Yes
Proteins	No	Yes	Yes	Yes	Yes	Yes
Salts	No	Yes	No	No	No	Yes
Suggested Agilent Product	Captiva syringe filters Captiva filter vials	Chem Elut S	Captiva ND	Bond Elut QuEChERS with d-EMR-Lipid and other dispersive	Captiva EMR-Lipid	Bond Elut Silica and Polymeric SPE

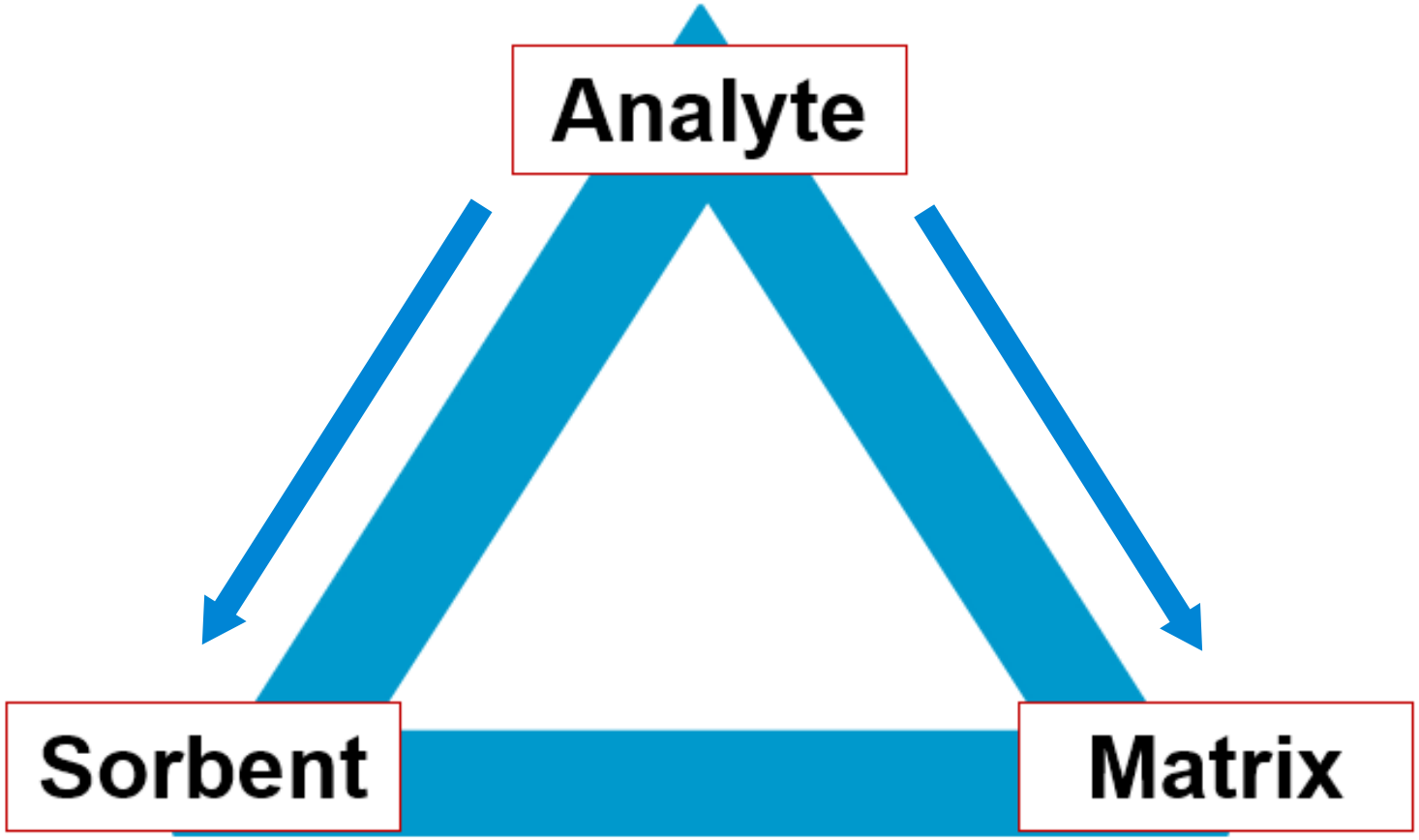
Solid Phase Extraction – Why?

- To remove matrix interferences, such as proteins, lipids, pigments, and salts
- To increase the concentration of analytes
- To do a solvent exchange





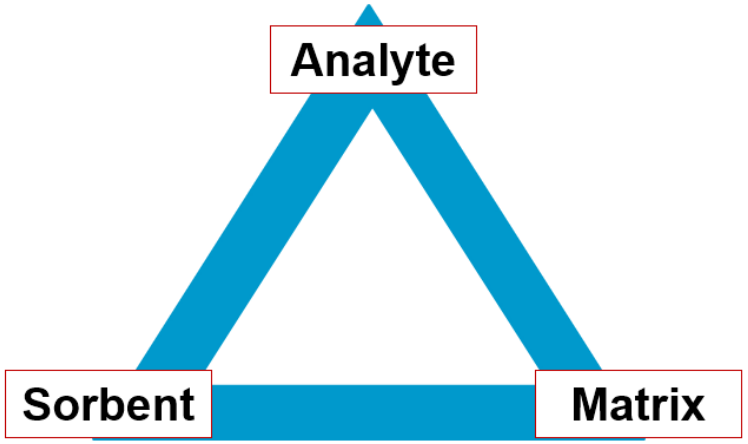
What Happens During Solid Phase Extraction?

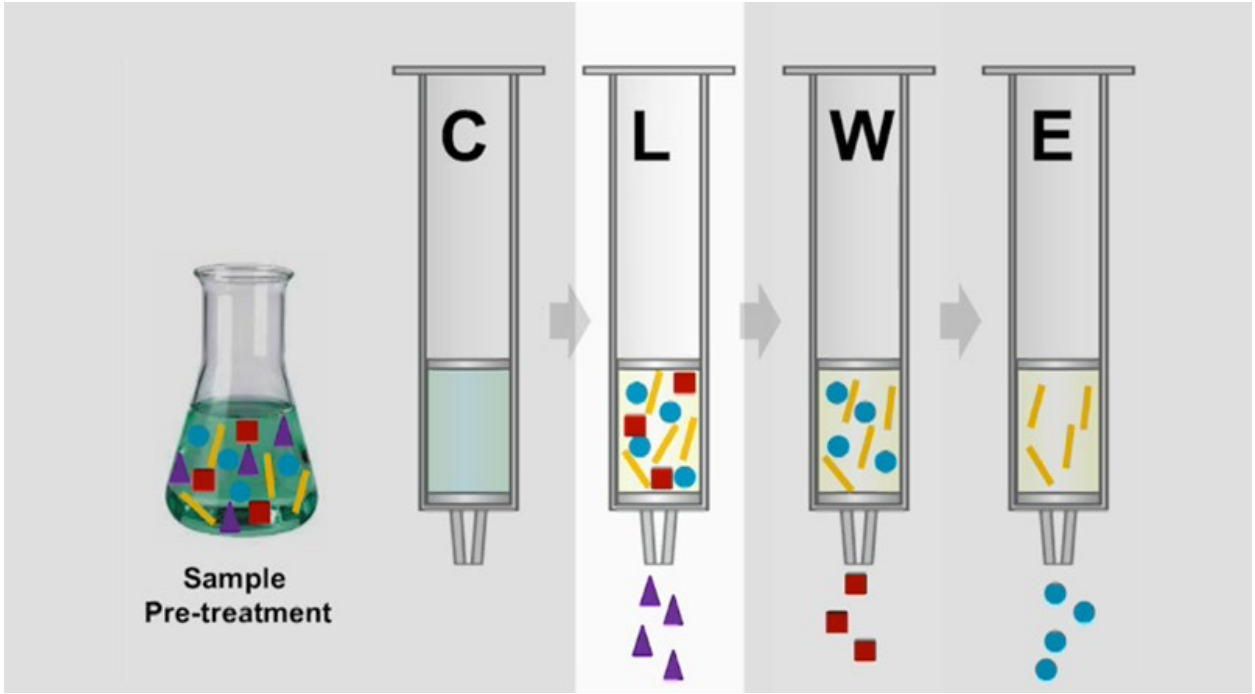


Solid Phase Extraction – How?

SPE workflow

- Sample pretreatment
- Condition and equilibrate
- Load
- Wash
- Elute





Selecting the Right SPE – How?

Do Your Research

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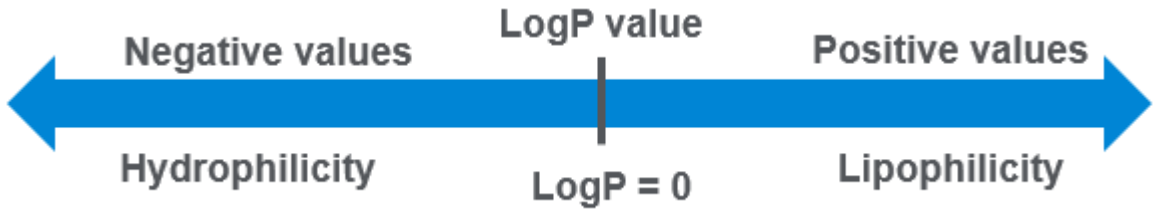
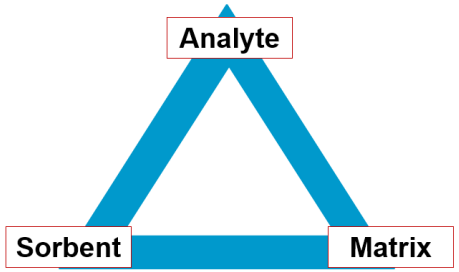
- You may not need to start from scratch. Use existing methods as the starting point for your method development
- Does any LC chromatographic data exist on the analytes?
- Carefully examine the molecular structure of the analyte, its solubility, and other tendencies
- Consider physical state of the sample, the matrix
- Consider the analysis technique and its appropriate sample solvent; if derivatization is needed, for example

Analyte Considerations

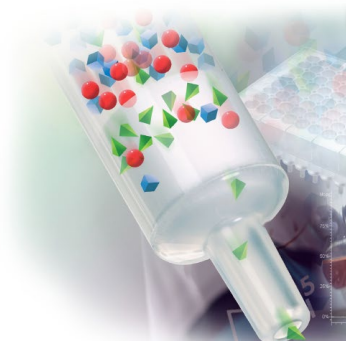
- Is the analyte nonpolar or polar (C, H, N, O)?
- Does the analyte contain any ionic groups?
- Is the analyte a small molecule or a large molecule?
- Is the analyte unstable in acid or base?
- What is the logP and pKa of the analyte?
- What is the approximate concentration of the analyte in sample?
- Any likelihood of the analyte undergoing nonspecific binding to glassware or plastic?
- Detection limit of the analytical system for the analyte

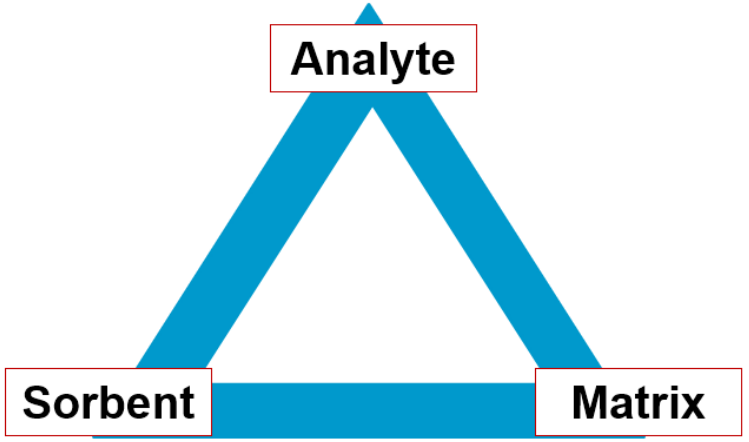




Matrix Considerations

- What is the sample matrix, is it polar, nonpolar?
- What are the major interferences in the matrix?
- What are the differences between interferences and analyte?
- Is any sample pretreatment such as pH adjustment necessary?
- Is the matrix viscose, does it need dilution?
- Is an internal standard required?



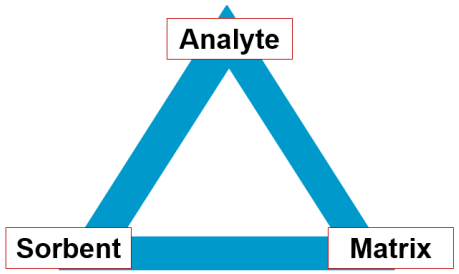


Sample Considerations

- Sample volume
- The number of samples to be processed
- If sample is solid, how do you process it and extract it?
- Cost per sample/sample processing time/number of steps/simplicity

SPE Extraction Mechanisms

- Hydrophobic interaction
- Polar interaction
- Ion Exchange
- Mixed mode (hydrophobic + ion exchange)
- Other: covalent



SPE Sorbents

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Sorbents

- Nonpolar
- Polar
- Cation exchange
- Anion exchange
- Mixed mode
- Covalent
- Specialty sorbents

Silica based

- More than 40 phases and selectivities
- Application-specific phases
- Wide range of published applications
- Method development/optimization may be required

Polymer based

- Simple generic methods
- Less method optimization required
- Faster flow
- Higher capacity
- Greater pH range

Bond Elut SPE – Available Phases

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Nonpolar

C18, C8, C2, C1

C18 variations in carbon load and endcapping
EnvirElut
CH – cyclohexyl
CN-E – endcapped cyanopropyl
PH – phenyl
Plexa, PPL, ENV, LMS, Focus, Nexus

Polar

PSA - primary and secondary amine
NH2 - aminopropyl
DEA - diethylaminopropyl
Diol - diol
SI - silica
CN - nonendcapped cyanopropyl

Cation Exchange

SCX – benzenesulfonic acid
PRS – propylsulfonic acid
CBA – carboxylic acid
Nexus WCX

Anion Exchange

SAX – quaternary amine
PSA – primary and secondary amine
NH2 – aminopropyl
DEA – diethylaminopropyl

Covalent

PBA – phenylboronic acid

Silica/Polymeric

Mixed Mode IEX/Nonpolar

Certify – SCX/C8
Certify II – SAX/C8
Plexa PCX
Plexa PAX
PFAS WAX

Specialty Phases

Lipid Extraction
AccuCAT
Atrazine
Mycotoxin

Alumina – aluminum oxide
Florisil – magnesium-silica
Carbon S – synthetic carbon
Carbon
Carbon/NH_2

Capacity and Void Volume of Sorbent Packed Bed

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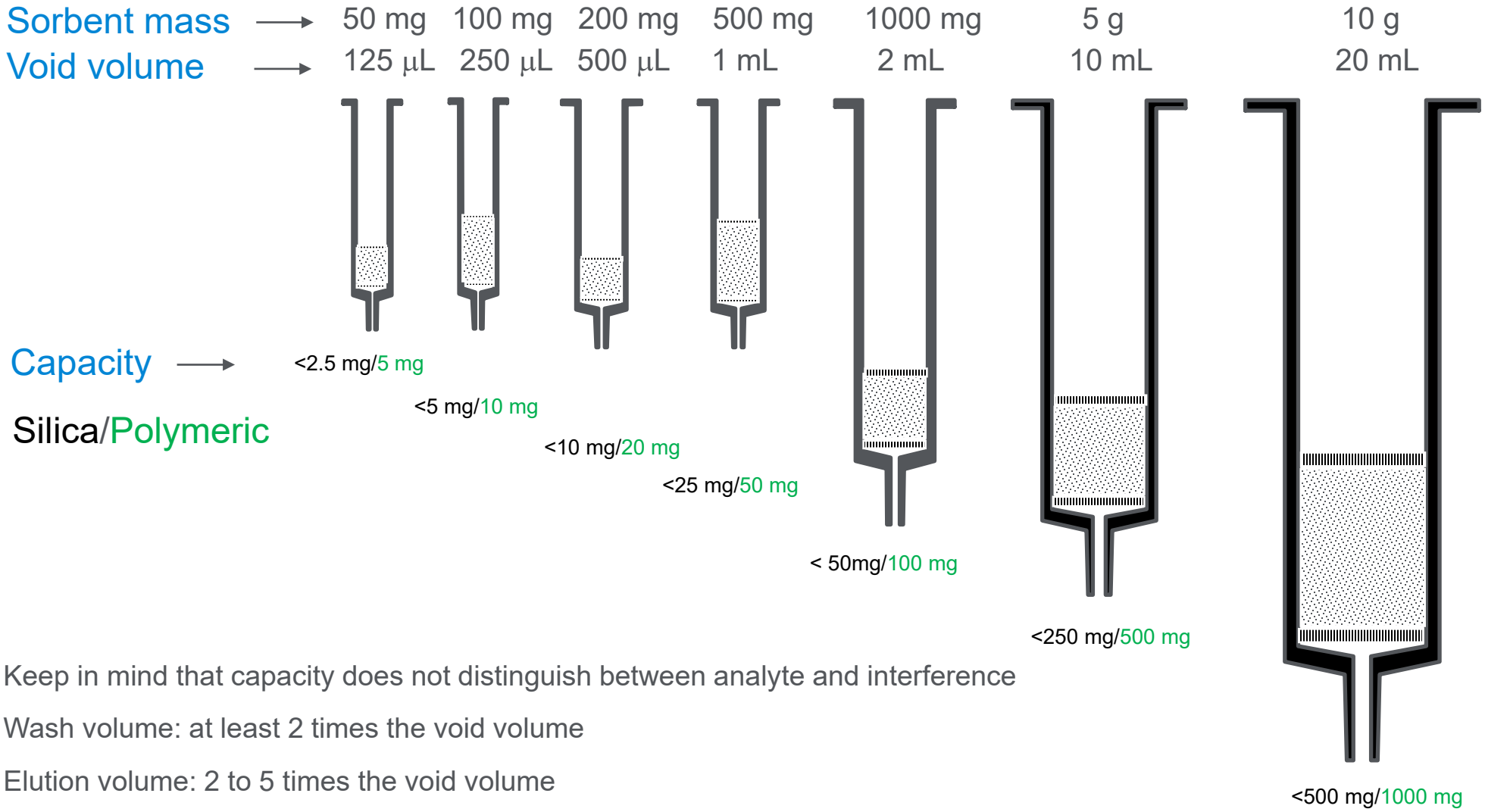

Keep in mind that capacity does not distinguish between analyte and interference

Wash volume: at least 2 times the void volume

Elution volume: 2 to 5 times the void volume

SPE Formats

- Open top cartridge
- Luer top cartridge (Bond Elut Jr)
- 96-well plate (round/square well)
- VersaPlate
- Pipette tip (OMIX)
- Bulk sorbent (Bondesil)

SPE Processing

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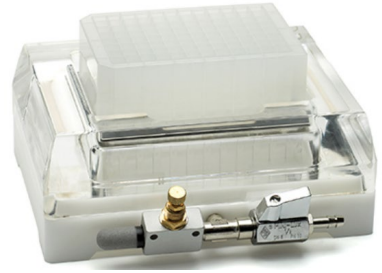
Vacuum manifolds

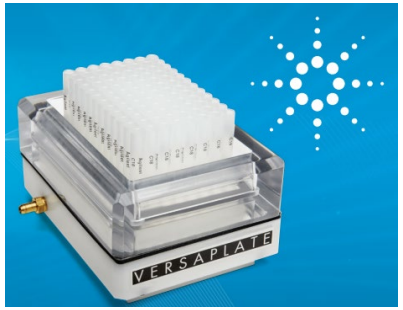
- Vac Elut 12
- Vac Elut 20
- Vac Elut SPS 24
- 96-well manifold
- VersaPlate manifold

Positive pressure manifolds

- PPM-48, for cartridges
- PPM-96, for well plates

Manual processing for cartridges





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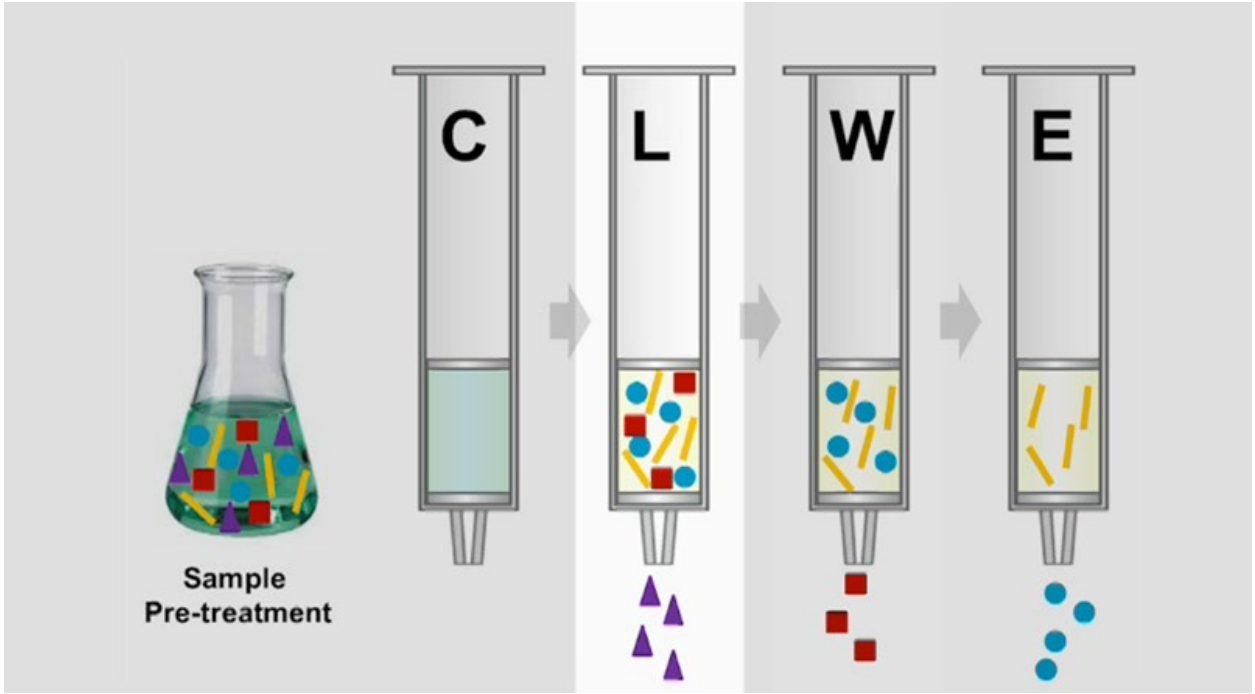
Developing SPE Methods and Troubleshooting

Developing SPE Methods and Troubleshooting

- Nonpolar
- Ion exchange
- Mixed mode



Nonpolar SPE

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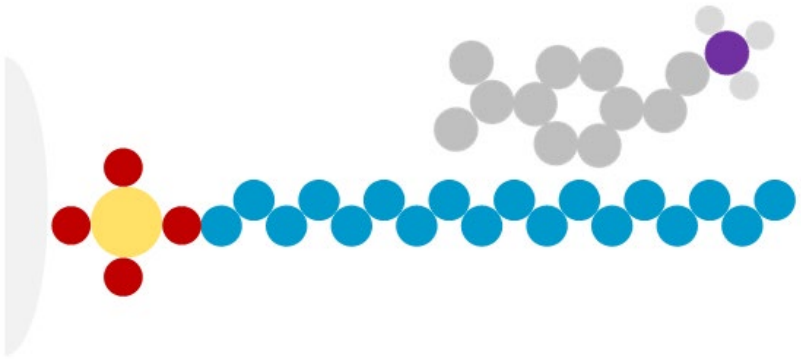
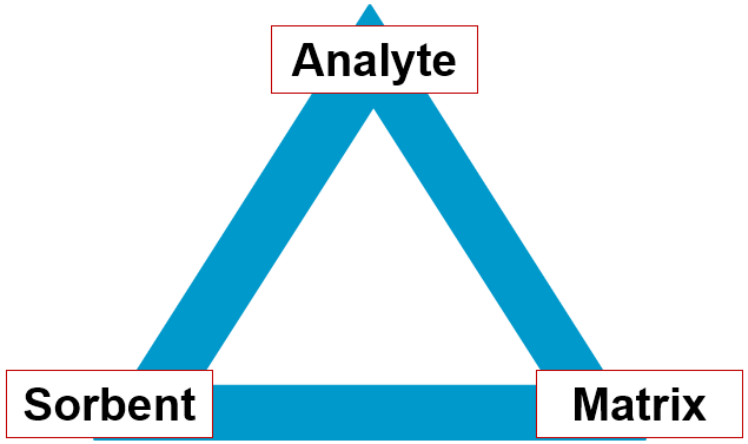
Compounds with nonpolar functional groups are extracted from polar solutions using nonpolar sorbents (C18, C8, CH, PH, CN-E).





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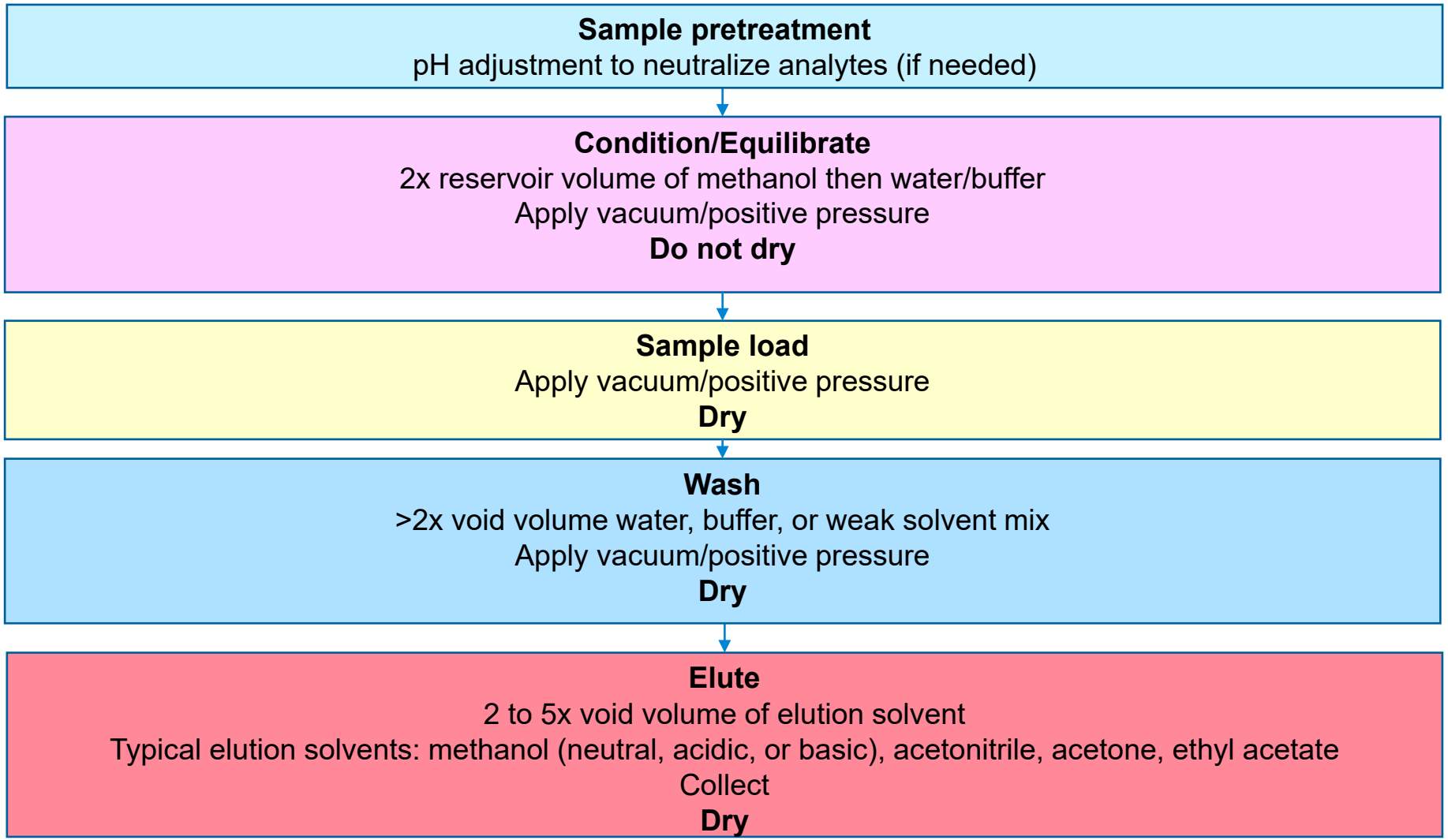
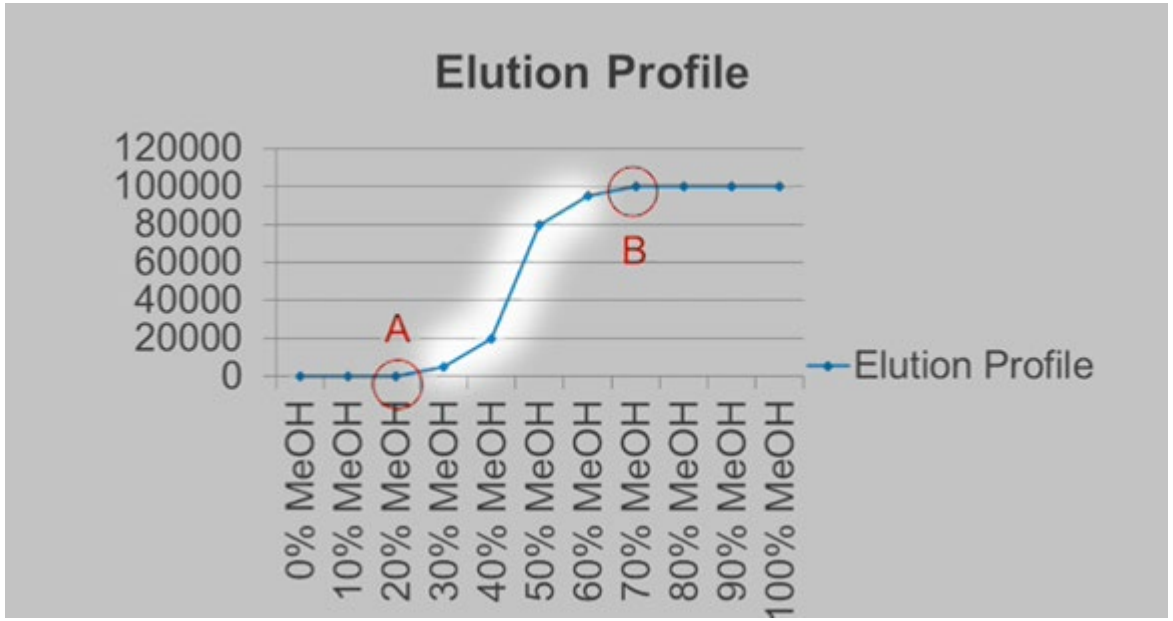
Nonpolar SPE
General extraction procedure

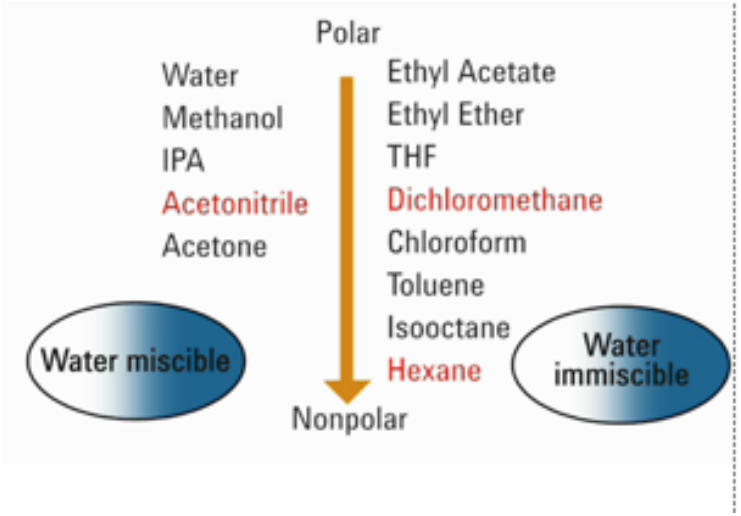


Nonpolar SPE

Which elution solvent to use?

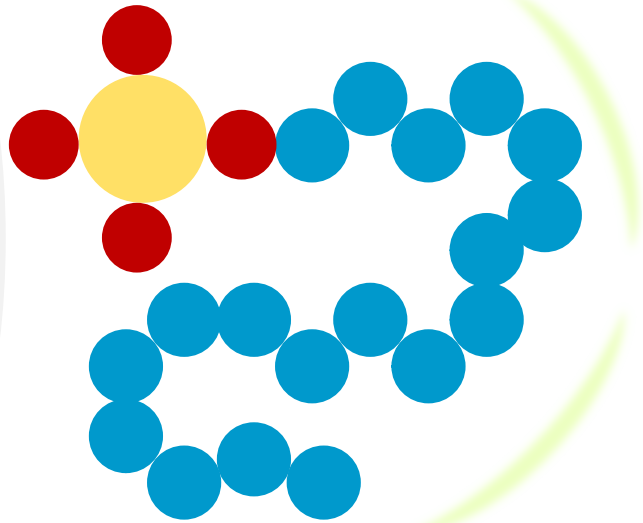
10 bottle optimization



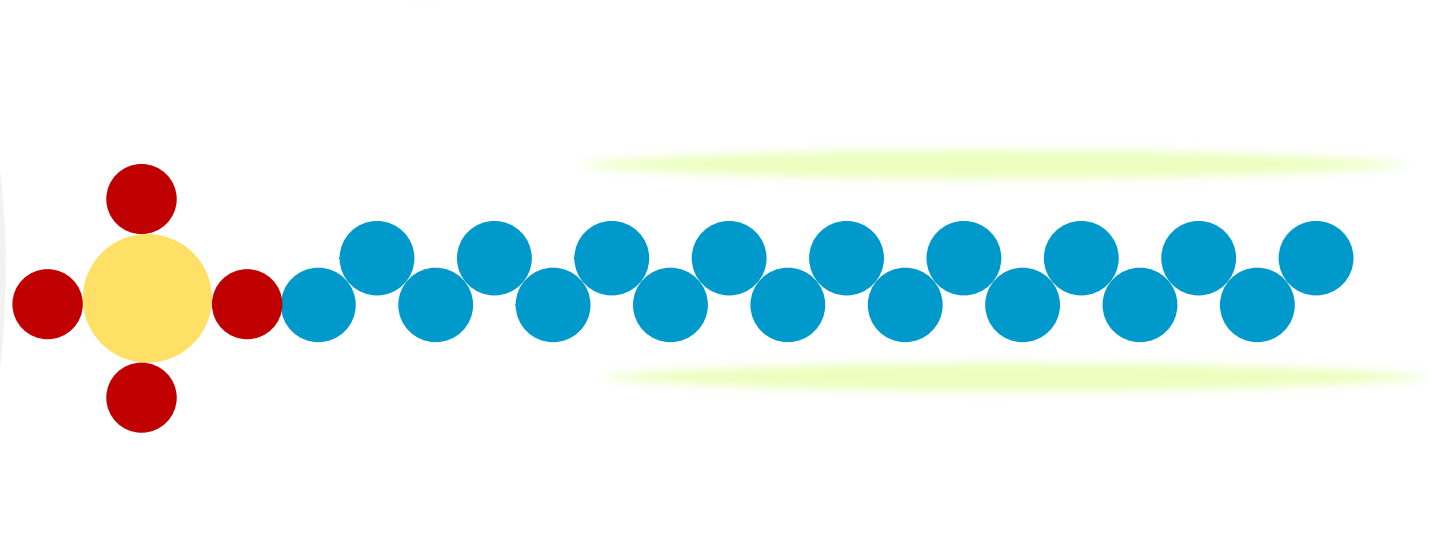


Goal: highest analyte recovery with the least number of interferences

Why is Sorbent Conditioning Important?



Phase collapse minimizes analyte interaction



Nonpolar SPE Example Method

Bond Elut PPL, excellent for extraction of polar analytes in drinking water

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Condition/Equilibrate
6 mL methanol
6 mL water

↓

Load
Install the reservoir to the SPE cartridge, and load 200 mL of water sample from the pretreatment.

↓

Wash
Wash with 10 mL water and, at the end, dry for 5 minutes with gentle vacuum.

↓

Elute 1
Elute with 6 mL 0.5% FA in 1:1 MeOH/ACN and apply vacuum for 10 minutes at the end to dry the sorbent.

↓

Elute 2
Elute with 6 mL 1% $NH_3 \cdot H_2O$ in 1:1 MeOH/ACN and apply vacuum to drain the cartridge until there is no visible liquid left. Mix the eluents from the two steps.

↓

Dry and reconstitute
Dry the eluent under N_2 in 35 °C water bath and reconstitute with 2 mL 10:90 MeOH/H_2O.

↓

LC/MS/MS analysis
Transfer the reconstituted sample to LC vails and inject for instrumentation analysis.

Bond Elut PPL, polymeric SPE, 500 mg, 6 mL, p/n: 2255001

Method for 39 PPCPs in drinking water

Nonpolar SPE Example Method

Bond Elut Plexa, an easy method for a wide range of analytes in drinking water

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Single SPE method for extraction of PAHs, chloropesticides, and triazines

Step	Method
1.	Prerinse cartridge with 6 mL EtOAc, followed by 6 mL DCM
2.	Condition with 10 mL MeOH
3.	Condition with 10 mL H_2O
4.	Load 800 mL water sample
5.	Dry sorbent with air for 10 minutes
6.	Soak and collect 2.5 mL fraction using ethyl acetate
7.	Collect 1 mL fraction using ethyl acetate
8.	Soak and collect 2.5 mL fraction using dichloromethane
9.	Collect 1 mL fraction using dichloromethane

Collect with slow flow, 5 mL/min

Bond Elut Plexa polymeric SPE, 200 mg, 6 mL, p/n: 12109206

List of Analytes Extracted from Drinking Water on Plexa

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Chloropesticides

- Alachlor
- Aldrin
- DDD o-p'
- DDD p-p'
- DDE o-p'
- DDE p-p'
- DDT p-p'
- DDT o-p'
- Dieldrin
- Endosulfan I (alfa)
- Endosulfan II (beta)
- Endosulfan sulfato
- Endrin
- HCH-alfa
- HCH-beta
- HCH-delta
- HCH-gamma
- Heptacloro
- Heptacloro Epoxido trans
- Hexaclorobenceno

Triazines

- Desispropylatrazine
- Desethylatrazine
- Cianazine
- Simazine
- Atrazine
- Terbutrine
- Propazine
- Tertbutylazine

PAHs

- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(a)pyrene
- Dibenzo(a,h)anthracene
- Benzo(ghi)perylene
- Indeno(1,2,3-cd)pyrene
- Chrysene

With Bond Elut Plexa, all three compound classes can be extracted using a single cartridge with a single SPE method.

Analyzed by

- HPLC-FL/UV for PAHs
- GC/MS for chloroPesticides
- LC/MS/MS for triazines

Nonpolar SPE
Troubleshooting

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Low recovery

Where is the analyte?

Analyze the effluent of each step (load, wash, and elution).

Nonpolar SPE
Troubleshooting

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Low recovery

Symptom: The effluent from the sample load step contains the analyte.

Cause: Inadequate retention

Solution:

- Make the analyte as neutral and hydrophobic as possible by pH adjustment of the sample
- Make the matrix as polar as possible
- Make sure that during conditioning/equilibration, the sorbent stays wet
- Reduce the flow rate, as retention is sometimes improved at lower flow rates. A typical flow rate during sample load is 2 to 4 mL/min.

Nonpolar SPE
Troubleshooting

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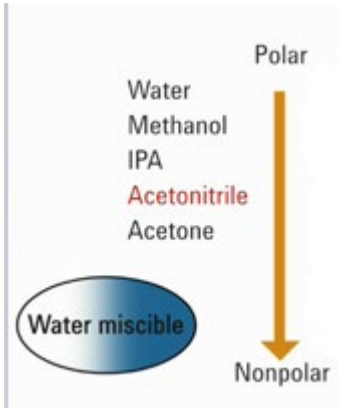
Low recovery



Symptom: The effluent from the wash step contains the analyte.

Cause: Wash solvent is too strong

Solution:

- Use a wash solvent like the pretreated sample solvent, with the same pH.
- If the wash solvent is a mix, use separate wash steps with each solvent to see which solvent is eluting the analyte. Then, use a less nonpolar solvent for that step.

Nonpolar SPE
Troubleshooting

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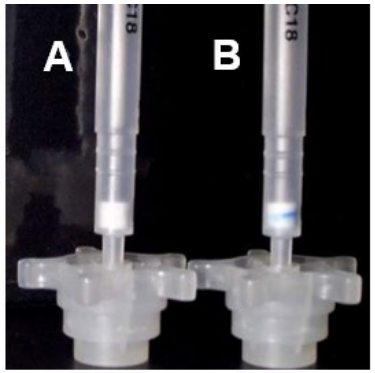
Low recovery

Symptom: The effluent from the elution step does not contains >90% of the analyte

Cause: Elution is not complete

Solution:

- Make sure the analyte is soluble in the selected elution solvent.
- Make sure that the cartridge is dried after the wash and elution steps
- Try soaking the sorbent with elution solvent.
- Apply the elution solvent in 2 to 3 smaller aliquots.
- Try a stronger elution solvent, or a combination of strong elution solvents
- If none of the above works, try using a less hydrophobic sorbent



A: 2 x 100 µL aliquots
B: 1 x 200 µL aliquot

Nonpolar SPE
Troubleshooting

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Dirty extracts

Symptom: Dirty extracts

Cause: Insufficient cleanup

Solution:

- Increase the organic ratio in the wash solvent or try a multistep wash approach
- Reduce the strength of the elution solvent
- Your current sorbent may be too universal. Use a more selective sorbent.
- Try stacking different sorbents or use a mixed-mode sorbent
- Prerinse the cartridge with the elution solvent

Nonpolar SPE
Troubleshooting

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Variable recovery

Symptom: Irreproducible results

Cause: Partially effective SPE method

Solution:

- Try a larger elution volume
- Make sure the sorbent is properly conditioned/equilibrated, and kept wet before sample load
- Try a stronger elution solvent or various buffer/solvent combinations
- Make sure the analyte is completely neutralized during sample pretreatment
- Lipids in the sample can reduce the retention capacity of the sorbent. Use a larger sorbent bed.

Ion Exchange SPE

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Analytes with ionic groups may be extracted from low-ionic strength aqueous solutions using a sorbent that has the opposite charge.

Bond Elut SCX
Bonded phase: benzene sulfonic acid

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Ion Exchange SPE

Strong ion: the ionic group is always charged. Changing the pH will not affect the charged state, for example, quaternary amine and sulfonates

Weak ion: the ionic group can be charged or neutral. Changing the pH will affect the charged state, for example, primary, secondary, tertiary amines, and carboxylic groups

Retention: when sorbent and analyte are oppositely charged

Elution: when one or both sorbent and analyte are neutral

Please note:

- Analytes with strong ions can be extracted on a weak ion exchanger. They will strongly retain on a strong ion exchanger and will not elute from it.
- Analytes with weak ions can be extracted on a weak ion exchanger or a strong ion exchanger.

Ion Exchange SPE

Important considerations for Ion Exchange

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Ensure analyte and sorbent are oppositely ionized

- Sample pretreatment for weak acids: pH = pK_a + 2
- Sample pretreatment for weak bases: pH = pK_a – 2

Ionic strength

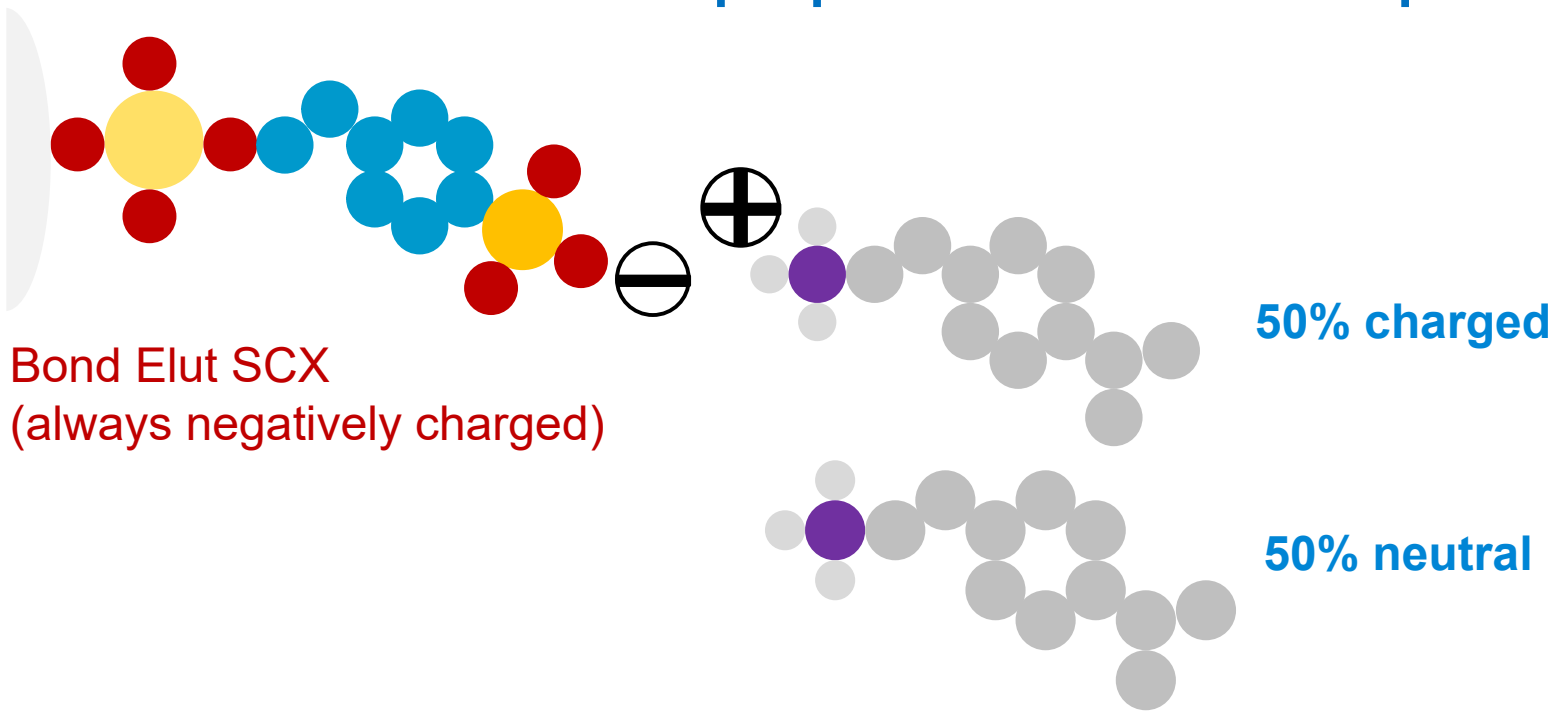
- Low for retention
- High for elution

Flow rate

- Slow flow

Example: Retention of a Weak Base on SCX Sorbent

When sample pH is the same as the pKa of the analyte



Bond Elut SCX
(always negatively charged)

50% charged

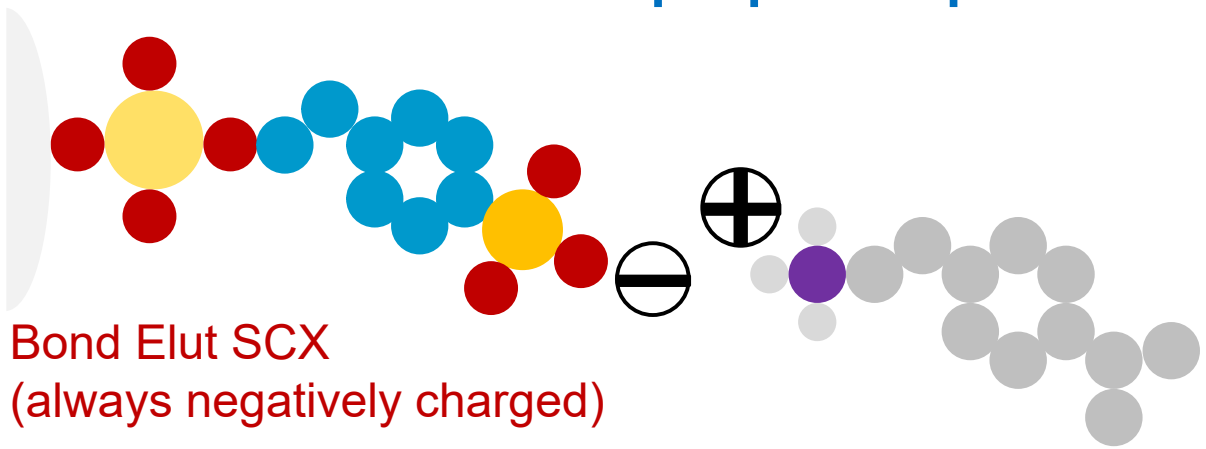
50% neutral

Weak cationic analyte, pKa 9.4

At pH 9.4 (equal to pKa of analyte), only 50% of the analyte is positively charged and will be retained on Bond Elut SCX.

Example: Retention of a Weak Base on SCX Sorbent

When sample pH is 2 pH units below the pKa of the analyte



Bond Elut SCX
(always negatively charged)

100% charged

Weak cationic analyte, pKa 9.4

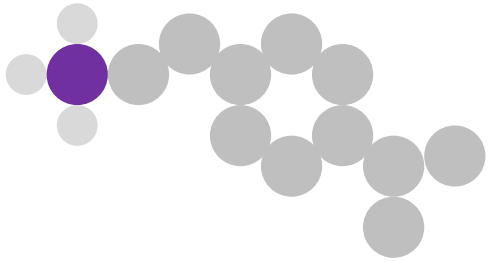
At pH 7.4 (2 units below the pKa of the analyte), 100% of the analyte will be positively charged and retained on Bond Elut SCX.

Example: Elution of a Weak Base from a SCX Sorbent

pH of the elution solvent is 2 pH units above the pKa of the analyte

Bond Elut SCX
(always negatively charged)

100% neutral



Weak cationic analyte, pKa 9.4

If the pH is 11.4 (2 units above the pKa of the analyte), 100% of the analyte will be neutral and elute from Bond Elut SCX.

Ion Exchange SPE

General extraction procedure

Sample pretreatment
pH adjustment to ionize analytes (if needed)
Dilute sample to reduce ionic strength (if needed)

↓

Condition/Equilibrate
2x reservoir volume of methanol then water/buffer with the same pH as the sample
Apply vacuum/positive pressure
Do not dry

↓

Sample load
Apply vacuum/positive pressure, flow rate <4 mL/min
Dry

↓

Wash
>2x void volume buffer with the same pH as the sample to maintain electrostatic retention
Apply vacuum/positive pressure
Dry

↓

Elute
2 to 5x void volume of elution solvent, flow rate <4 mL/min
Typical: buffer (pH that neutralizes the analytes), high ionic strength buffer, buffer with higher selectivity ion
Collect
Dry

Ion Exchange SPE
Troubleshooting

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Low recovery

- Inadequate retention: adjust sample pH so that both the analyte and sorbent are fully charged with opposite charges.
- Inadequate capacity: try a larger sorbent mass
- Sample solvent is too high in ionic strength: dilute the sample with water or desalt the sample
- Flow rate is too fast: during sample load and elution, reduce the flow rate to ensure enough contact time for the ion exchange to occur.
- Add some organic solvent to the elution solvent to overcome secondary interactions with the sorbent.
- If the analyte cannot be completely eluted from a strong ion exchange sorbent, try a weak ion exchange sorbent.

Mixed Mode SPE

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Polymer based

- Bond Elut Plexa PCX: PSDVB + SCX (benzene sulfonic acid)
- Bond Elut Plexa PAX: PSDVB + SAX (quaternary amine)

Silica based

- Bond Elut Certify (SCX + C8)*
- Bond Elut Certify II (SAX + C8)*

**For SPE methods on Bond Elut Certify and Certify II, see Certify Methods Manual: https://www.agilent.com/cs/library/Brochures/Bond%20Elut%20Certify%20MethodsManual.pdf*

Mixed Mode SPE

General extraction procedure

Sample pretreatment
pH adjustment to ionize analytes (if needed)

↓

Condition/Equilibrate
2x reservoir volume of methanol then buffer with the same pH as the sample
Apply vacuum/positive pressure
Do not dry

↓

Sample load
Apply vacuum/positive pressure, flow rate <4 mL/min
Dry

↓

Wash
>2x void volume aqueous solvent with the same pH as the sample to maintain electrostatic retention
Apply vacuum/positive pressure
Dry

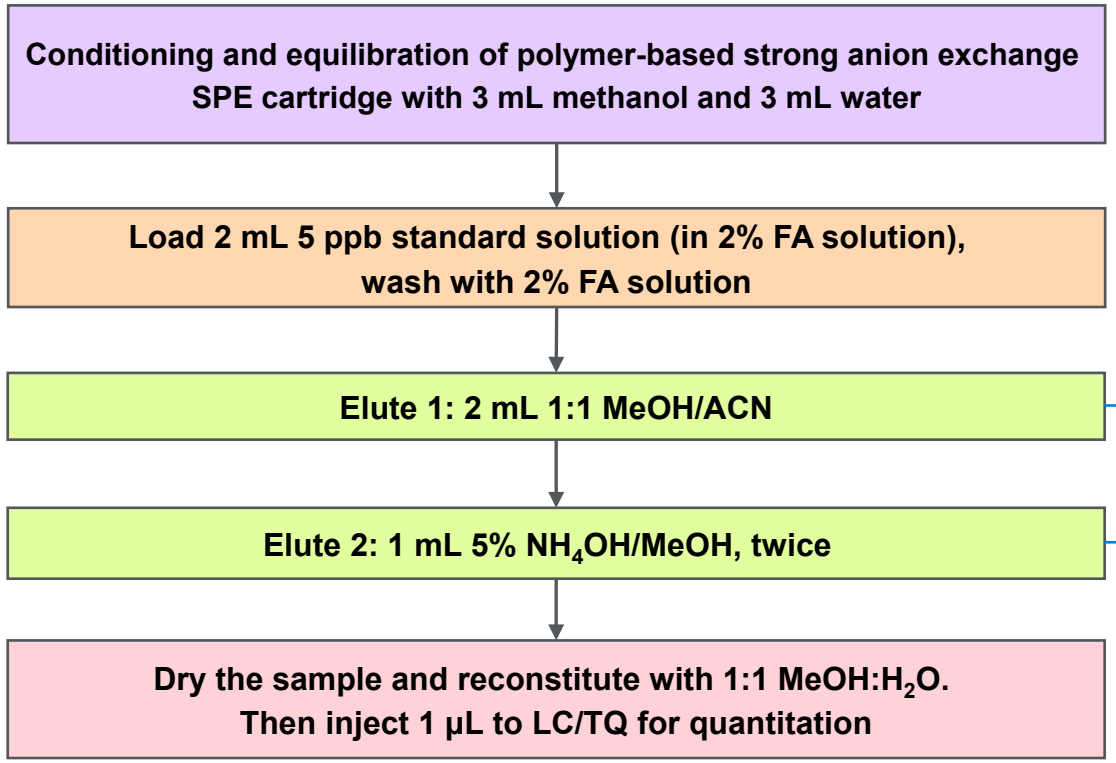
↓

Elute
2 to 5x void volume of elution solvent, flow rate <4 mL/min
Typical: Mix of organic and buffer (pH that neutralizes the analytes)
Collect
Dry

See Appendix for generic methods of Bond Elut Plexa PCX, Plexa PAX, and Plexa

Mixed Mode SPE Example Method

Bond Elut Plexa PCX, fractionation of acidic/neutrals and weak bases



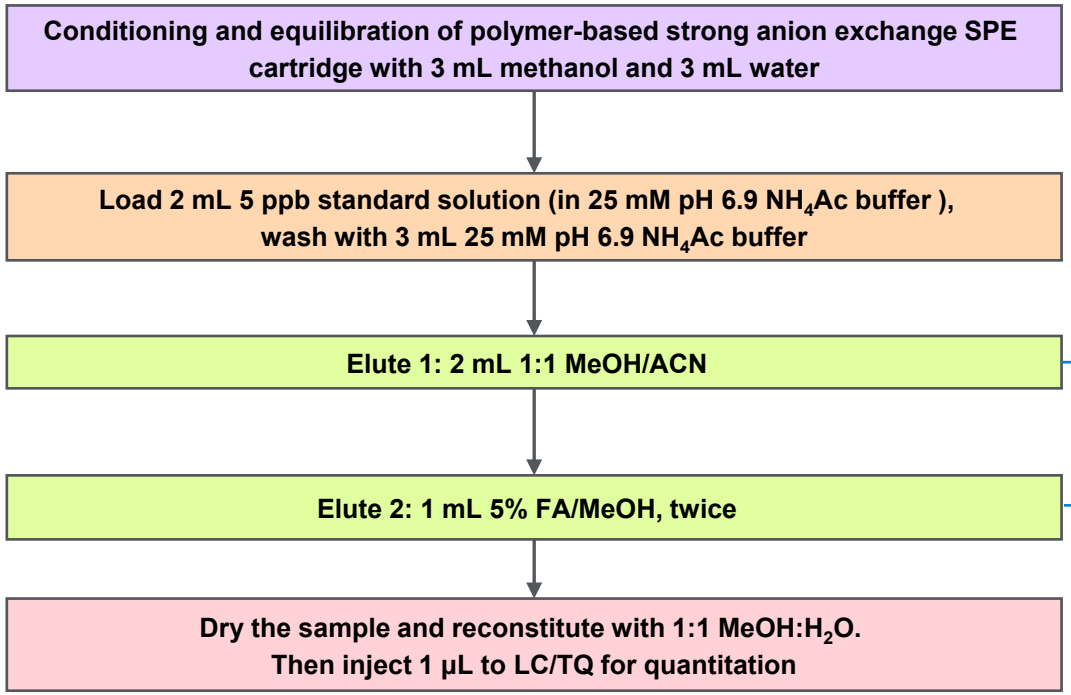
Target: acidic or neutral targets
Interaction: hydrophobic interaction

Target: weak bases and zwitterions
Interaction: ion exchange with benzene sulphonic acid

Bond Elut Plexa PCX, 60 mg, 3 mL, p/n: 12108603

Mixed-Mode SPE Example Method

Bond Elut Plexa PAX, fractionation of basic/neutrals and weak acids



Target: basic or neutral targets.
Interaction: hydrophobic interaction.

Target: weak acids and zwitterion.
Interaction: ion exchange with quaternary amine.

Bond Elut Plexa PAX, 60 mg, 3 mL, p/n: 12107603

Mixed-Mode SPE Example Method

Bond Elut PFAS WAX, extraction of PFAS in drinking water (EPA method 533)

Extraction procedure

1. Place SPE cartridges and 15 mL centrifuge collection tubes on Agilent Vac Elut SPS 24 manifold. Rotate the cowling to the waste position.
2. Rinse each SPE cartridge with 10 mL MeOH.
3. Add 60 mL reservoirs and adapters to each SPE cartridge.
4. Rinse each cartridge with 10 mL of 0.1 M phosphate buffer. Close the stopcock and add an additional 3 mL of 0.1 M phosphate buffer.
5. Fill the reservoirs with 60 mL of sample, and adjust flow rate to approximately 5 mL/min (vacuum pressure 3 to 5 inHg).
6. Repeat the filling step until the 250 mL sample volume has been transferred.
7. Rinse the bottles, reservoirs, and cartridges with 10 mL of 1 g/L ammonium acetate into the reservoirs.
8. Dry the cartridges for 5 minutes at a vacuum pressure of 15 to 20 inHg.
9. Rotate the cowling on the Vac Elut SPS 24 manifold to the collect position.
10. Elute the cartridges by rinsing the sample bottles, reservoirs, and cartridges with 5 mL of 2% ammonium hydroxide in MeOH.
11. Repeat the elution with a second 5 mL portion of 2% ammonium hydroxide in MeOH.
12. Evaporate the 10 mL extracts under a gentle stream of nitrogen at 55 to 60 °C until dry.
13. Reconstitute extract in 1 mL of 80/20 MeOH/water and analyze.

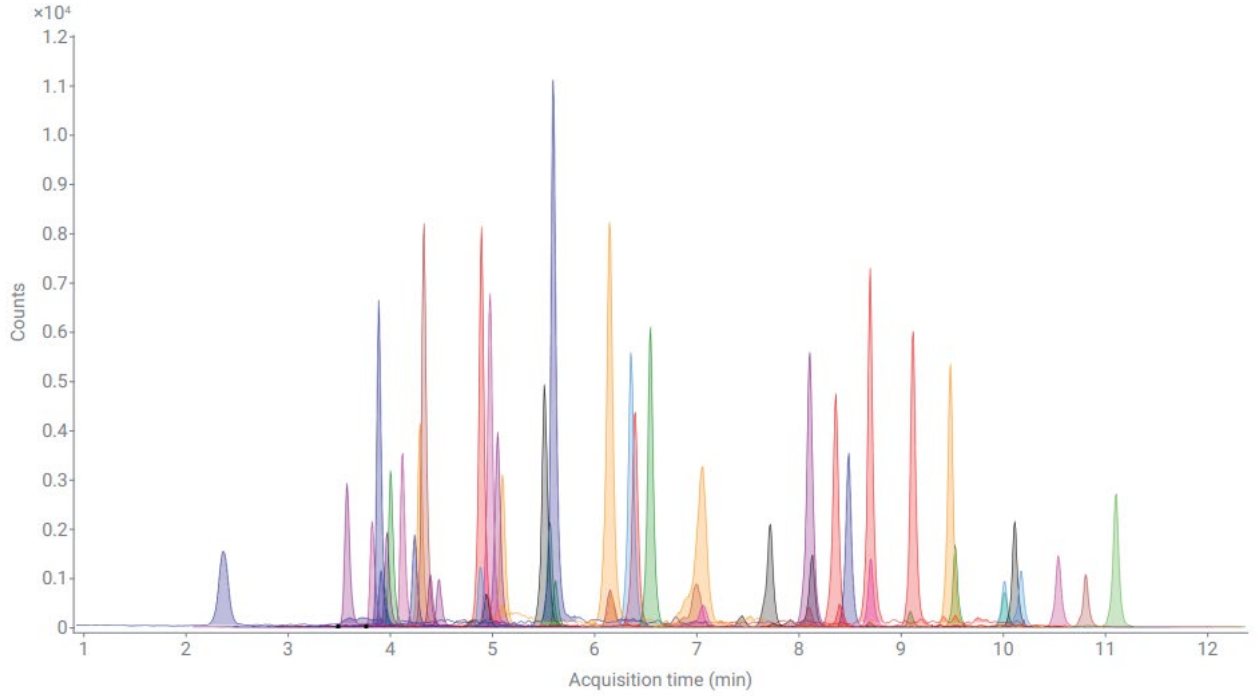


Figure 2. Target quant ion chromatogram for a standard at 5 ng/mL for most compounds (retention times listed in Appendixes A and B).

Bond Elut PFAS WAX, 500 mg, 6 mL, 30/pk, p/n: 5610-2152

Application note: 5994-4960EN

What's New?

Recently introduced Agilent sample preparation products

Bond Elut Lipid Extraction SPE

- Great alternative to liquid-liquid extraction for extraction of lipidomic samples
- Provides better reproducibility and streamlined sample preparation for lipid analysis
- Requires smaller volumes of precious sample compared to liquid-liquid extraction

Description	Quantity	Part Number
Agilent Bond Elut Lipid Extraction, 1 mL cartridge	100/pk	5610-2041
Agilent Bond Elut Lipid Extraction, 96-well plate	1 plate	5610-2042
Agilent Bond Elut Lipid Extraction, 96-well plate	5 plates	5610-2043

Method guide for 1 mL cartridge: 5994-1627EN

Method guide for 96-well plate: 5994-1690EN

Bond Elut PFAS WAX SPE

Agilent InfinityLab

Property	Specification
Base Polymer	Poly(styrene-co-divinylbenzene) (PSDVB)
Functionalized	Diamino ligand
Chemistry	Weak anion exchange (WAX) and hydrophobic retention
WAX pKa	> 8
Particle size	45 um

Part Number	Description
5610-2150	Bond Elut PFAS WAX, 150 mg, 6 mL, 30/pk
5610-2151	Bond Elut PFAS WAX, 200 mg, 6 mL, 30/pk
5610-2152	Bond Elut PFAS WAX, 500 mg, 6 mL, 30/pk

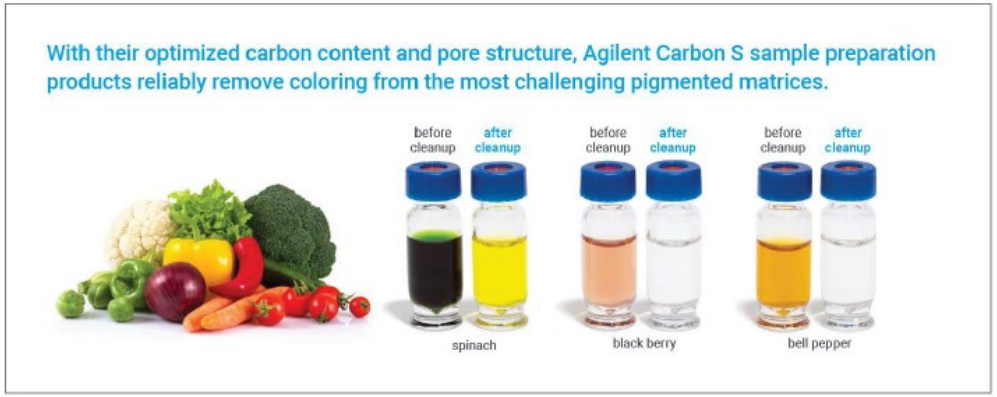
Bond Elut PFAS WAX brochure: [5994-4996EN]

Carbon S

- Advanced hybrid carbon material with optimized carbon content and pore structure
- Great for removing pigments from highly pigmented food and environmental samples
- Superior results to GCB-based products, it does not cause loss of planar pesticides like GCB.
- Simplified workflow and improved recovery
- Offered in variety of formats:
  - Quechers, dSPE mixes
  - Bond Elut SPE, packed cartridges
  - Captiva EMR filtration cartridges with carbon S
  - Bulk powder

Product pages on agilent.com

- Captiva EMR | Agilent
- QuEChERS Dispersive Kits | Agilent
- Bond Elut Carbon S and Carbon S/NH_2 | Agilent



See appendix for part numbers of Carbon S products

Carbon S

Captiva EMR with Carbon S, selection guide

Captiva EMR-HCF1(with NH2) & HCF2 (with PSA)

High Chlorophyll Fresh

- Spinach, Arugula, Chard etc.

Captiva EMR-GPF

General Pigmented Fresh

- Berries, Peppers, Broccoli etc.

Captiva EMR-GPD

General Pigmented Dry

- Spices, seasoning, Herbal medicine

Captiva EMR-LPD

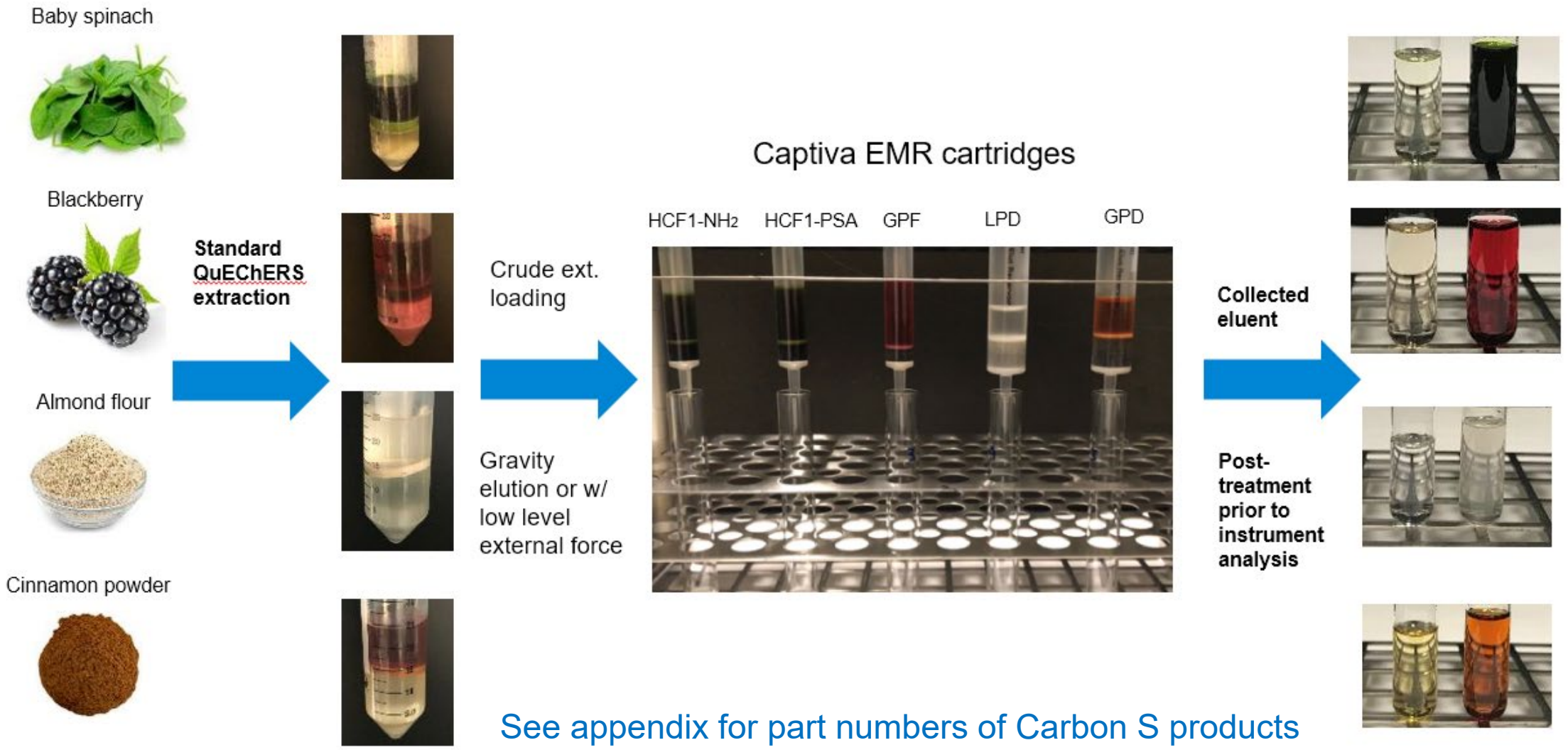
Low Pigmented Dry

- Nuts, tobacco, light pigmented spices

[Captiva EMR | Agilent]

Carbon S

Captiva EMR with Carbon S, simplified passthrough workflow for pesticides



See appendix for part numbers of Carbon S products

Captiva Filter Vials – Now Available in Preslit Version Too

Agilent InfinityLab

Ordering information

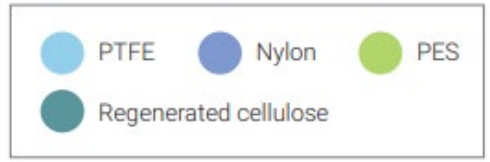
Description	Part Number (Nonslit Septa)	Part Number (Preslit Septa)
0.45 µm PTFE filter vial, 100/pack	5191-5933	5610-2122
0.20 µm PTFE filter vial, 100/pack	5191-5934	5610-2123
0.45 µm Nylon filter vial, 100/pack	5191-5935	5610-2118
0.20 µm Nylon filter vial, 100/pack	5191-5936	5610-2119
0.45 µm RC filter vial, 100/pack	5191-5939	5610-2124
0.20 µm RC filter vial, 100/pack	5191-5940	5610-2125
0.45 µm PES filter vial, 100/pack	5191-5941	5610-2120
0.20 µm PES filter vial, 100/pack	5191-5942	5610-2121
Vial closure tool	5191-5943	

Captiva Filter Vial flyer: 5994-0567EN

Color-coded for your convenience

Pore size identified by septum color

- 0.2 µm: white
- 0.45 µm: red



Easy as 1-2-3

1. Fill

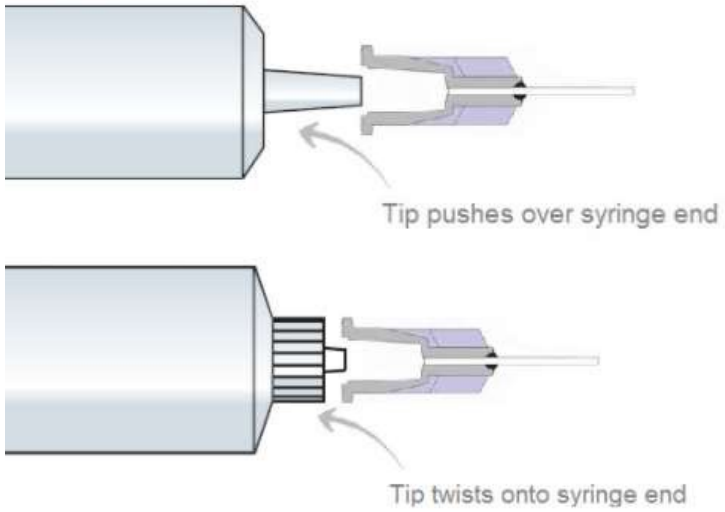
2. Cover

3. Plunge

Agilent

Disposable Syringes – More Choices

- Agilent offers five different sizes of disposable syringes: 20 mL, 10 mL, 5 mL, 2 mL, and 1 mL.
- Disposable syringes are available in nonsterile and sterile individually packaged options.
- Choose from Luer lock or Luer slip options to best suit your laboratory's needs.



Disposable syringe part numbers

Summary

Summary

Agilent InfinityLab

Approach SPE method development systematically

- Do your research, get to know the properties of target analytes and samples
- Investigate the analysis technique, solvent compatibility, detection limit
- Narrow down choices of SPE sorbent, formats, and processing equipment
- When troubleshooting, change one parameter at a time
- Ask for help:
  - spp-support@agilent.com
  - 800-227-9770, option 3, then option 3, then option 3

Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for former Prozyme products

Available in the U.S. and Canada, 8–5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

advancebio.glycan@agilent.com

Web chat: Product pages of agilent.com

Thank You!

Appendix

Generic Methods, Bond Elut Plexa, Plexa PAX, and Plexa PCX

Agilent InfinityLab

Generic method recommendations

	Acids	Neutrals		Bases
Analyte	LogP>1.0 pK_a < 5	LogP > 1.5 pKa 3-5	 pK_a 6-10	LogP > 0.8 pK_a 6-10
	Plexa PAX	Plexa (Acid load method)	Plexa (Base load method)	Plexa PCX
Sample Pre-treatment	2% NH_4OH	1% HCO_2H	2% NH_4OH	2% H_3PO_4
Sorbent Condition	100% MeOH	100% MeOH		100% MeOH
Equilibration	100% H_2O	100% H_2O		100% H_2O
Load	Apply pre-treated sample			
Wash	100% H_2O	5% MeOH in H_2O		2% HCO_2H in H_2O
Elution 1/Wash 2	100% MeOH *Neutrals*	100% MeOH *Neutrals*		1:1 MeOH/ACN *Acids, Neutrals*
Elution 2	5% HCO_2H in MeOH *Acids*			5% NH_3 in 1:1 MeOH/ACN *Bases*
Analysis	Prepare extracts for instrumental analysis			

Note: This user guide is a convenient starting point for any SPE method development. Further optimization may be required to adjust the method to your application needs.

Generic Methods, Bond Elut Plexa, Plexa PAX, and Plexa PCX

Agilent InfinityLab

Method development and troubleshooting for plasma samples

Bond Elut Plexa PAX

Bond Elut Plexa PAX contains a strong anion exchange functionality. Simple generic methodology and excellent batch to batch reproducibility offer robust anion exchange SPE workflow.

Strong Anion Exchange SPE for Acidic Analytes	
Sorbent Condition	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pre-treatment	Dilute 1:3 with 300 µL: 2% NH_4OH in H_2O
Washes	1. 500 µL H_2O 2. 500 µL MeOH
Elution	2 x 250 µL 5% HCO_2H in MeOH

Volumes stated for all methods are for a 30 mg, 1 mL SPE format device.

pH adjustment – To improve ion exchange interactions on Plexa PAX, ionize analytes prior to loading. For acidic analytes the pH should be at least 2 pH units above the pK_a.

Bond Elut Plexa

Bond Elut Plexa is a non-polar divinylbenzene-based neutral polymeric sorbent. This sorbent is the best choice for non-ionic extraction of a wide range of acidic, neutral and basic analytes from different matrices.

Non-Polar SPE for neutrals and moderately acidic or basic analytes	
Sorbent Condition	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pre-treatment	Dilute 1:3 with 300 µL: 2% NH_4OH *(neutrals and bases)* 1% HCO_2H in H_2O *(acids)*
Washes	500 µL 5 % MeOH in H_2O
Elution	2 x 250 µL MeOH

pH adjustment – To improve hydrophobic interaction on Plexa, neutralize analytes prior to loading. Basic analytes should be at least 2 pH units above the pK_a. Acidic analytes should be 2 pH units below the pK_a.

Bond Elut Plexa PCX

Bond Elut Plexa PCX is a cation exchanger with mixed mode sorbent characteristics and is therefore suitable for the extraction and clean-up of polar and non-polar bases from biofluids.

Strong Cation Exchange SPE for Basic Analytes	
Sorbent Condition	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pre-treatment	Dilute 1:3 with 300 µL: 2% H_3PO_4 in H_2O
Washes	1. 500 µL 2% HCO_2H in H_2O 2. 500 µL MeOH:ACN (1:1, v/v)
Elution	2 x 250 µL 5% NH_3 (28-30%) in MeOH: ACN (1:1, v/v)

pH adjustment – To improve ion exchange interactions on Plexa PCX, ionize analytes prior to loading. Basic analytes should be at least 2 pH units below the pK_a. Acidification is also necessary to disrupt analyte-protein interaction.

Volumes stated for all the above methods are for a 30 mg, 1 mL SPE format device.

Generic Methods, Bond Elut Plexa, Plexa PAX, and Plexa PCX

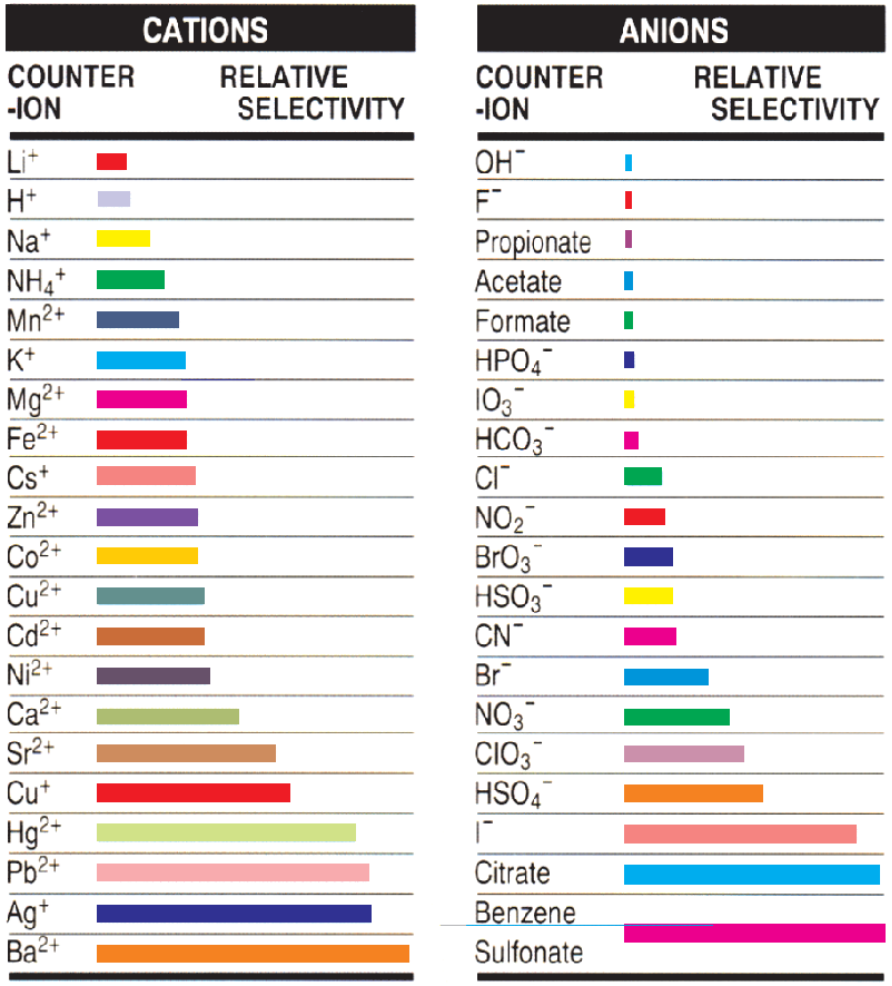
Agilent InfinityLab

Troubleshooting for plasma samples

Troubleshooting	Bond Elut Plexa	Bond Elut Plexa PCX	Plexa PAX
Analyte(s) eluting in the wash step(s)	• Reduce volume of washing step • Reduce concentration of organics in the wash step		
	• Rinse with either 2% NH_3 for basic analytes or 1% formic acid for acids to ensure hydrophobic interactions • Increase sorbent bed mass	• Increase sorbent bed mass for increased ion exchange capacity	
Inadequate Elution (Eluent does not contain >90% of the analyte.)	• Decrease flow rate, (1 mL/min is recommended) • Check solubility of analyte in the eluent • Increase strength of elution solvent • Increase the eluent volume or use multiple aliquots of eluent		
	• Add modifier (depending on analyte type) to the elution solvent, thereby promoting ionization	• Use up to 10% ammonia (28-30%) in solvents such as MeOH and ACN	• Use up to 10% formic acid in MeOH for anion exchange elution

Ion Exchange SPE

Relative selectivity of common counterions



CATIONS	
COUNTER -ION	RELATIVE SELECTIVITY
Li^+	
H^+	
Na^+	
NH_4^+	
Mn^{2+}	
K^+	
Mg^{2+}	
Fe^{2+}	
Cs^+	
Zn^{2+}	
Co^{2+}	
Cu^{2+}	
Cd^{2+}	
Ni^{2+}	
Ca^{2+}	
Sr^{2+}	
Cu^+	
Hg^{2+}	
Pb^{2+}	
Ag^+	
Ba^{2+}	

ANIONS	
COUNTER -ION	RELATIVE SELECTIVITY
OH^-	
F^-	
Propionate	
Acetate	
Formate	
HPO_4^-	
IO_3^-	
HCO_3^-	
Cl^-	
NO_2^-	
BrO_3^-	
HSO_3^-	
CN^-	
Br^-	
NO_3^-	
ClO_3^-	
HSO_4^-	
I^-	
Citrate	
Benzene Sulfonate	

Carbon S products
Part numbers

Agilent InfinityLab

QuEChERS dSPE kits

Description	GCB Part Number	Carbon S Part Number
QuEChERS dSPE TFDA 15 mL, PSA, C18, MgSO4, 50/pk, w/Carbon S	5982-6664	5610-2054
QuEChERS dSPE TFDA 15 mL, animal origin, 50/pk, w/Carbon S	5982-6665	5610-2055
QuEChERS dSPE, ChP TCM, 50/pk, w/Carbon S	5610-2048	5610-2056
QuEChERS dSPE GB 23200.113, tea and spice, 50/pk, w/Carbon S	5982-6670	5610-2057
QuEChERS dSPE 2 mL, universal kit, 100/pk, w/Carbon S	5982-0028	5610-2058
QuEChERS dSPE 2 mL, universal kit w/ceramic homogenizers, 100/pk, w/Carbon S	5982-0028CH	5610-2059
QuEChERS dSPE 15 mL, universal kit, 50/pk, w/Carbon S	5982-0029	5610-2060
QuEChERS dSPE 15 mL, universal kit, 50/pk, w/ceramic homogenizers, w/Carbon S	5982-0029CH	5610-2061
QuEChERS dSPE 2 mL, pigment sample (AOAC like), 100/pk, w/Carbon S	5982-5222	5610-2062
QuEChERS dSPE 2 mL, pigment sample (AOAC like), w/ceramic homogenizers, 100/pk, w/Carbon S	5982-5222CH	5610-2063
QuEChERS dSPE 15 mL, pigment sample (AOAC like), 50/pk, w/Carbon S	5982-5258	5610-2064
QuEChERS dSPE 15 mL, pigment sample (AOAC like), w/ceramic homogenizers, 50/pk, w/Carbon S	5982-5258CH	5610-2065
QuEChERS dSPE 2 mL, fat + pigments (AOAC like), 100/pk, w/Carbon S	5982-5421	5610-2066
QuEChERS dSPE 2 mL, fat + pigments (AOAC like), w/ceramic homogenizers, 100/pk, w/Carbon S	5982-5421CH	5610-2067
QuEChERS dSPE 15 mL, fat + pigments (AOAC like), 50/pk, w/Carbon S	5982-5456	5610-2068
QuEChERS dSPE 15 mL, fat + pigments (AOAC like), w/ceramic homogenizers, 50/pk, w/Carbon S	5982-5456CH	5610-2069
QuEChERS dSPE 2 mL, pigment sample (EN like), 100/pk, w/Carbon S	5982-5221	5610-2070
QuEChERS dSPE 2 mL, pigment sample (EN like), w/ceramic homogenizers, 100/pk, w/Carbon S	5982-5221CH	5610-2071
QuEChERS dSPE 15 mL, pigment sample (EN like), 50/pk, w/Carbon S	5982-5256	5610-2072
QuEChERS dSPE 15 mL, pigment sample (EN like), w/ceramic homogenizers, 50/pk, w/Carbon S	5982-5256CH	5610-2073
QuEChERS dSPE 2 mL, high pigment (EN like), 100/pk, w/Carbon S	5982-5321	5610-2074
QuEChERS dSPE 2 mL, high pigment (EN like), w/ceramic homogenizers, 100/pk, w/Carbon S	5982-5321CH	5610-2075
QuEChERS dSPE 15 mL, high pigment (EN like), 50/pk, w/Carbon S	5982-5356	5610-2076
QuEChERS dSPE 15 mL, high pigment (EN like), w/ceramic homogenizers, 50/pk, w/Carbon S	5982-5356CH	5610-2077

Carbon S products
Part numbers

Agilent InfinityLab

Bond Elut SPE

Description	GCB Part Number	Carbon S Part Number
Bond Elut 50 mg, 1 mL, 100/pk, w/Carbon S	126414	5610-2078
Bond Elut 100 mg, 1 mL, 100/pk, w/Carbon S	126418	5610-2079
Bond Elut Jr 250 mg, 100/pk, w/Carbon S	446424	5610-2080
Bond Elut Jr 400 mg, 100/pk, w/Carbon S	466430	5610-2081
Bond Elut 250 mg, 6 mL, 30/pk, w/Carbon S	12102201 or 5982-4432	5610-2082
Bond Elut 500 mg, 6 mL, 30/pk, w/Carbon S	12252201 or 5982-4465	5610-2083
Bond Elut/NH2 500 mg, 6 mL, 30/pk, w/Carbon S	12252202 or 3664325032	5610-2084
Bond Elut 300/NH2 500 mg, 6 mL, 30/pk, w/Carbon S	2264265032 or 5982-4569	5610-2085
Bond Elut 250 mg/PSA 250 mg, 3 mL, 50/pk, w/Carbon S	12102042C250 or 5982-4567	5610-2086
Bond Elut 500 mg/PSA 500 mg, 6 mL, 30/pk, w/Carbon S	12102042C500 or 5982-4568	5610-2087

Carbon S products
Part numbers

Agilent InfinityLab

Enhanced Matrix Removal

Description	Carbon S Part Number
Enhanced Matrix Removal high chlorophyll fresh 1 (EMR-HCF1), with NH2, 3 mL, 50/pk	5610-2088
Enhanced Matrix Removal high chlorophyll fresh 2 (EMR-HCF2), with PSA, 3 mL, 50/pk	5610-2089
Enhanced Matrix Removal general pigmented fresh (EMR-GPF), 3 mL, 50/pk	5610-2090
Enhanced Matrix Removal general pigmented dry (EMR-GPD), 6 mL, 30/pk	5610-2091
Enhanced Matrix Removal low pigment dry (EMR-LPD), 6 mL, 30/pk	5610-2092

Bulk Carbon S

Description	Current Part Number	New Part Number
Carbon S bulk, 25 g	5982-4482	5610-2093
Carbon S bulk, 100 g	64100G	5610-2094
Carbon S bulk, 10 g	6410G	5610-2095

Carbon S products
Part numbers

Agilent InfinityLab

Sample packs

Part Number	Product description
5610-2096	Bond Elut Carbon, 250mg, 6ml, 3/pk Sample pack of 5610-2082
5610-2097	Bond Elut Carbon, 500mg, 6ml, 3/pk Sample pack of 5610-2083
5610-2098	BE Carbon 500mg/PSA 500mg, 6ml, 3/pk Sample pack of 5610-2087
5610-2099	QuEChERS dispersive SPE kit, ChP TCM, 5/pk Sample pack of 5610-2056
5610-2100	Dispersive, 15ml, Universal kit 5/pk Sample pack Sample pack of 5610-2060
5610-2101	Dispersive SPE 15ml, Pigment Sample EN, 5/pk Sample pack of 5610-2072
5610-2102	Dispersive SPE 15ml, Fat + Pigments AOAC, 5/pk Sample pack of 5610-2068
5610-2103	TFDA QuEChERS dSPE 15mLPSA C18 GCB MgSO4, 5/pk Sample pack of 5610-2054
5610-2104	QuEChERS dSPE GB 23200.113 Tea and Spice, 5/pk Sample pack of 5610-2057
5610-2105	Fresh matrix Enhanced Matrix Removal, sample pack 3-5610-2088, 3-5610-2089 and 3-5610-2090
5610-2106	Dry matrix Enhanced Matrix Removal, sample pack 3-5610-2091 and 3-5610-2092

Disposable Syringes
Part numbers

Agilent InfinityLab

Product Description	Part number	Syringes per package
1 mL sterile Luer slip	5610-2107	100
ValueLab 1 mL non-sterile bulk Luer slip	5610-2108	7000
2 mL (3 mL) Luer Lock Sterile	5610-2109	100
2 mL (3 mL) Luer lock bulk	5610-2110	6300
ValueLab 2 mL non-sterile bulk Luer slip	5610-2111	6300
5 mL (6 mL) Luer Lock Sterile	9301-6476	100
5 mL (6 mL) Luer Lock bulk	5610-2112	3600
ValueLab 5 mL non-sterile bulk Luer slip	5610-2113	3600
10 mL (12 mL) Luer Lock Sterile	9301-6474	100
10 mL (12 mL) Luer lock bulk	5610-2114	2000
ValueLab 10 mL non-sterile bulk Luer slip	5610-2115	2000
20 mL (24 mL) Luer Lock Sterile	5190-5103	100
20 mL (24 mL) Luer lock bulk	5610-2116	1000
ValueLab 20 mL non-sterile bulk Luer slip	5610-2117	1000