Simplifying Sample Introduction

Rachael Simon
Product Manager, GC Supplies

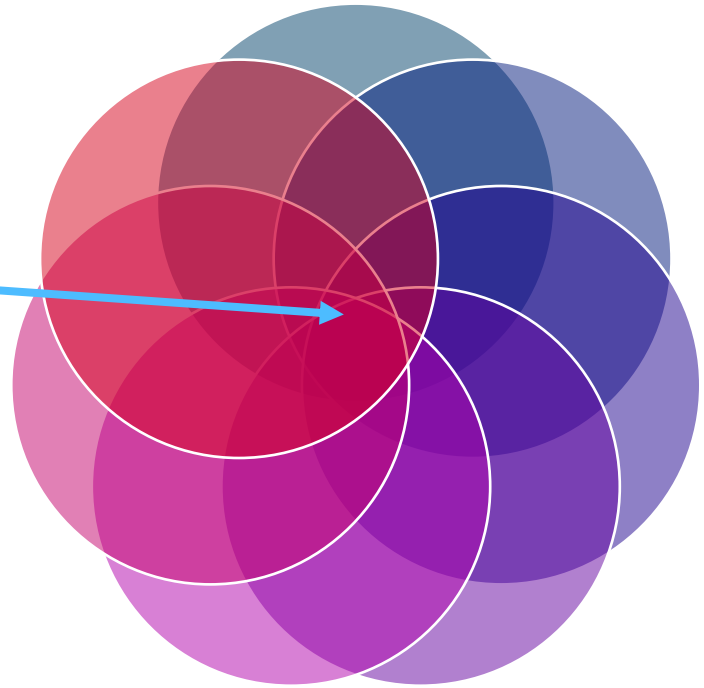
Agilent
Trusted Answers

Sample Introduction

A complex process dependent on many variables.

Some of the keys to a successful injection:

“The Perfect Injection”



Minimal adsorption

Narrow sample band

Correct column installation

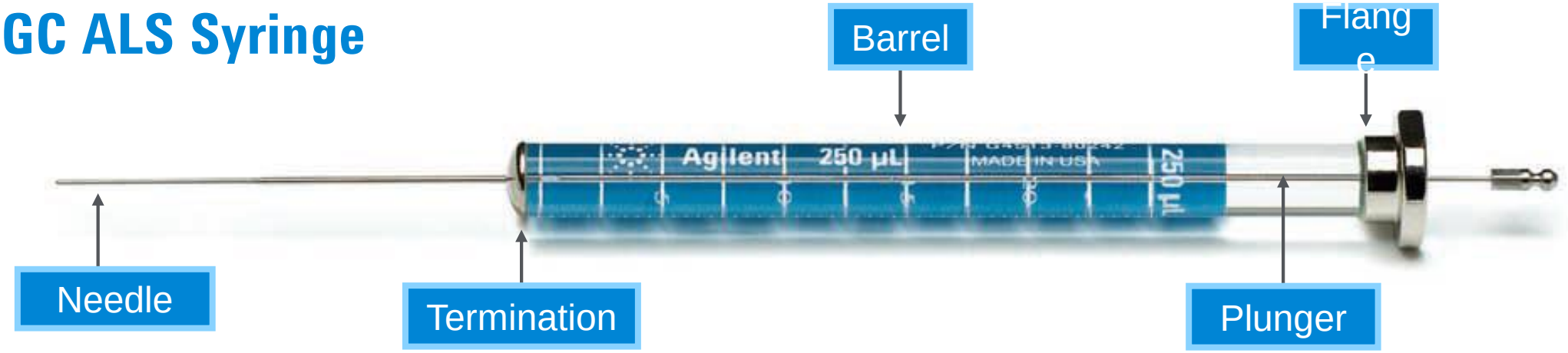
Just enough **(clean)** sample

No discrimination

No carryover

Speedy injection

Anatomy of a GC ALS Syringe



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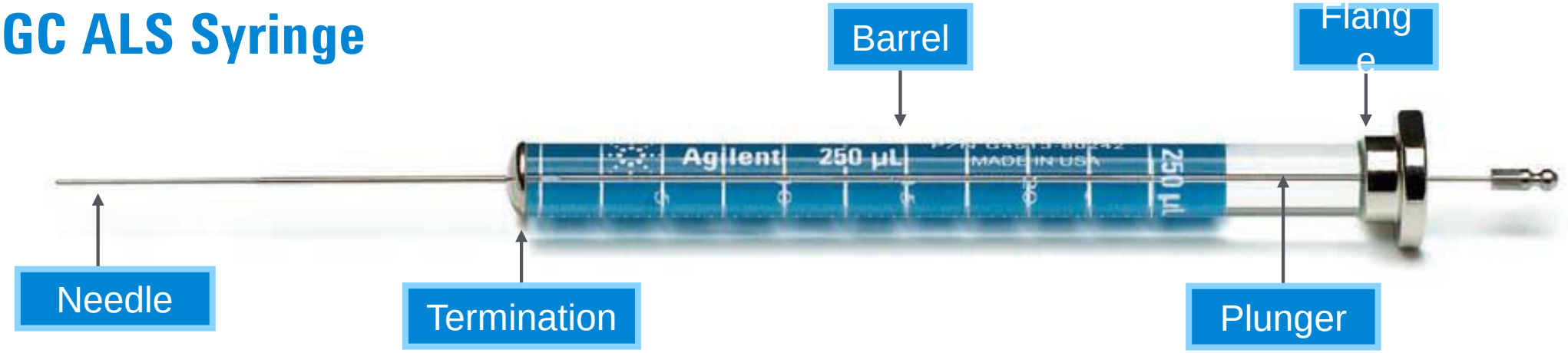
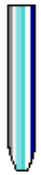
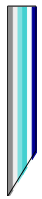




Cone Tip/PS AS (shown)
Used in Agilent autosamplers for optimum performance and reliability by reducing septum coring,

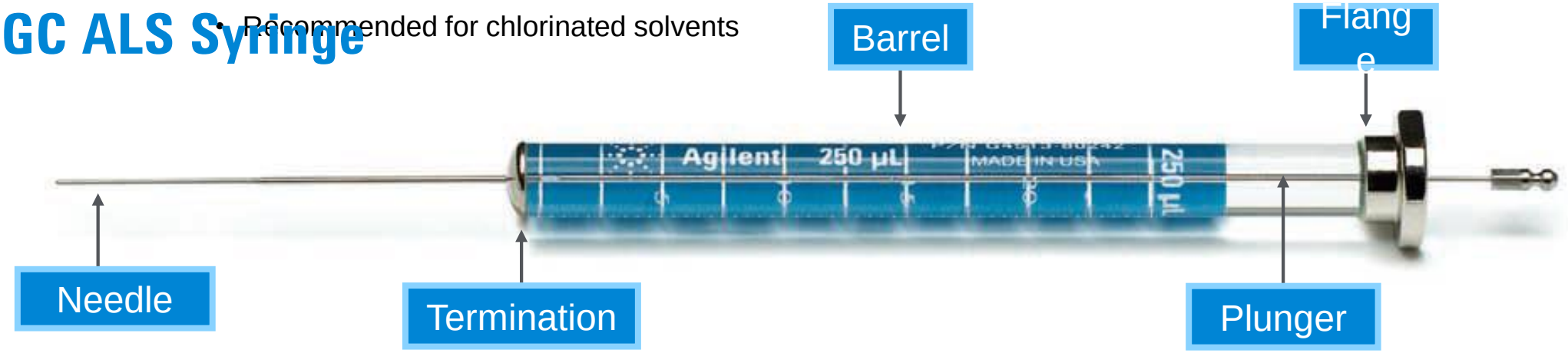


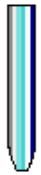
Bevel Tip/PS 2
General purpose, excellent choice for transferring liquids from ampoules or vials. For manual GC injections, a bevel tip is preferred for optimum septum penetration with minimal coring.

Side Hole Tip/PS 5
Recommended for thin gauged septa and large volume- or gas injections.

Anatomy of a GC ALS Syringe

- Recommended for chlorinated solvents





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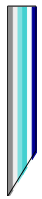

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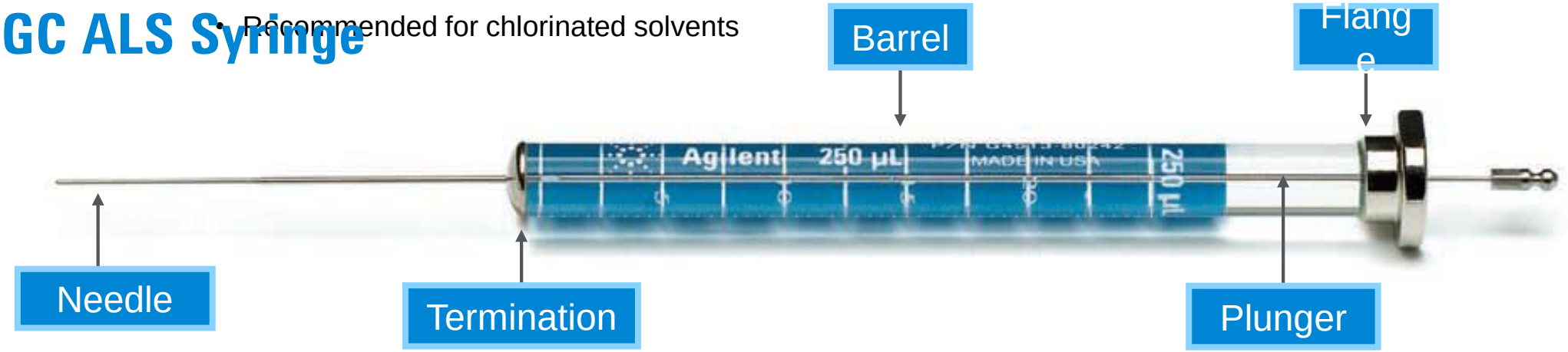

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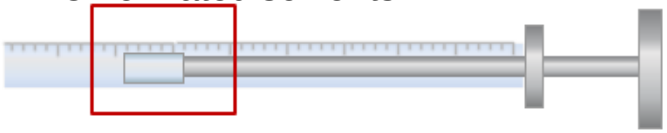
Standard plungers
- Fit tightly within syringe barrel
- Limit loss of volatile sample
- Individually fitted to the syringe
- Not replaceable/Not interchangeable
- Recommended for analysis of liquid samples

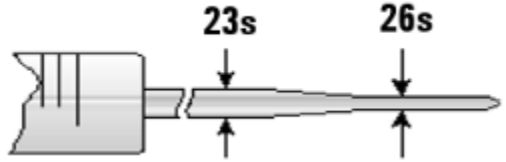
PTFE-tipped (shown)
- Limit sample deposit adsorption
- **Forms gas-tight seal**
- Replaceable
- Requires maintenance to maintain PTFE sea
- Recommended for:
  - "Dirty" samples
  - Highly volatile samples
  - Gas injections
  - Chlorinated solvents



Syringe Selection Tips

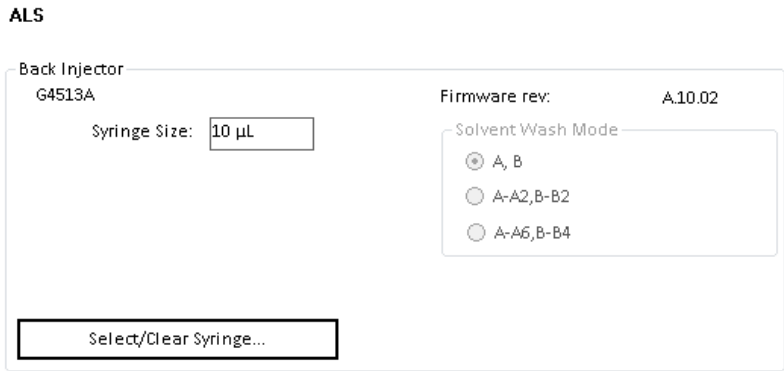
best bet

- 10µL cone-tip, 23/26s tapered needle with PTFE tipped plunger for most SSL and MMI applications
- Taper provides strength of larger needle while minimizing puncture size in septum



- Ensure proper syringe is configured in software
- Gold vs. blue syringes
  - Use blue when:
    - Analyzing trace samples
    - Plunger lifetime is a concern
    - Wear on inlet septa is a concern
  - Use gold when:
    - Price is a concern
    - Analyzing heavily concentrated samples

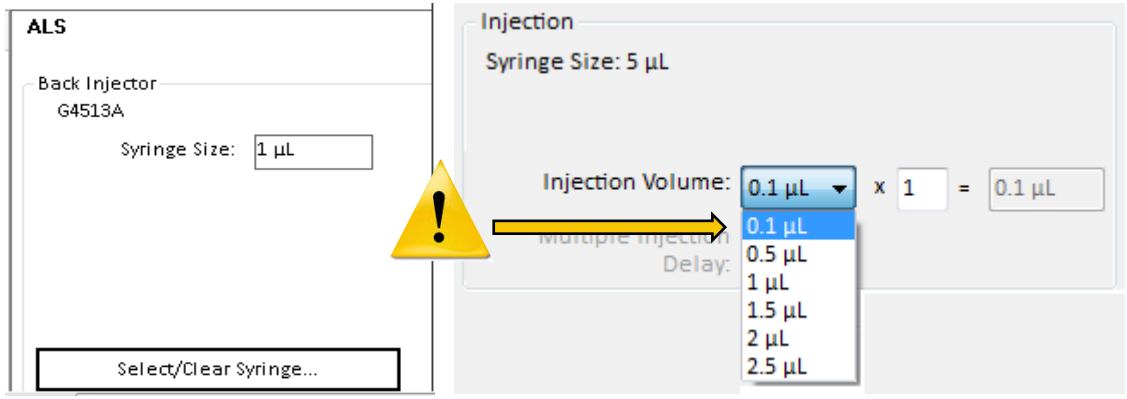
Needle Gauge	Inlet Type* Column ID						
	SSL any	PP any	MMI any	PTV any	COC 250 µm	COC 320 µm	COC 530 µm
23s	●	●	●	●	●	●	●
26s	●	●	●	●	●	●	●
23/26s tapered	●	●	●	●	●	●	●
26s/32 tapered	●	●	●	●	●	●	●

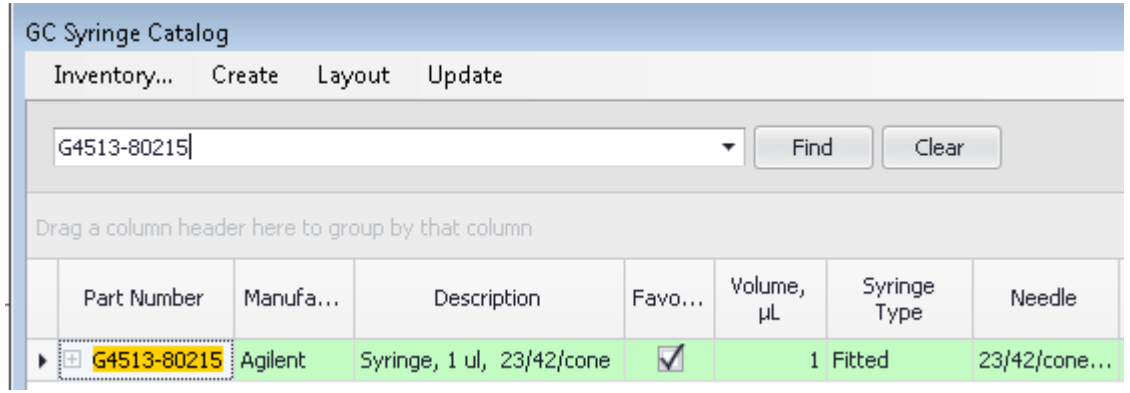


Syringe Selection Tips: Microvolume Injections and Syringes

- 5µL syringes are ideal for small volume injections BUT:
  - Typically shorter lifetime (narrow plunger diameter → bends easier)
  - Do NOT use in solvent saver mode (too much strain on plunger)
  - Not available with PTFE tip (plunger sensitive to PTFE friction)
    - Why not? PTFE can't be accurately machined at that narrow a diameter

- Microvolume syringes
  - **Blue line nanovolume syringes are half-marked!**
    - Need to configure ALS with 2x syringe volume
    - Otherwise risk getting half the response

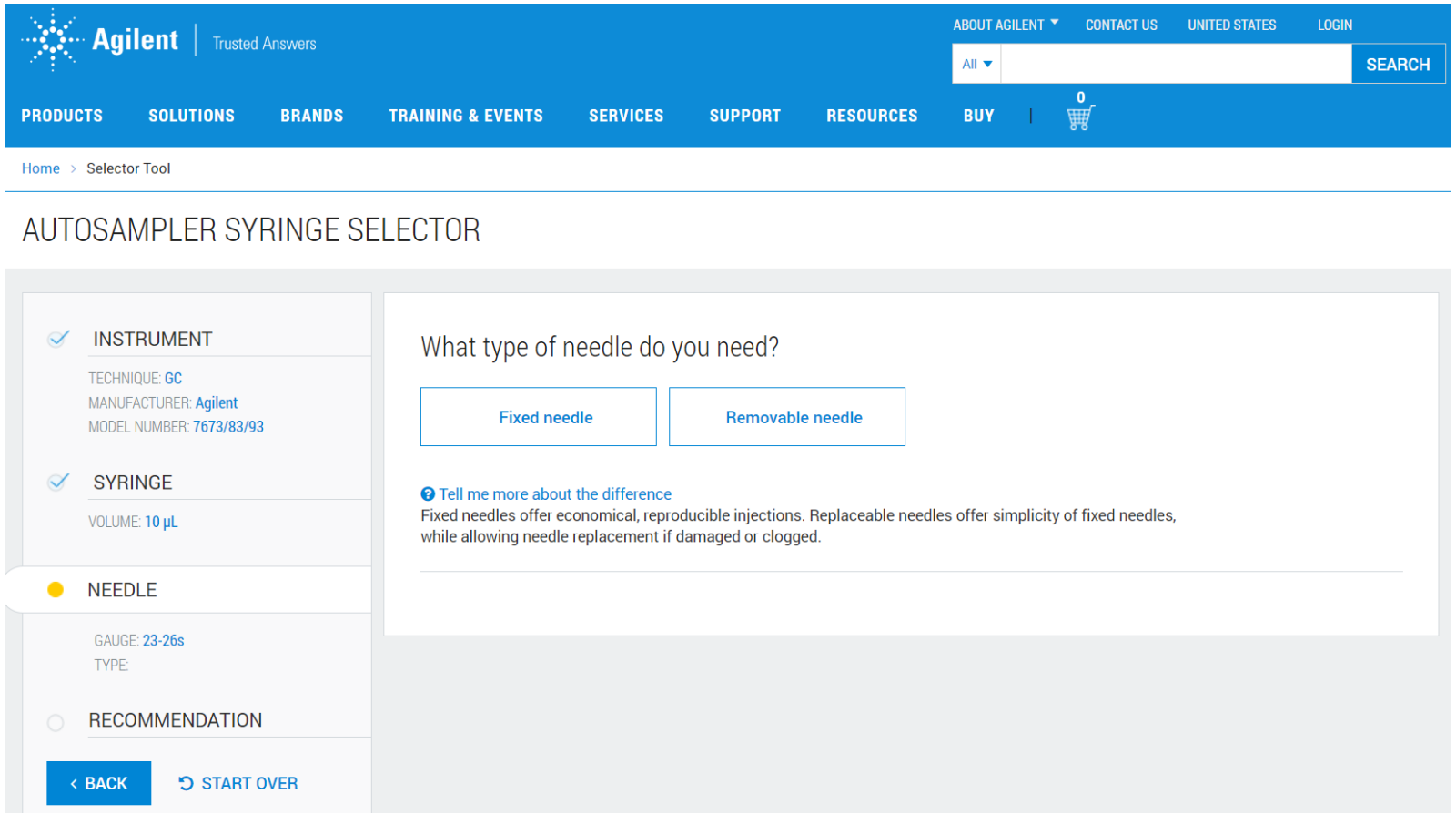




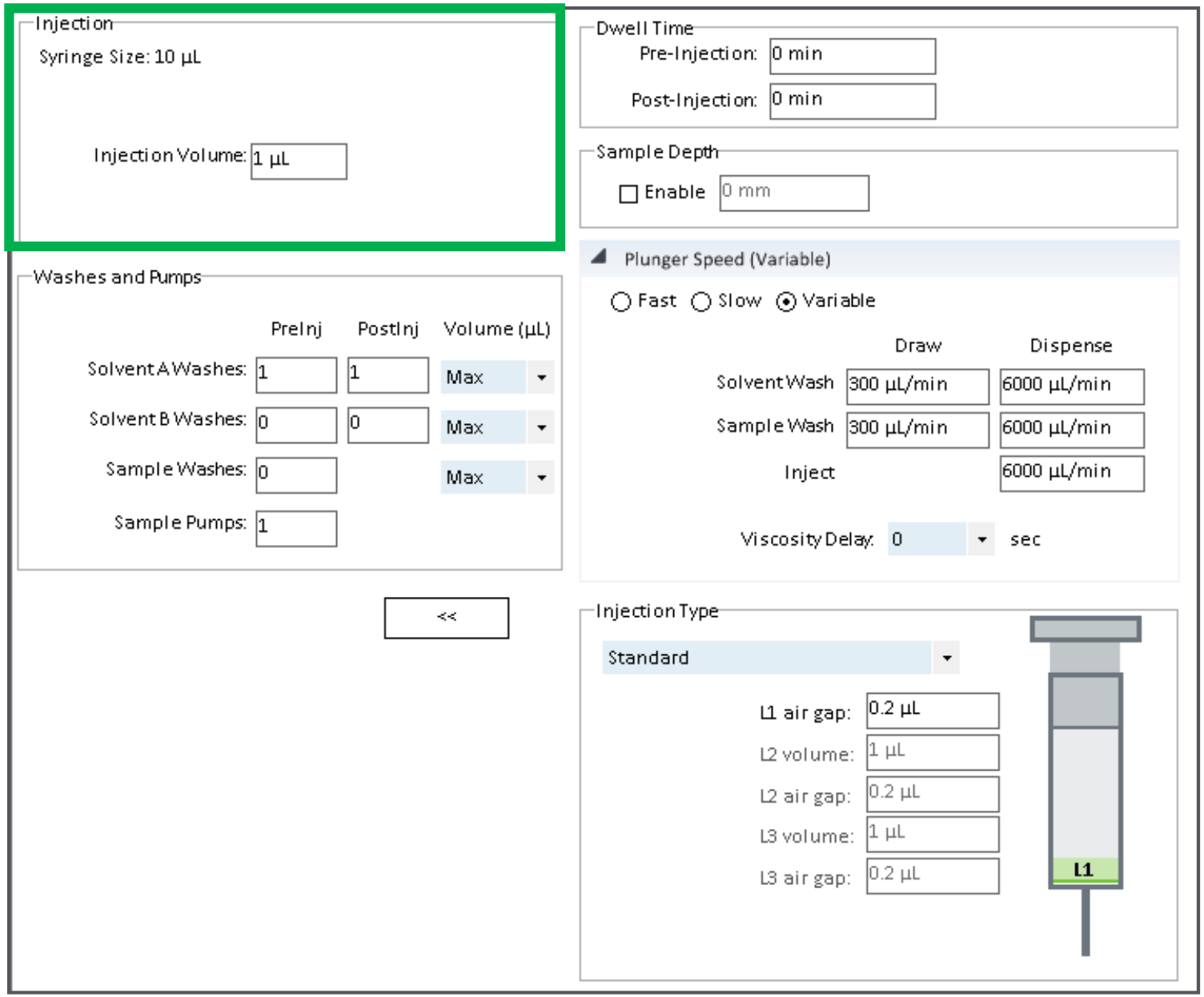
Need help?

Check out our online syringe selector tool

https://www.agilent.com/search/gn/syringe-selector



ALS Method Parameters

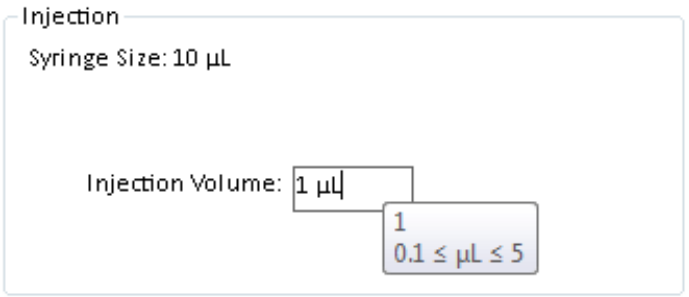


Injection Volume

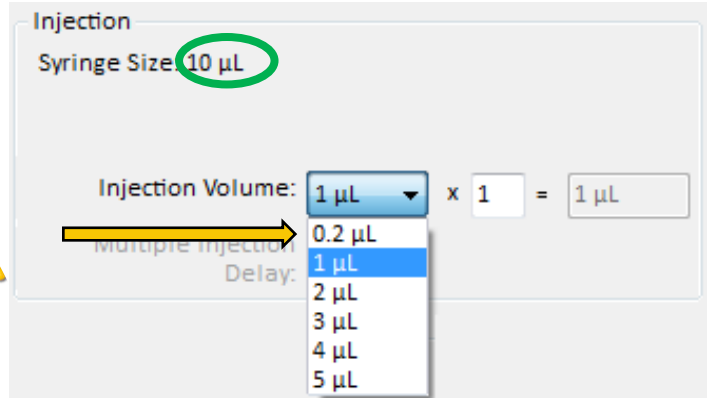
Syringe capacity:

- Avoid injection volumes below the lowest 10% of syringe capacity
  - Injection will work, but may reproducibility may suffer
- ALS software automatically limits max injection volume to 50% of the configured syringe volume
  - 10 µL syringe → recommend 1 to 5 µL injection size

New GC driver
Injection volume selection



Old GC driver
Injection volume selection



Starting points for injection volume

Goal: Inject **as little sample as possible** to meet detection limit

- Ensure compatibility between solvent type, volume, and inlet liner
  - Use vapor volume calculator
- Injection volumes for most organic solvents should be within 1 – 2 µL or less
  - Split vs. splitless
- Avoid injecting water- coefficient of expansion is too high
- Higher injection volumes:
  - dirty samples → more maintenance
  - Concentrated samples → overloading

Split ≤ 1µL

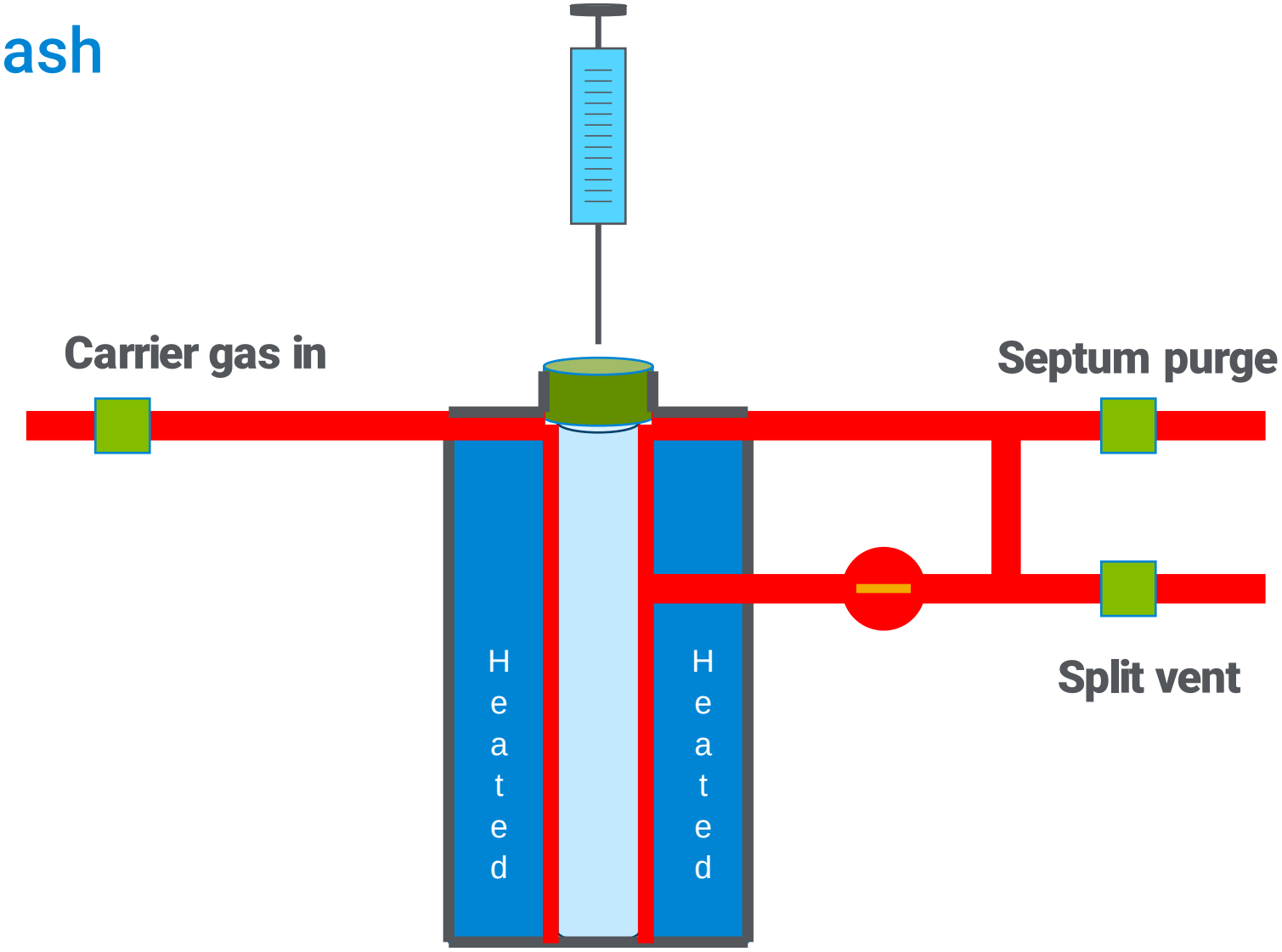
Splitless ≤ 2µL

More Maintenance

Tip: Download our vapor volume calculator to determine the highest volume compatible with your liner

https://www.agilent.com/en/support/gas-chromatography/gccalculators

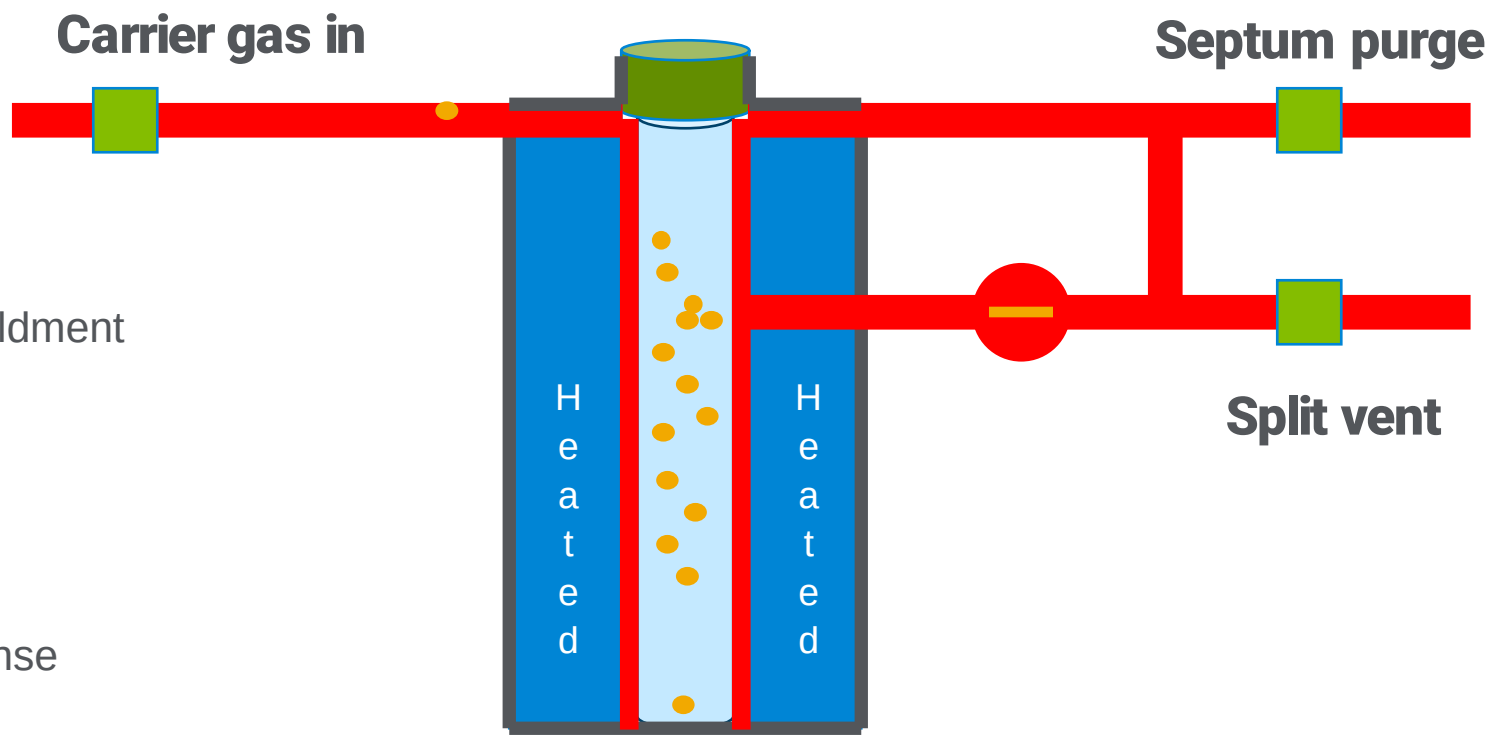
Sample Backflash



Sample Backflash

How To Avoid/Minimize

- Reduce injection volume
- Larger liner volume
- Use pressure pulse
- Tapered liner
- Choose the
- UltiMetal Plus Inlet weldment



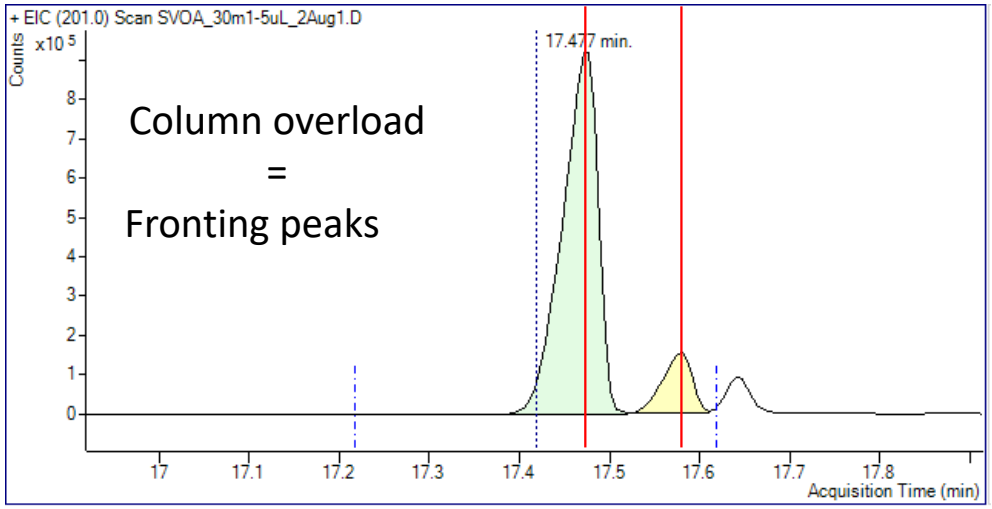
Negative Effects

- Tailing
- Low compound response
- Complete compound absorption
- Poor reproducibility

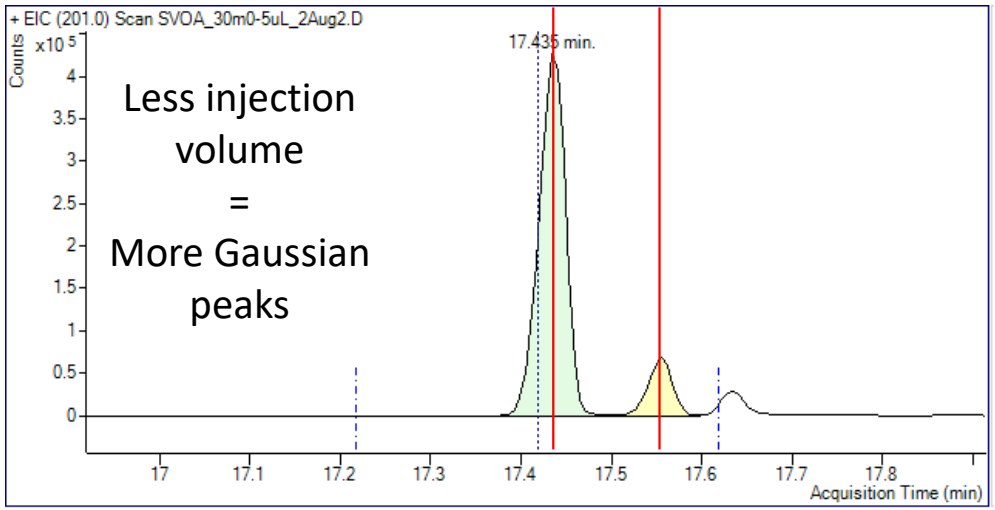
Chromatographic signs that your injection volume is too high

- **Overloading**
  - **Watch for highly concentrated samples**
  - **Don’t inject too much sample**

1 µL injection



0.5 µL injection



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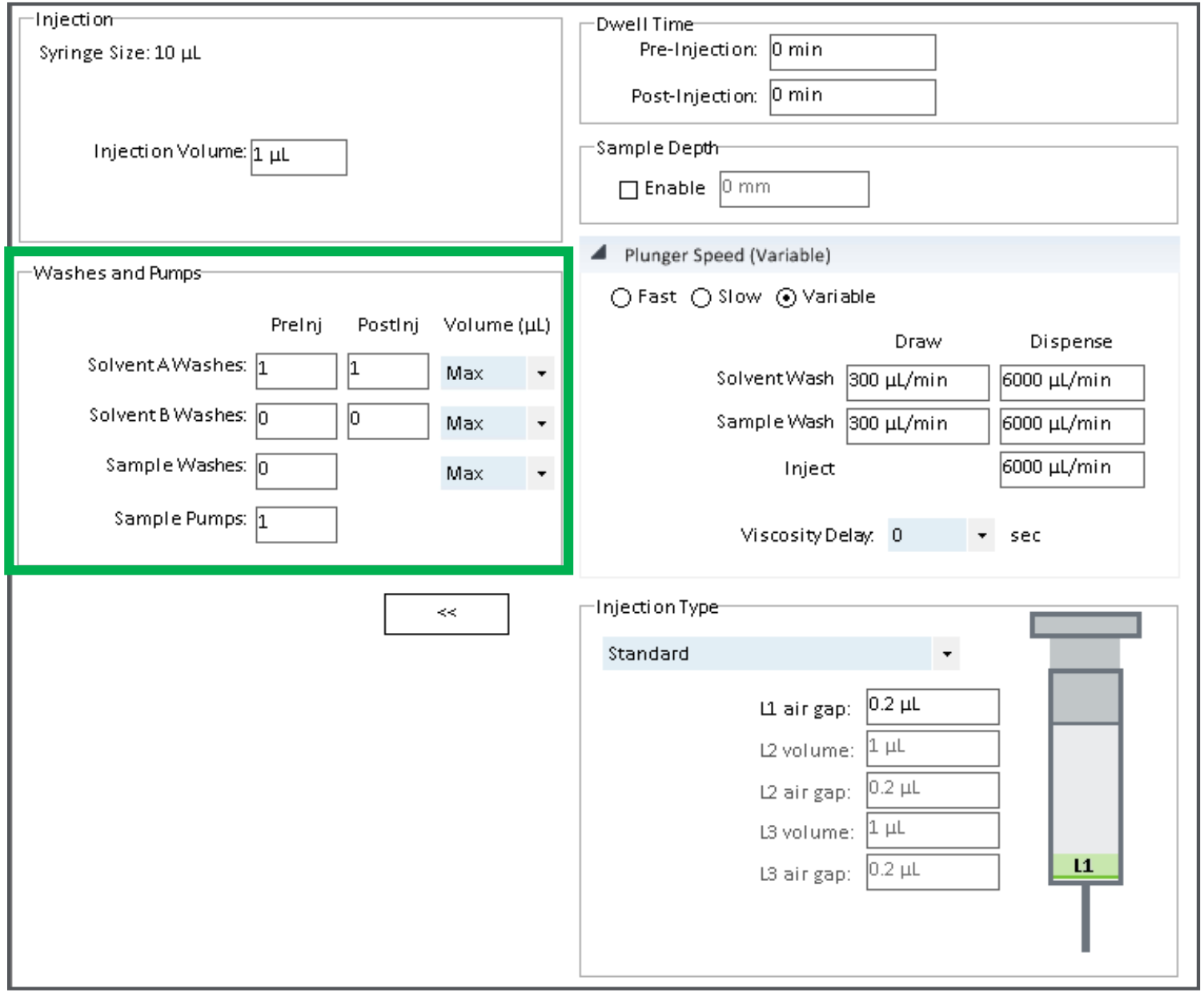
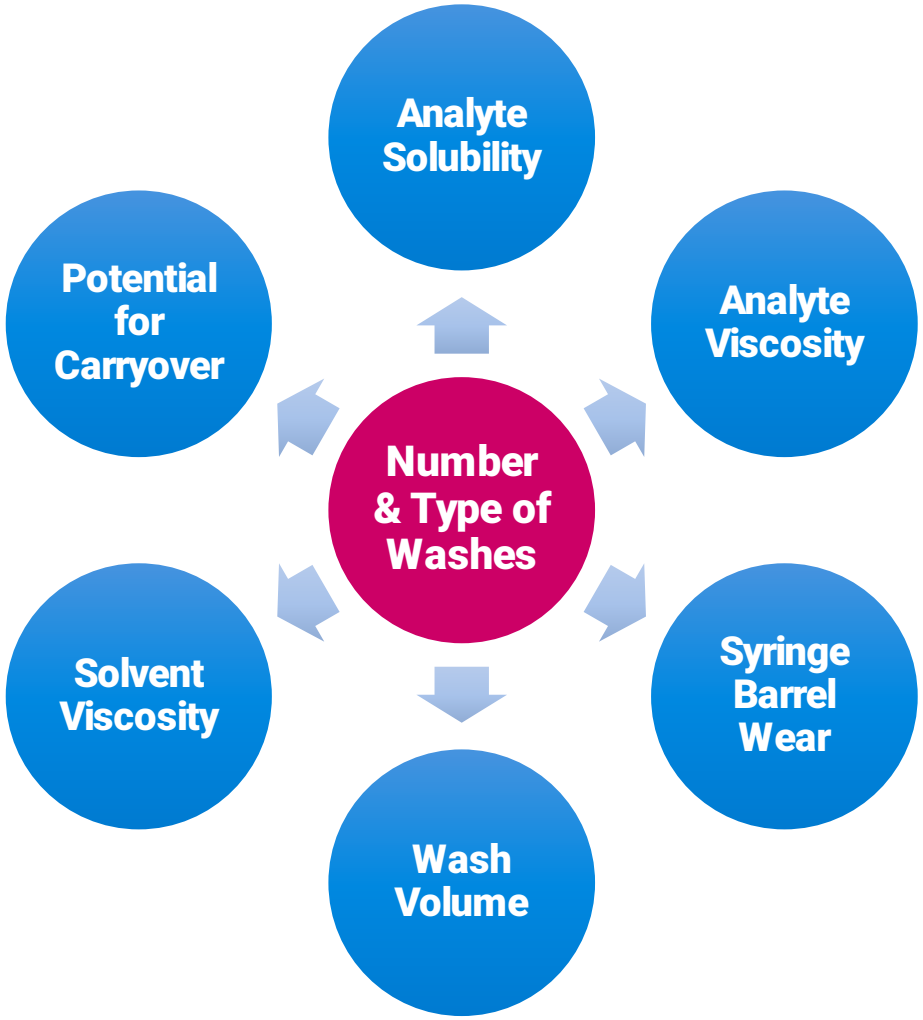


Washes and Pumps: Solvents

- Ideally, 4 max volume (80%) washes reduces carryover to one part in 10,000



Washes and Pumps

	PreInj	PostInj	Volume (µL)
Solvent A Washes:	4	4	Max
Solvent B Washes:	0	0	Max
Sample Washes:	1		Max
Sample Pumps:	3		

Washes and Pumps: How much wash solvent are you using?

$$\#\ injections\ * \frac{(\#pre + \#post)\ washes}{injection} * wash\ volume = wash\ solvent\ used$$

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≤ 2 mL 

> 2 mL 

- Use wash vial B
- Reduce wash volume
- Reduce # of injections

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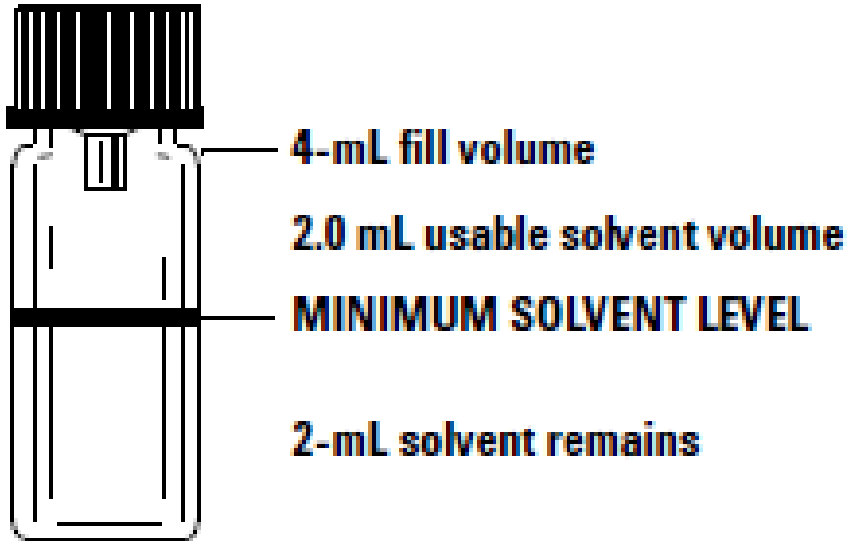

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	Name	Vial	Type	Level
1	Blank	1	Blank	
2	0.1 ug/L	2	Cal	1
3	0.5 ug/L	3	Cal	2
4	1 ug/L	4	Cal	3
5	5 ug/L	5	Cal	4
6	10 ug/L	6	Cal	5
7	Blank	1	Blank	
8	09272017-01	7	Sample	
9	09272017-01-SP	8	MatrixSpike	
10	Bake	99	Blank	
11	Blank	1	Blank	
12	09272017-02	9	Sample	
13	09272017-02-SP	10	MatrixBlank	
14	Bake	99	Blank	
15	Blank	1	Blank	
16	09272017-03	11	Sample	
17	09272017-03-SP	12	MatrixBlank	
18	Bake	99	Blank	
19	Blank	1	Blank	
20	09272017-04	13	Sample	
21	09272017-04-SP	14	MatrixBlank	
22	Bake	99	Blank	

22 line sequence

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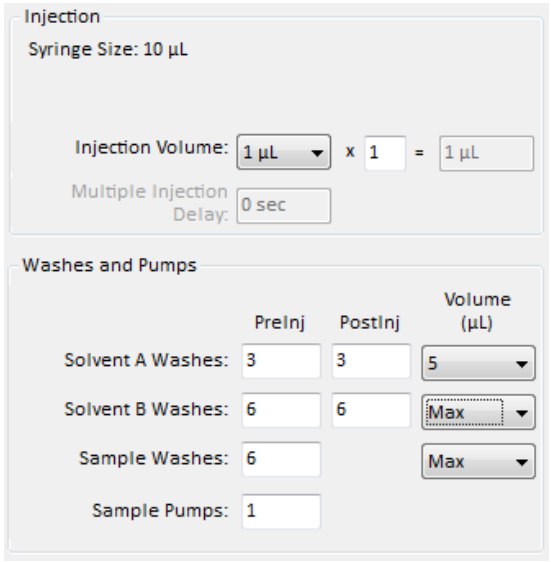

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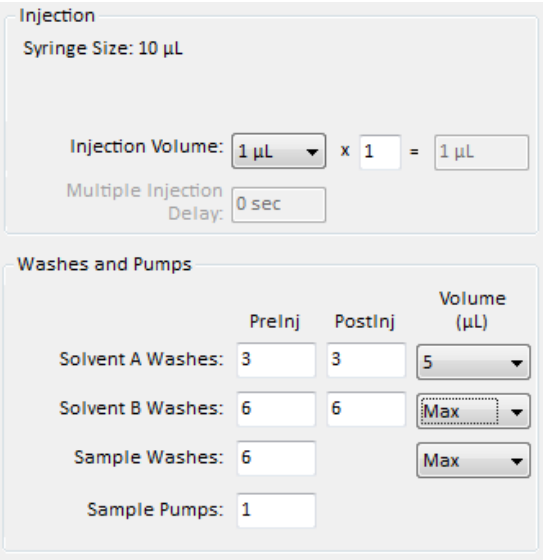

A = 6 washes

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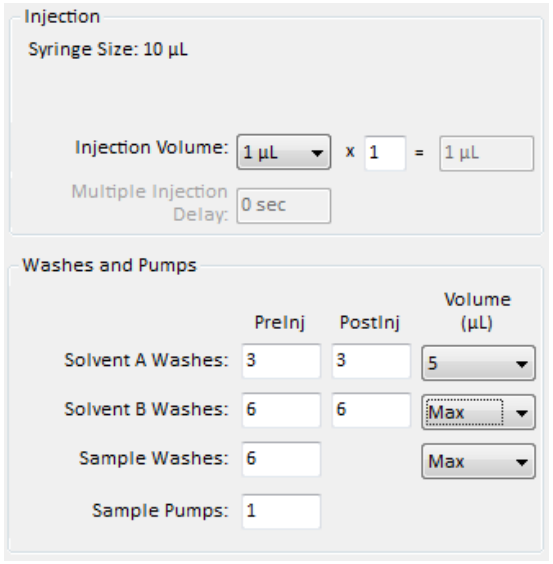

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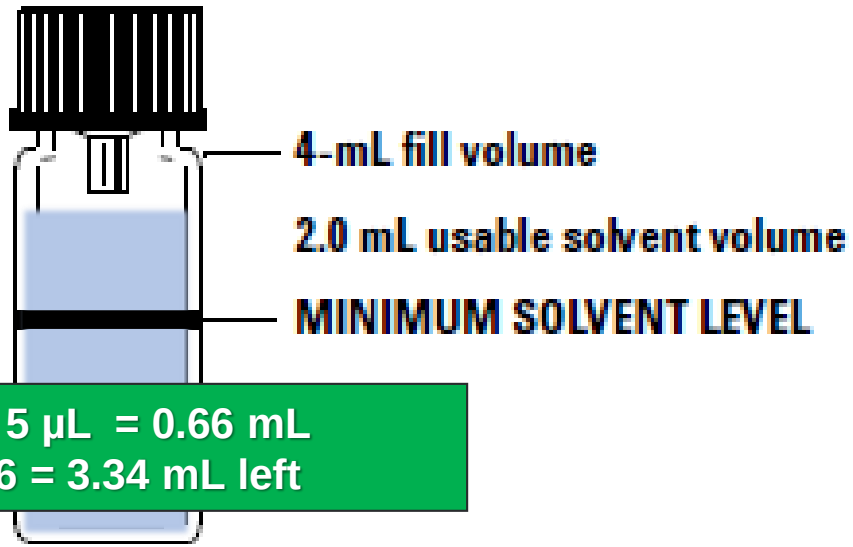
Solvent A



22 * 6 * 5 µL = 0.66 mL
4 – 0.66 = 3.34 mL left

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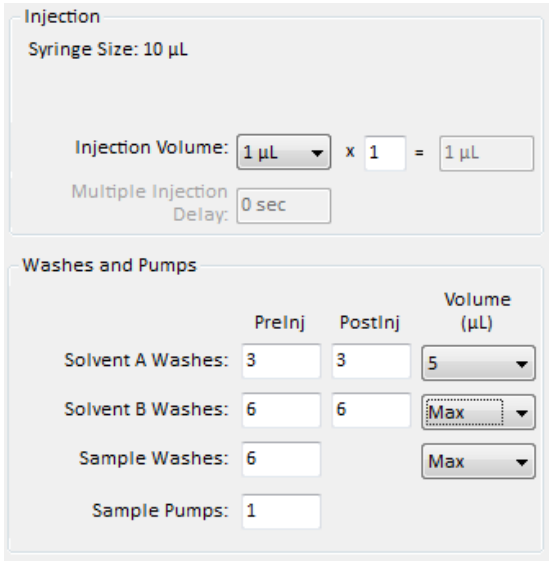
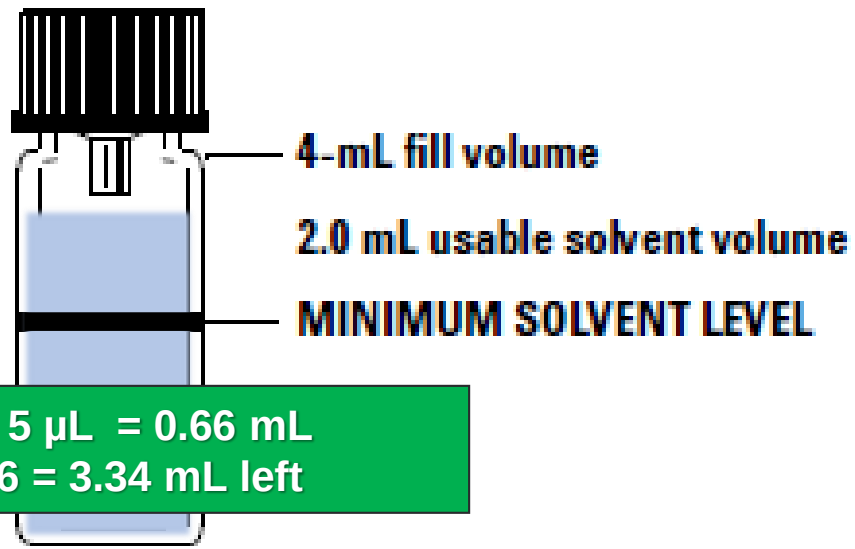


A = 6 washes

B = 12 washes

Solvent A



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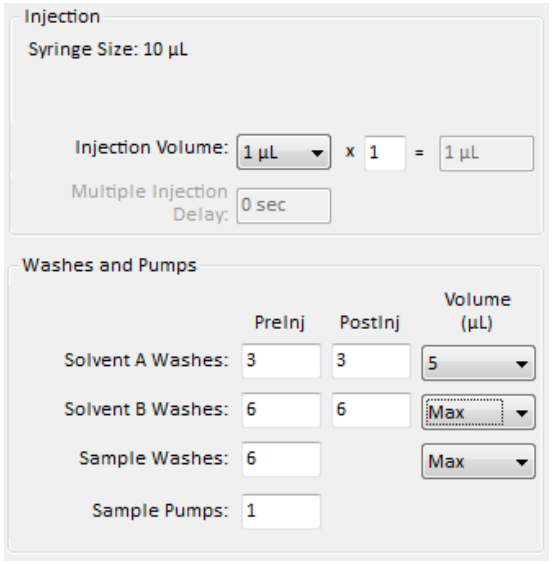

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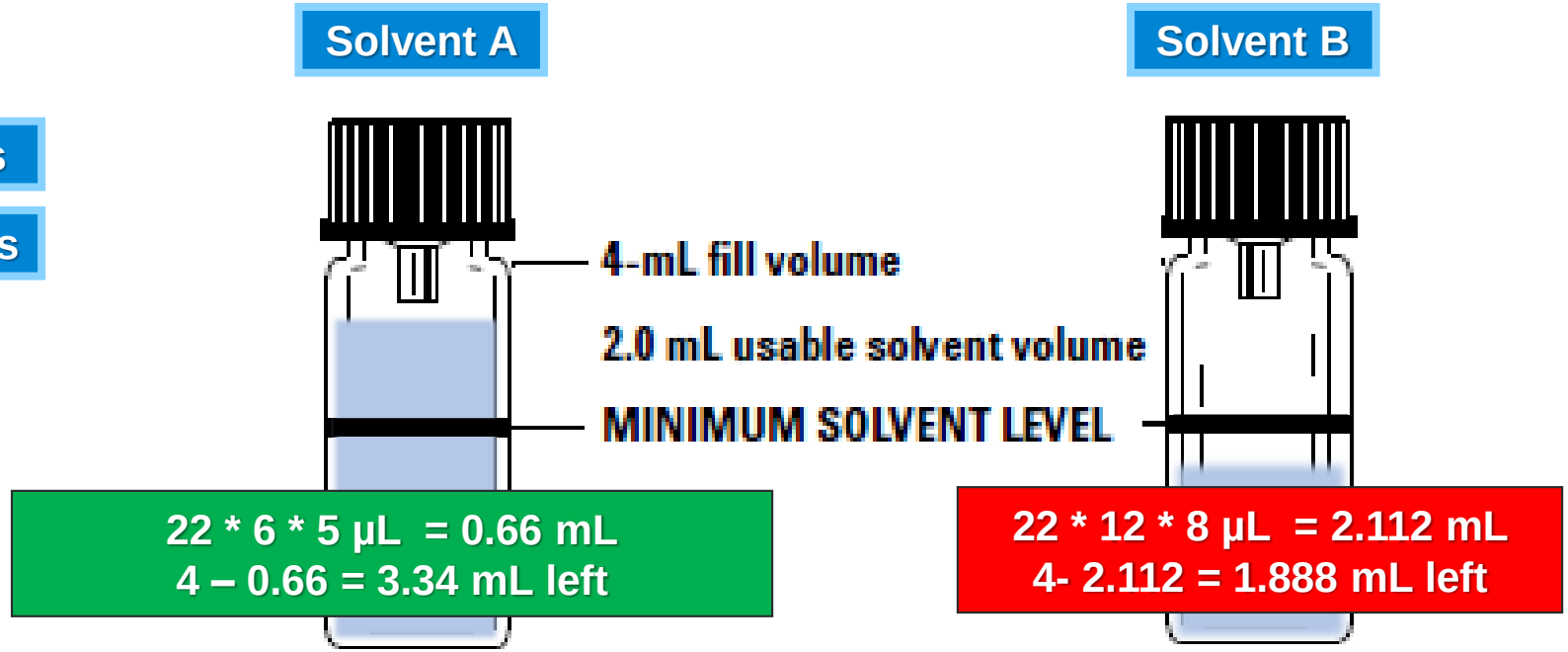
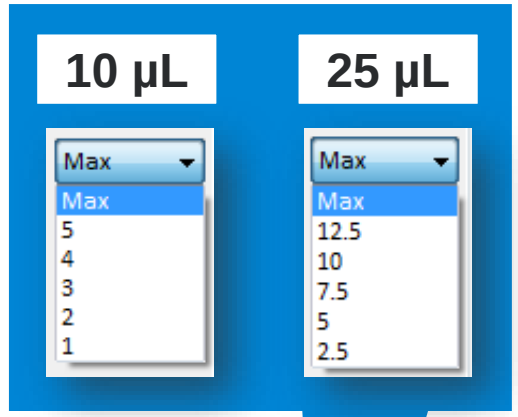
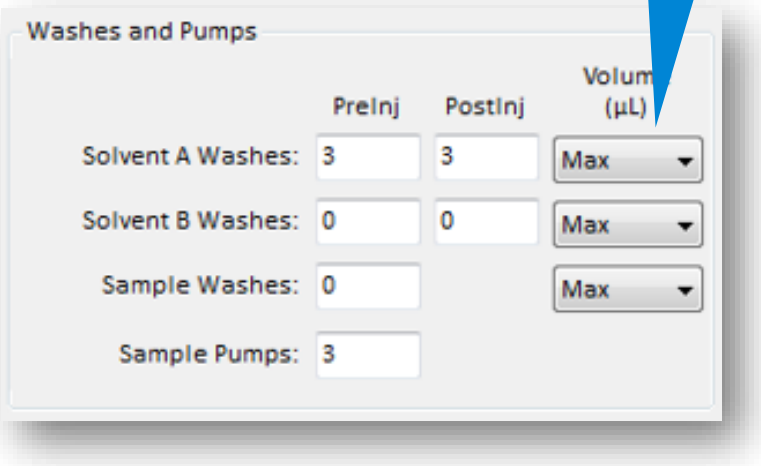


Washes and Pumps: Solvents

- High wash application? Try solvent saver
  - **MUST use PTFE-tipped syringe**
    - Fitted syringes lubricate sufficiently, causing premature failure
  - 10%, 20%, 30%, 40%, and 50% of syringe size (µL)
    - Wash volume will automatically be configured upon syringe size selection

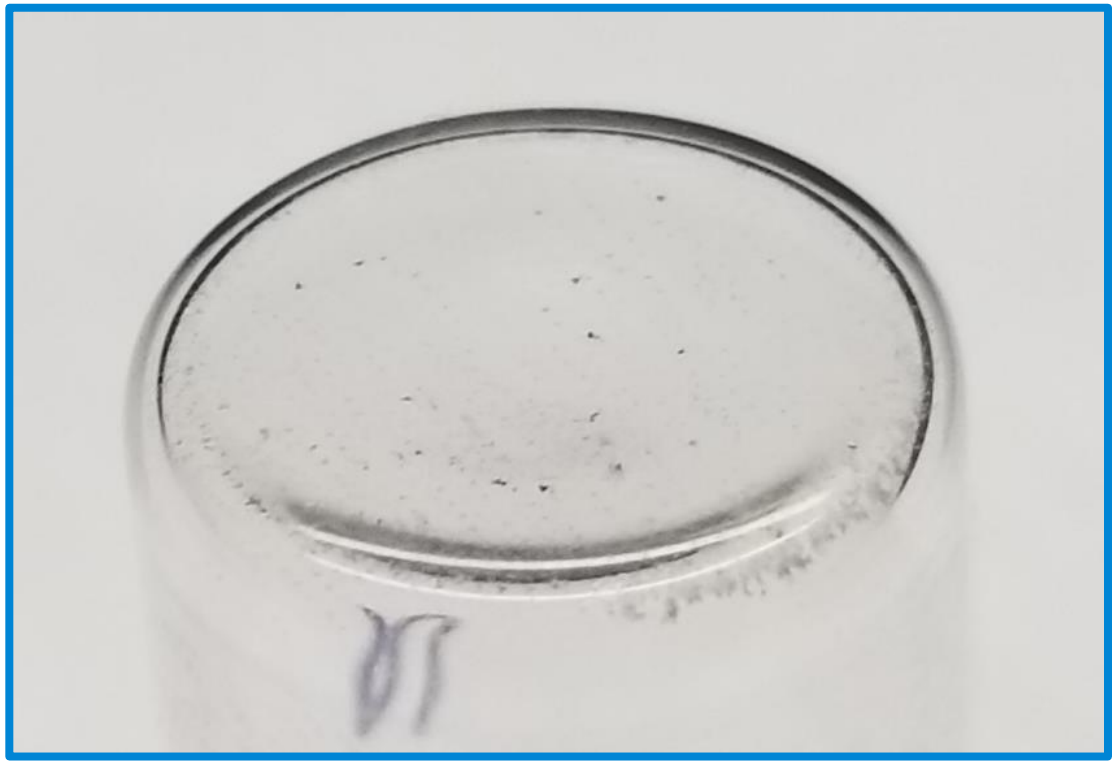
  - Steps:
    - Syringe draws in solvent to specified amount
    - Syringe and needle rise from solvent bottle
    - Plunger rises to the 80% mark, rinsing syringe barrel with solvent, then air
    - Solvent and air discharged into waste bottle
- **Don't let the wash vial run dry**



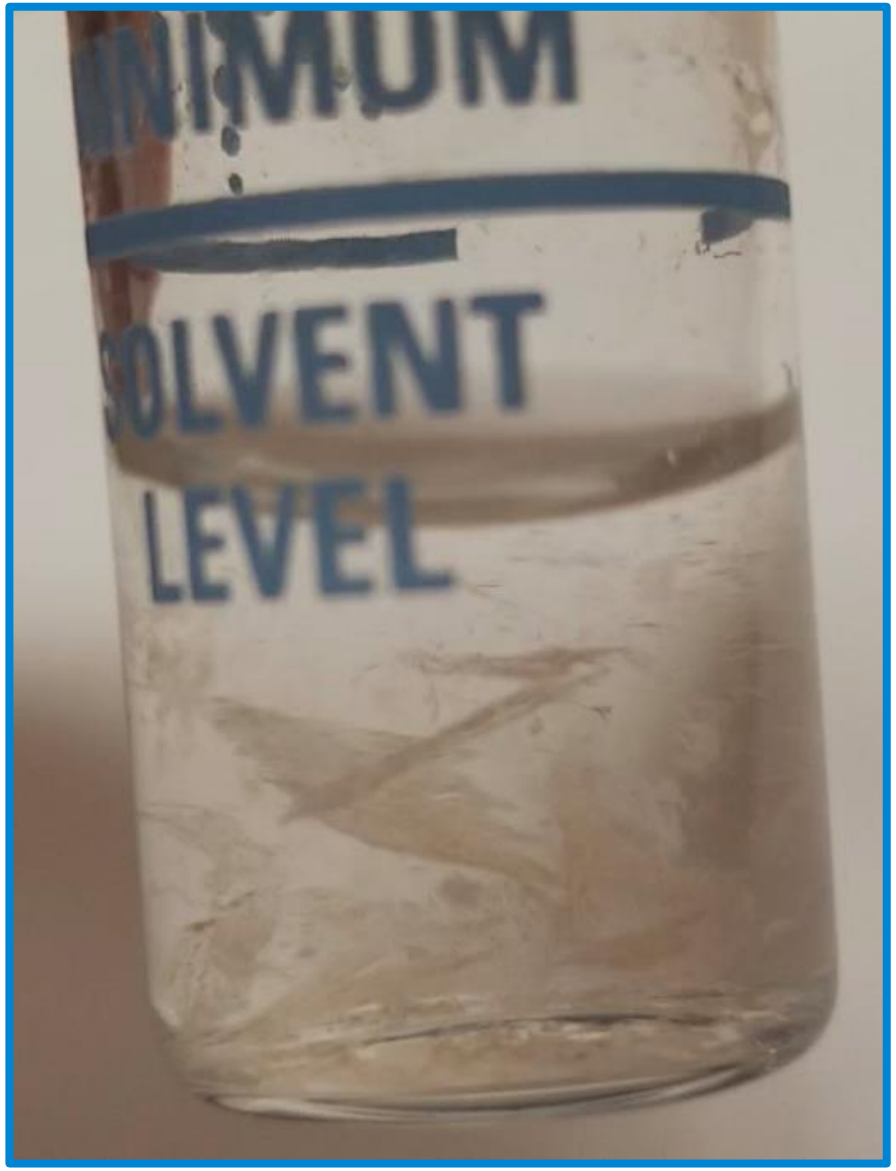


Washes and Pumps: Solvents

- Frequently clean or replace wash vials
  - Traces of previous samples will accumulate over time
  - Do not refill or “top-off” the vial, instead empty, rinse, and replace solvent
  - Use a cotton swab to remove particulates from the glass surface



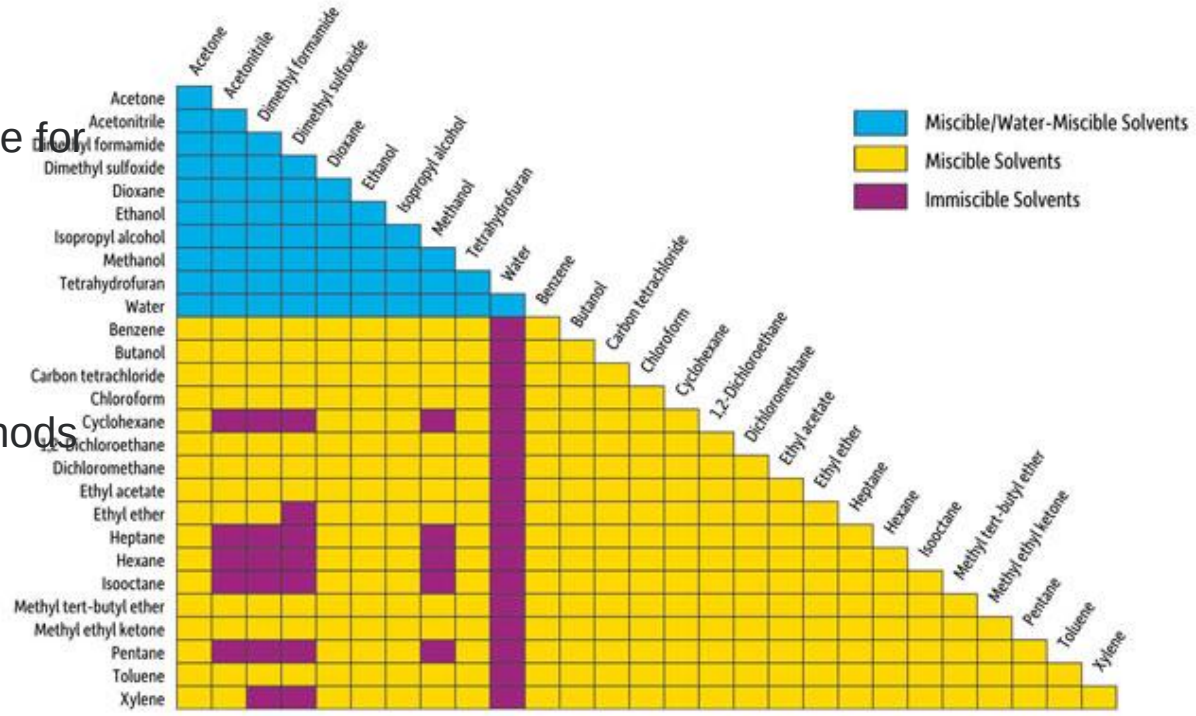
Contaminated wash vial bottom

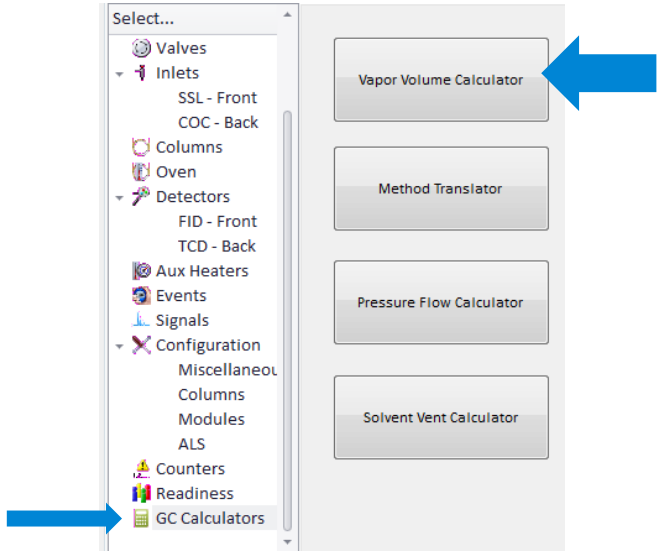


Contaminated wash solvent

Washes and Pumps: Solvents

- Choose a (or a series of) wash solvent(s) that make(s) sense for the analysis
  - Is the analyte soluble in the solvent?
  - If wash solvent ≠ sample solvent, are they miscible?
  - Do not use acidic or alkaline solvents with syringes
  - What other solvents are used/analytes determined in methods on that same GC?
- Use both A and B wash vials
  - 2nd wash vial will be cleaner than first
  - 2nd wash vial should never be water (rust)



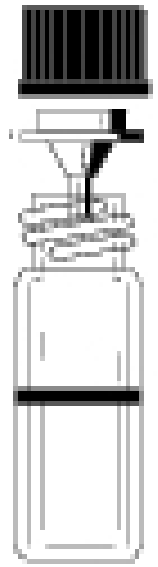


best bet

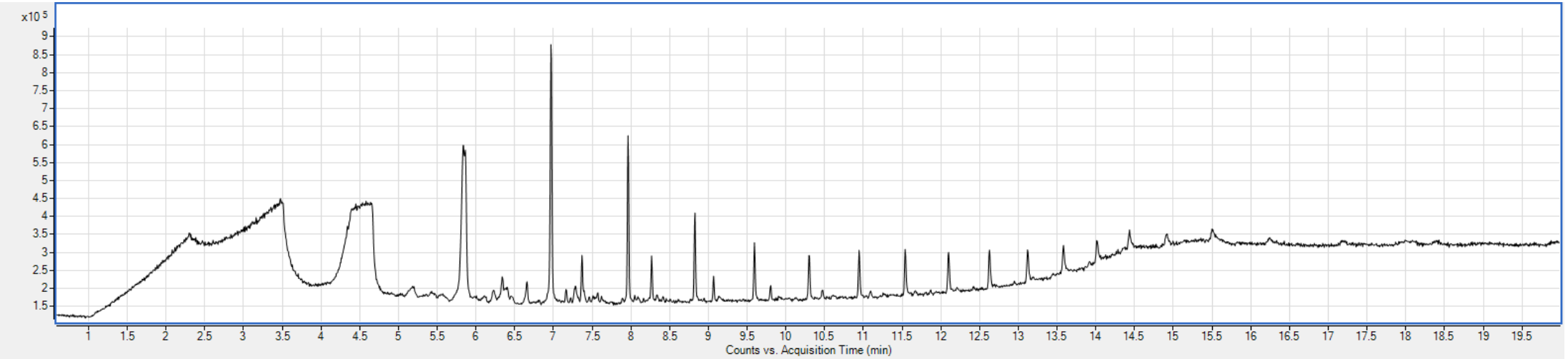
Avoid viscous solvents, and solvents with high vapor expansion volumes. Use the vapor volume calculator to make sure it will not overload the inlet liner.

Washes and Pumps: Solvents

- Diffusion caps are important.
  - Reduce volatile solvent diffusion
  - Better alternative than using vial septa, which could core, contaminate wash solvent vial → septum bleed peaks

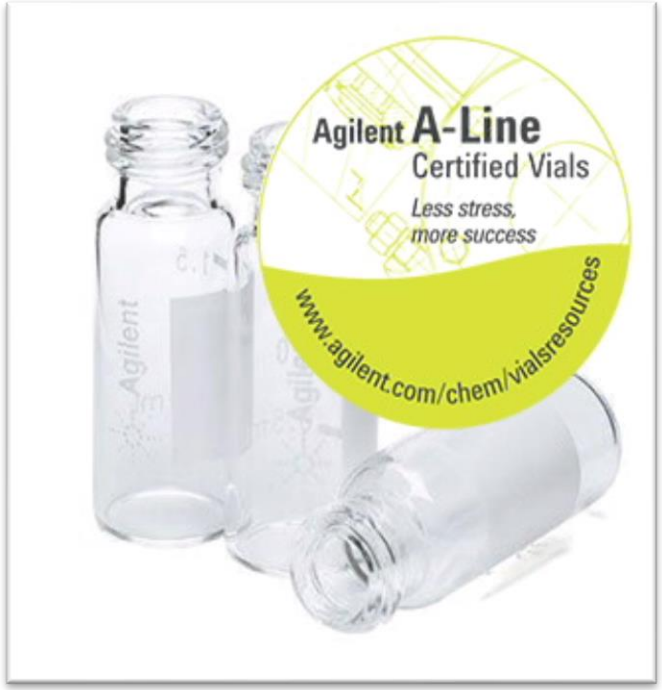


5182-0551, 4 mL wash vials with fill markings and caps 25/pk
07673-40180, Diffusion inserts with black open top screw caps 12/pk



Use the right vial

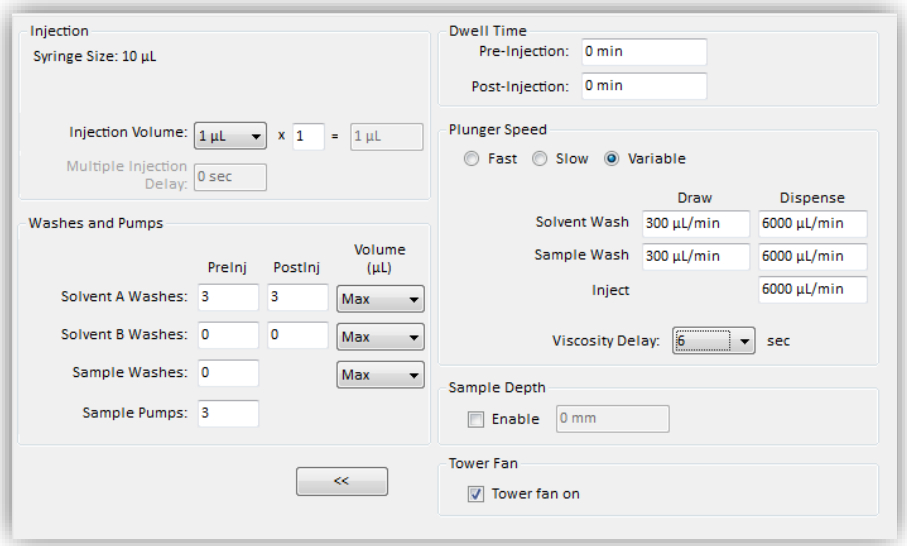
- **Choose high quality vials and caps**
  - **Poorly constructed vial septa → siloxanes → bleed peaks**
  - **Low quality vial → leach contaminants into sample**
  - **Choose the right cap/septa for your solvent**



	High performance septa	Thin PTFE	PTFE/Silicone*	PTFE/Silicone/PTFE*	PTFE/Red rubber	Flouroelastomer	Butyl
Temperature range	40 °C to 300 °C**	Up to 260 °C	–40 °C to 200 °C	–40 °C to 200 °C	–40 °C to 90 °C	–40 °C to 260 °C	–50 °C to 150 °C
Use for multiple injections	No	No	Yes	Yes	No	No	No
Price	More expensive	Very economical	Economical	Most expensive	Very economical	Economical	Economical
Resistance to coring	Excellent	None	Excellent	Excellent	None	None	None
Recommended for storage	No	No	Yes	Yes	No	No	No
Best for	High temperature headspace applications	Superior chemical inertness, short cycle times, and single injections	Most common HPLC and GC analyses, not as resistant to coring as P/S/P	Superior performance for ultra trace analysis, repeat injections, and internal standards	Chlorosilanes, more economical option for single injections	Chlorinated solvents, higher temperatures	Organic solvents, acetic acids, impermeable to gases

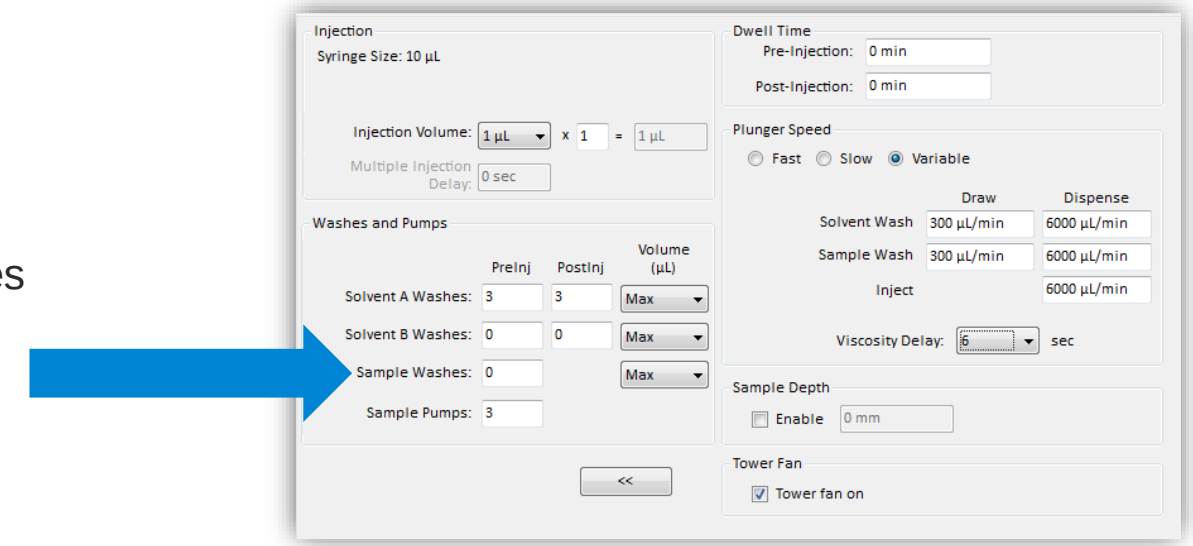
* Agilent silicone is platinum cured (versus peroxide cured), making it more inert and less likely to interact with samples.
** For up to 1 hour.

Washes and Pumps: Samples



Washes and Pumps: Samples

- **Sample washes**
  - Primes syringe barrel with sample, discards into waste bottle
  - Improves reproducibility
  - Exercise caution if sequence includes reduced volume samples



Washes and Pumps: Samples

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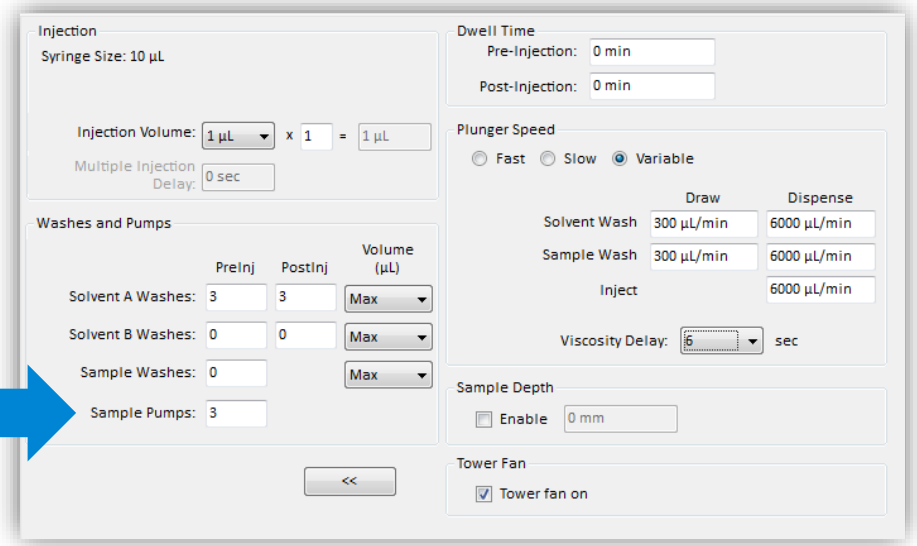
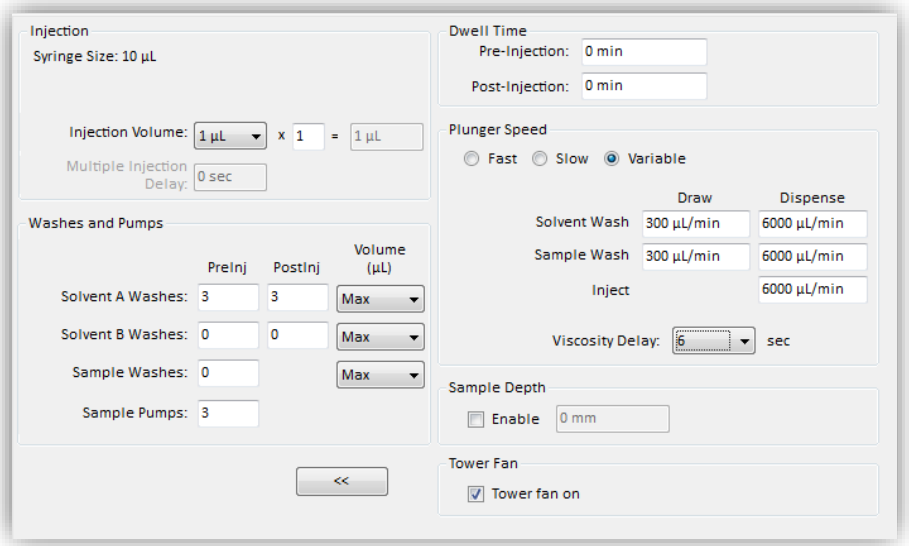
  - Primes syringe barrel with sample, discards into waste bottle
  - Improves reproducibility
  - Exercise caution if sequence includes reduced volume samples
- **Sample pumps**
  - Draws sample into syringe, discards into same vial
  - Eliminates air bubbles → improves reproducibility
  - Important for volatile samples (leaves film on inner needle wall)
  - Exercise caution if using viscous samples or solvents
  - If needle contains solvent from previous wash, can dilute sample (use sample washes)
  - Don't overdo it
    - 3-5 pumps is usually adequate
    - Excessive pumping can reduce plunger lifetime



Washes and Pumps: Samples



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  - If needle contains solvent from previous wash, can dilute sample (use sample washes)
  - Don't overdo it
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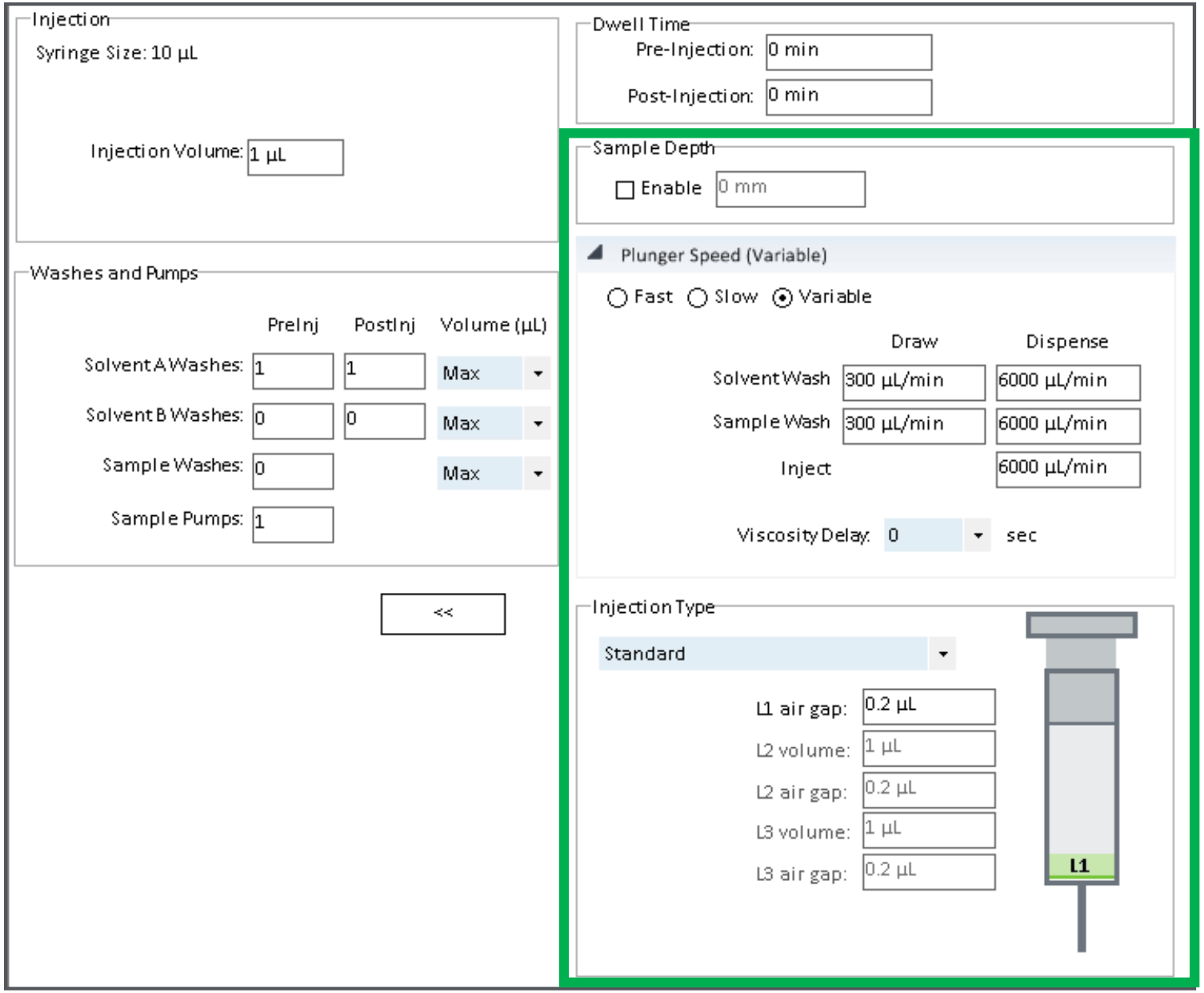
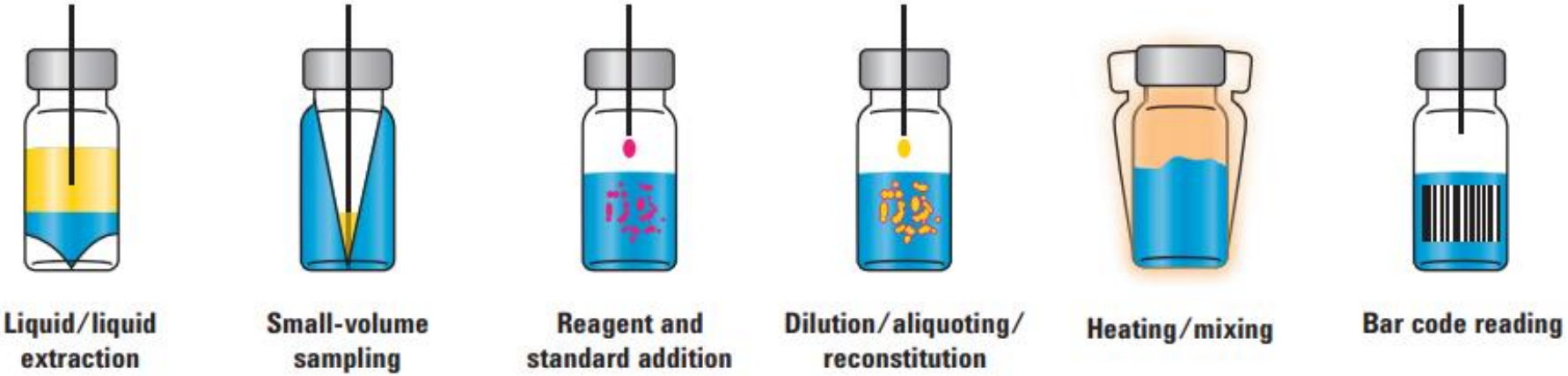
- Fill sample vial up to shoulder
  - Leaving small headspace prevents cavitation, vacuum formation
  - Improves reproducibility
  - Use microvial inserts to help assure good sampling depth for needle and to conserve sample

Advanced Method Parameters

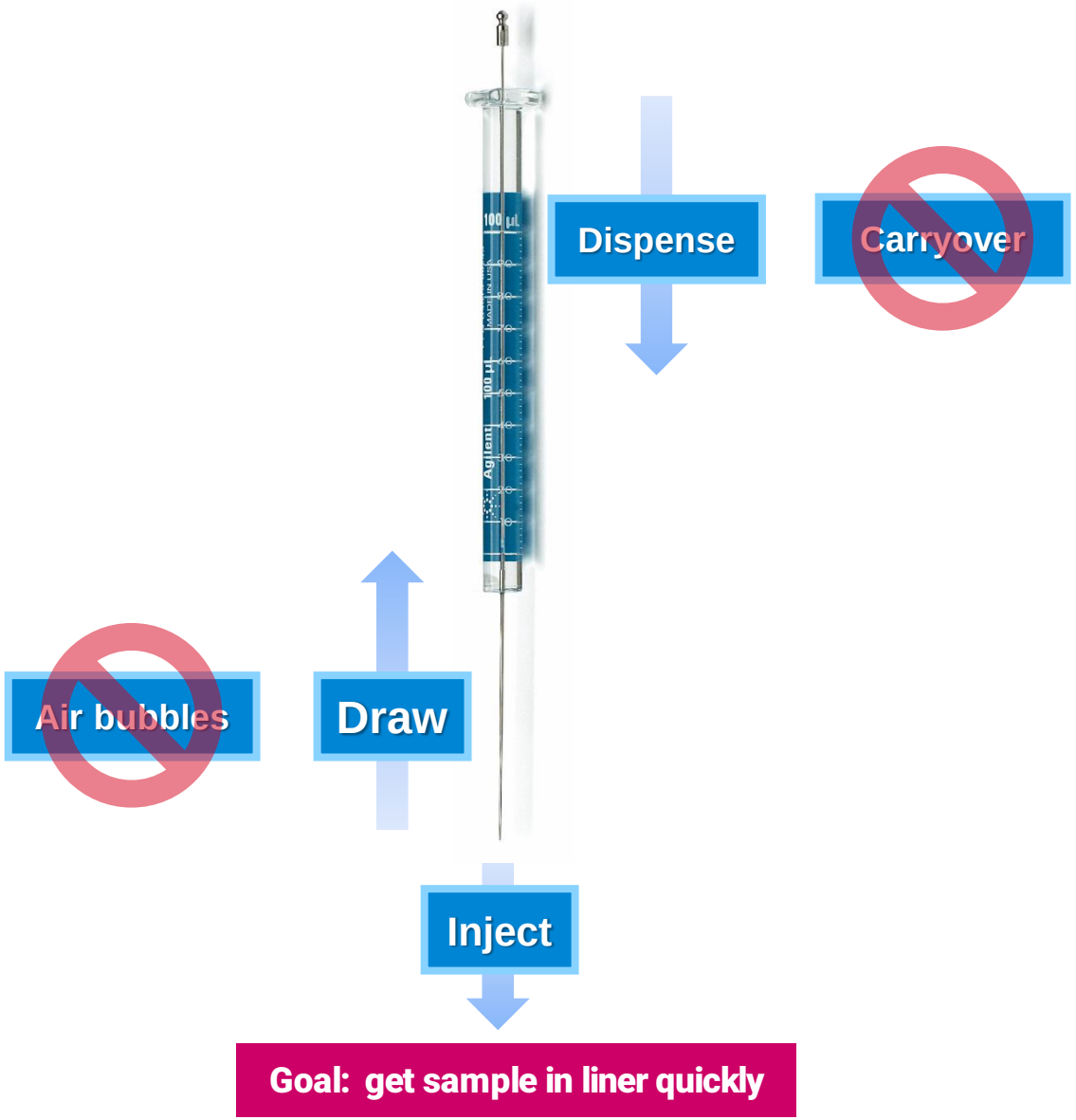


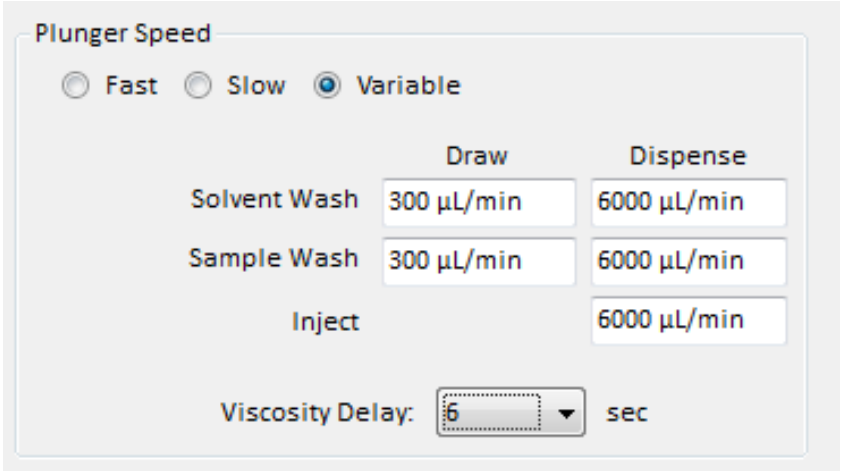
Sample Depth

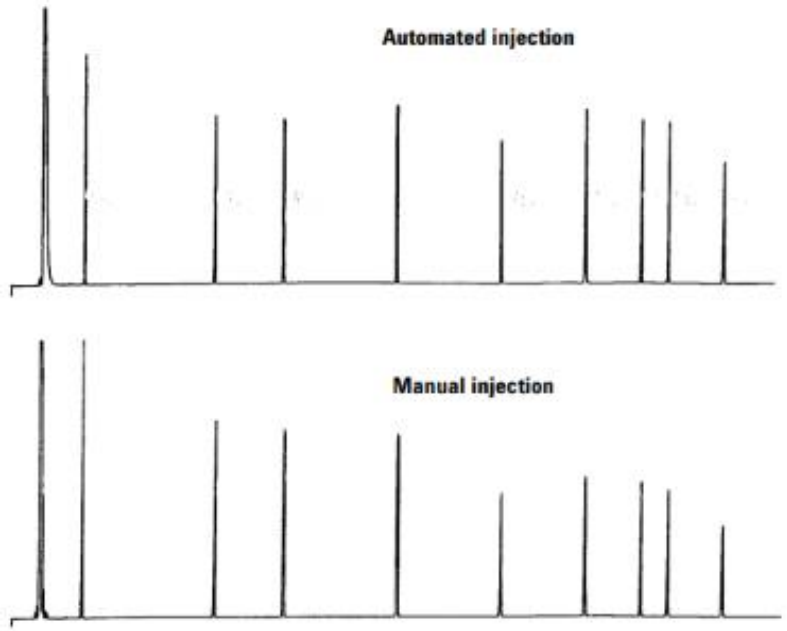
- Recommend default (3.6 mm from bottom of vial)
- Can change to sample from different heights in the vial
  - So -2 mm setpoint will sample 1.6 mm from vial bottom
  - Range is (-2 mm to 30 mm)
- Example uses:
  - analyzing samples that contain sediment (although properly filtering the sample is ideal)
  - Sampling from higher in the sample vial in liquid-liquid extractions
  - Small volume sampling
    - Exercise caution when using sample offsets in combination with vial inserts or conical vials
  - Ambient headspace analysis



Plunger Speed and Viscosity Delay







Plunger Speed and Viscosity Delay

Fast/Variable

µL/min µL/min µL/min

- Speed setpoints depend on configured syringe size
- Fast/Variable
  - Same parameters to start, but fast is fixed
  - Best starting point for almost all hot S/SL applications
    - Slower draw ensures efficient sampling, prevents air bubbles
    - Fast dispense and inject to ensure rapid, complete transfer to inlet

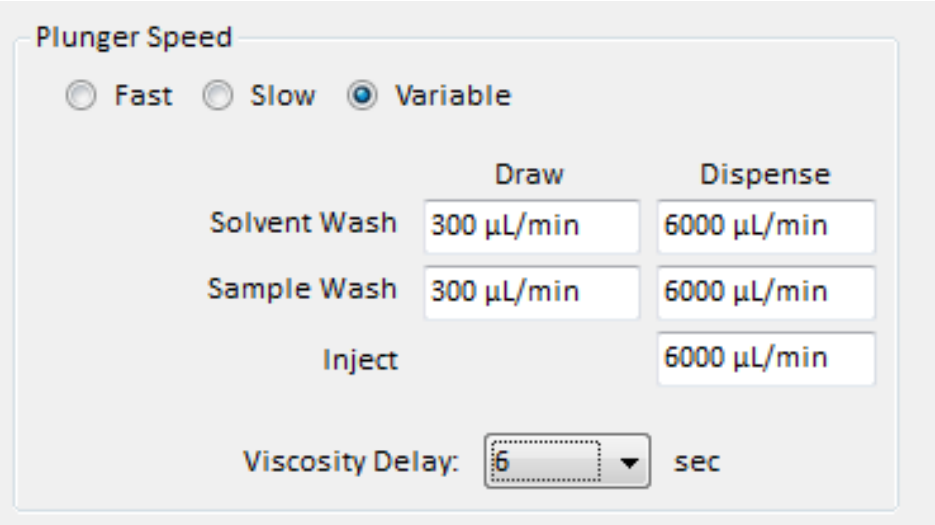
Slow

µL/min µL/min µL/min

- Slow
  - Slows inject rate only (draw and dispense rates remain fast)
  - Use for MMI/PTV/COC inlets
  - Too slow → broad or split peaks
    - Occurs when volatile compounds leave needle before plunger depressed

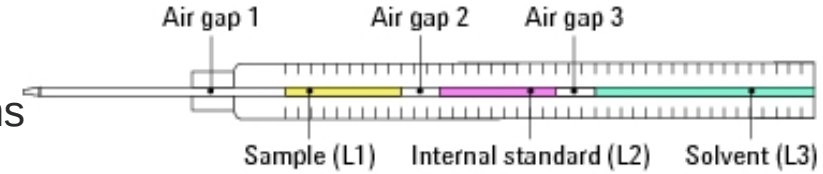
Plunger Speed and Viscosity Delay

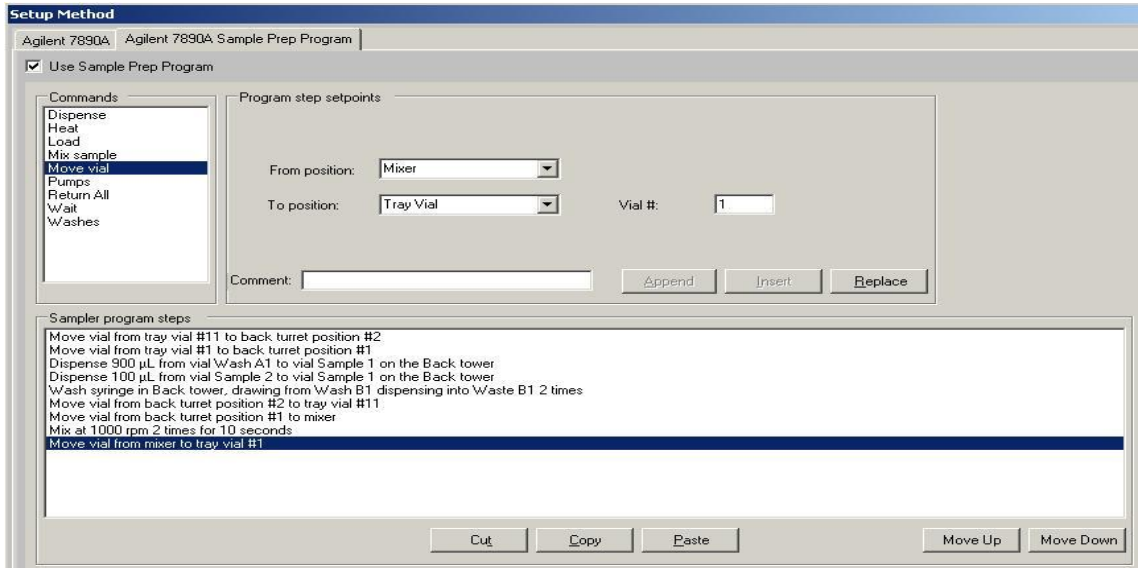
- Viscosity Delay
  - Time (sec) plunger pauses between pump and injection
  - Allows additional time for viscous samples to flow into syringe during pump
  - Use for viscous solvents like isooctane
  - Use for highly volatile solvents dichloromethane (to prevent cavitation)
  - A 2 second viscosity delay can be beneficial for many applications
    - Including GC OQ, GC/MS OQ, and GC/MS IDL checkout parameters



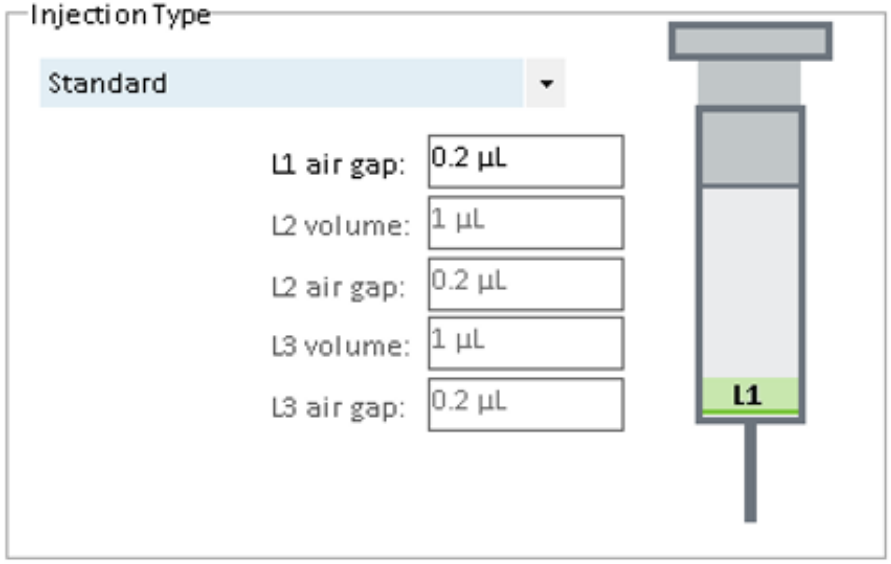
Injection types and Automated Sample Preparation

- Injection Types
  - Standard
  - Sandwich injections
  - Layered injections
  - Multiple injections

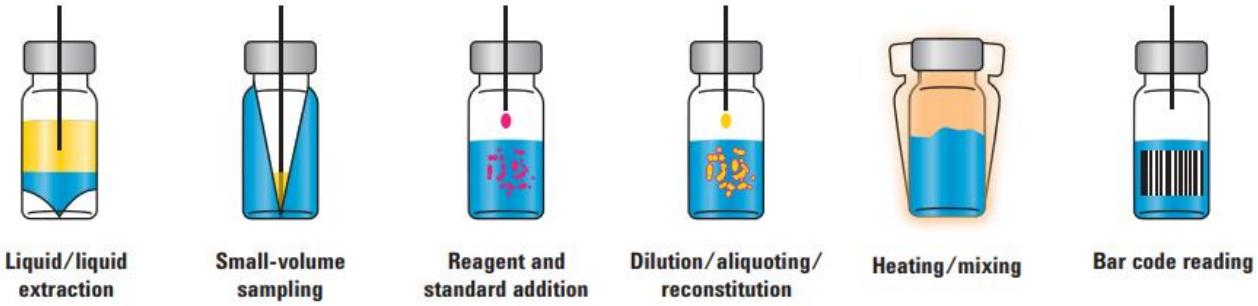




- Air gap
  - 0.2 µL default
  - Helps retain sample in syringe before injection

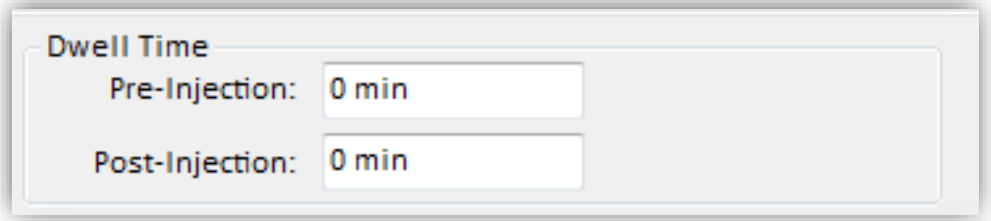


Interested? Let us know and we can devote a webinar to this topic

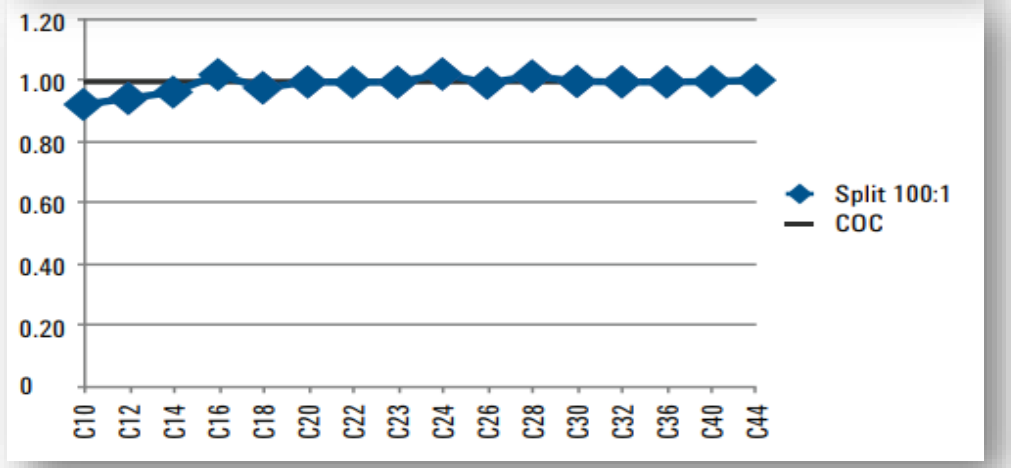


Dwell Time

- Types
  - Pre dwell time
    - Mins. needle remains in inlet before injection
  - Post dwell time
    - Mins. needle remains in inlet after injection
  - Example uses:
    - longer dwell time to simulate manual injection process
    - longer dwell time to warm syringe needle to inject viscous sample
- **Fast** injections are better, especially in split mode
  - Better precision and accuracy
  - Prevents broad or tailing peaks
  - Minimizes thermal degradation and analyte discrimination
  - Ensures narrow peaks
  - 7693 ALS injects in under 100 milliseconds



Carbon #	Split Area % RSD	Splitless Area % RSD	On Column Area % RSD
10	0.20	0.26	0.33
12	0.20	0.27	0.36
14	0.20	0.27	0.40
16	0.21	0.30	0.41
18	0.23	0.28	0.27
20	0.25	0.28	0.41
22	0.28	0.28	0.41
24	0.30	0.28	0.42
32	0.39	0.30	0.41
36	0.29	0.35	0.41
40	0.34	0.27	0.42
44	0.27	0.33	0.42



Contact Agilent Chemistries and Supplies Technical Support

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

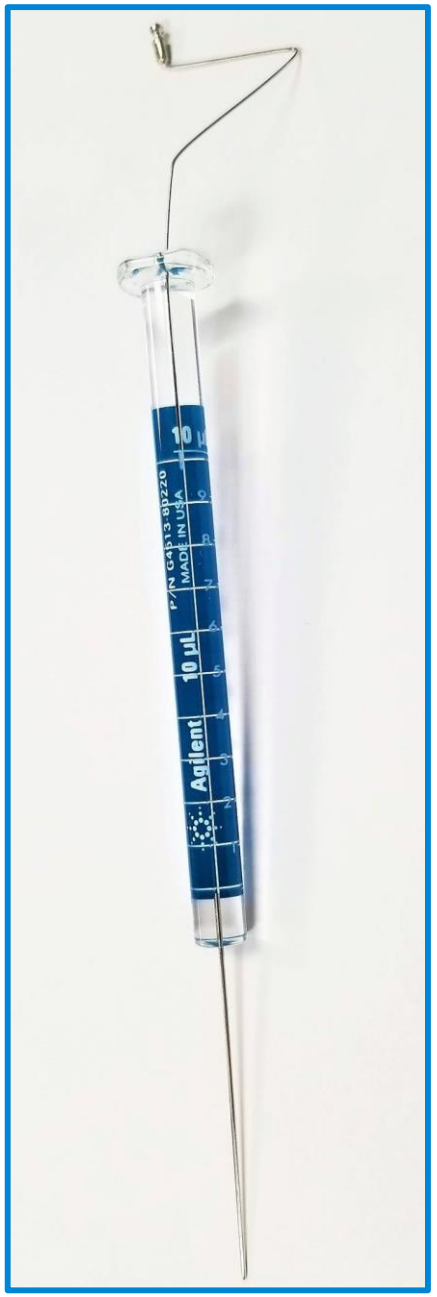
- Option 1 for GC/GCMS Columns and Supplies
- Option 2 for LC/LCMS Columns and Supplies
- Option 3 for Sample Preparation, Filtration and QuEChERS
- Option 4 for Spectroscopy Supplies

- gc-column-support@Agilent.com
- lc-column-support@agilent.com
- spp-support@agilent.com
- spectro-supplies-support@agilent.com

Troubleshooting

Troubleshooting

Problem: Bent Plunger or Stuck Syringe



Possible Cause(s):

- Particles such as dust, salts, metal, leftover sample, or glass can fill the narrow gap between the plunger shaft and the inside wall of the barrel.
- Overtightened septum nut compresses septa, causing excessive resistance during injection

Suggested Action(s):

- Switch to a syringe with PTFE-tipped plunger
- Avoid using 5µL syringes where possible
- If plunger movement feels “gritty”, carefully remove plunger from barrel, flush with solvent, and wipe dry with lint-free cloth. Carefully reinsert plunger into barrel. Finally, submerge needle tip into container of solvent and cycle plunger to pull solvent into and out of the barrel.
- Never cycle the plunger in a dry syringe
- Do not “mix & match” plungers and barrels
- Immediately clean syringes after use
- Loosen septum nut

Troubleshooting

Problem: Bent needle

Possible Cause(s):

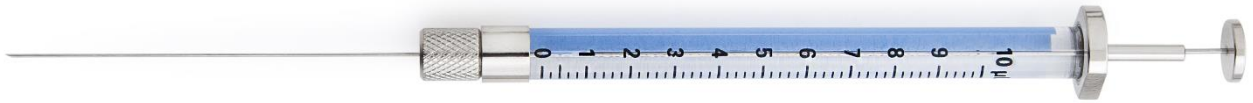
- Improper needle alignment
- Narrow gauge needles (26g) bend more easily than larger gauge (23g) needles
- Needles tend to bend when inserted into sample vial, not the inlet. This can be caused by septa that are too “rough”
- Needles bent during installation into the autosampler are more likely to bend when pushed through the sample vial cap septum.

Suggested Action(s):

- Use syringes with 23 to 26 gauge tapered needles
- Realign autosampler
- Verify septum nut is not over-tightened

Troubleshooting

Problem: No peaks

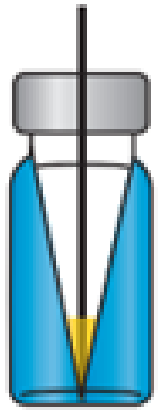


Possible Cause(s):

- Syringe plunger malfunction
- Plugged needle
- Sample level in vial too low
- Sample too viscous

Suggested Action(s):

- Clean or replace syringe
- Check sample level, refill or use low-volume vial insert
- Split ALS from rest of system by manually injecting sample to ensure peaks present
- Increase viscosity delay time
- Increase sample dilution factor
- Check sample depth setting in method



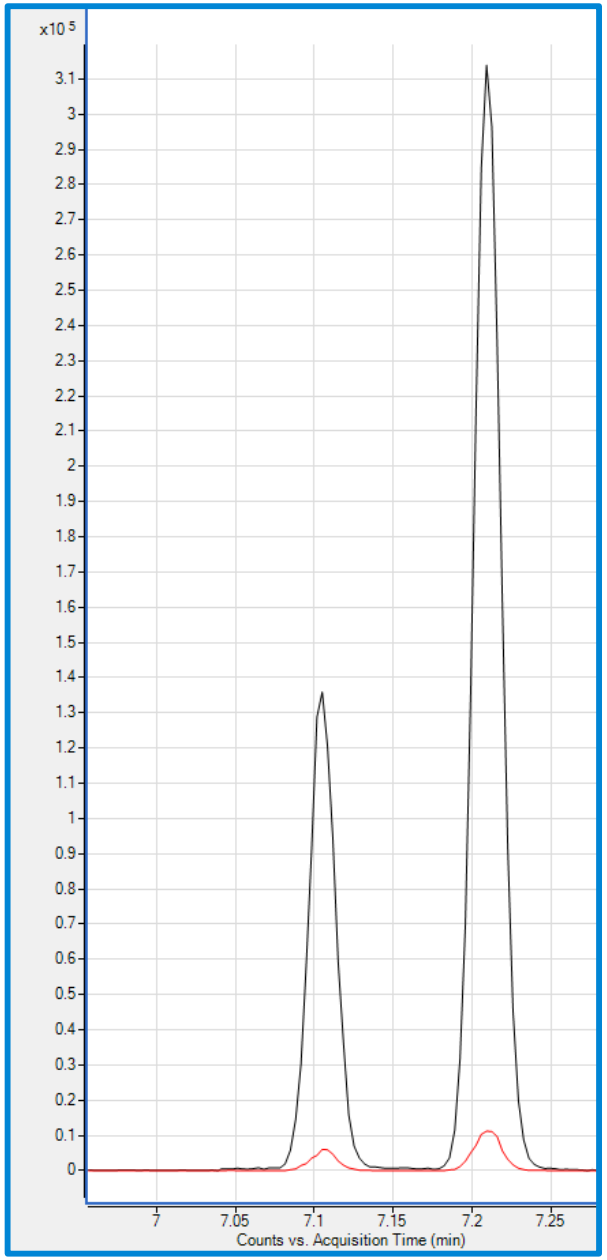
Troubleshooting

Problem: Sample carryover

Possible Cause(s):

- Insufficient number or type of washes
- Solvent wash vial empty
- Syringe worn or dirty
- Sample/solvent mismatch
- Dirty ALS needle guide
- Dirty septum nut

ALS needle guide- G4513-40525



Suggested Action(s):

- Increase number or type of washes
- Rinse with a variety of solvents
- Rinse and refill solvent wash vial
- Clean or replace syringe
- Ensure samples and solvents, from one vial to the next, are miscible
- Occasionally replace needle guide (aka “needle foot”)
- Check septum nut for sample residue

Troubleshooting

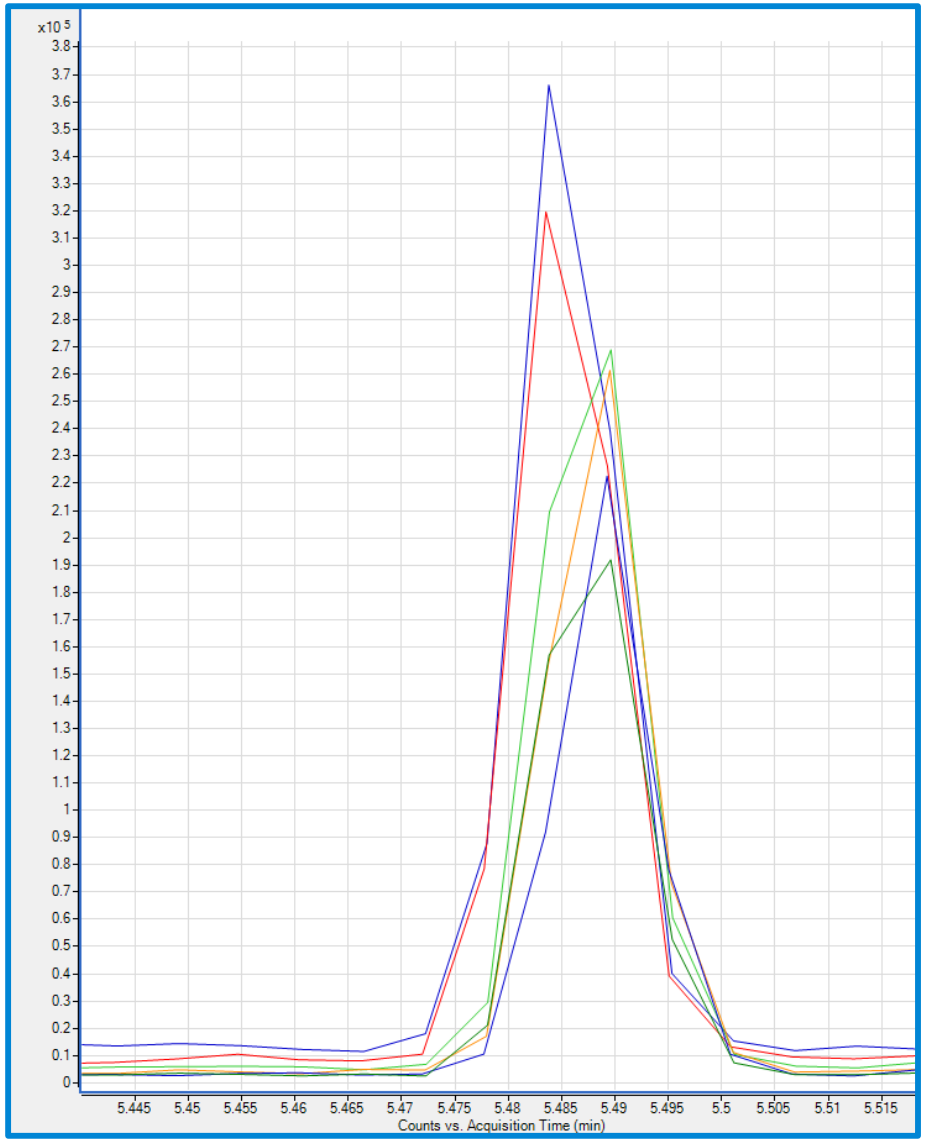
Problem: Poor Reproducibility

Possible Cause(s):

- Poor plunger seal
- Syringe worn or dirty
- Glass walls of syringe are scratched

Suggested Action(s):

- Clean or replace syringe
- Rinse and refill solvent wash vial
- Do not allow sample to crystallize inside syringe between injections
- Make sure solvents being used are miscible and compatible with the syringe

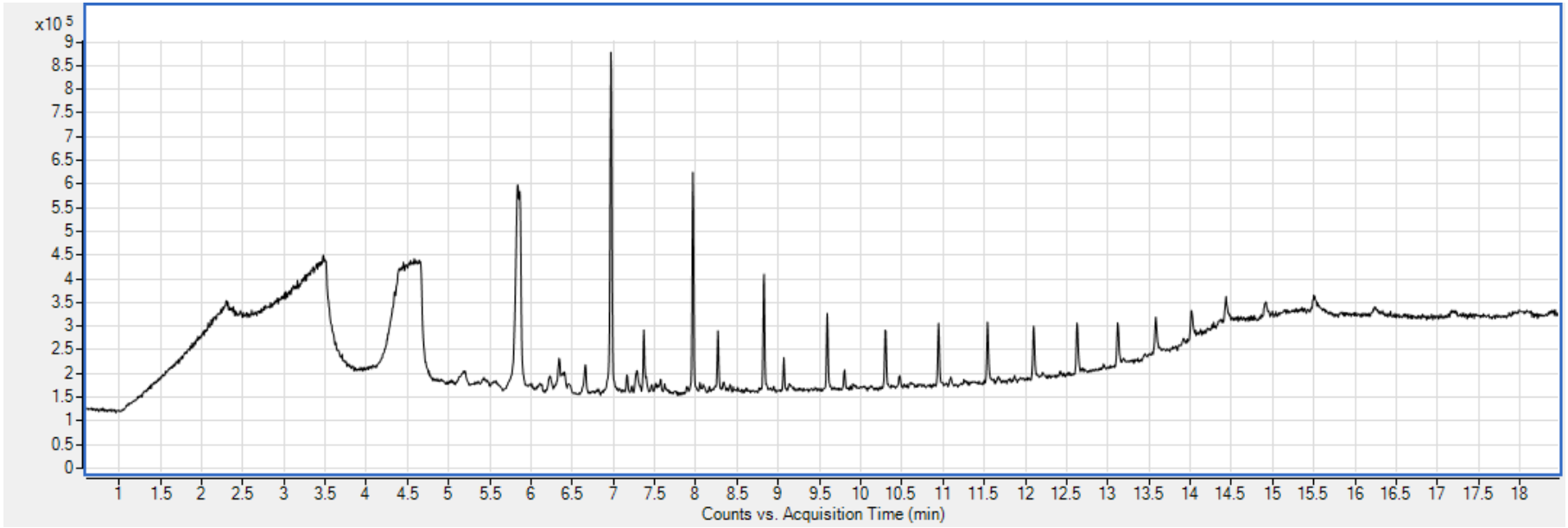


Septum maintenance: Septum coring

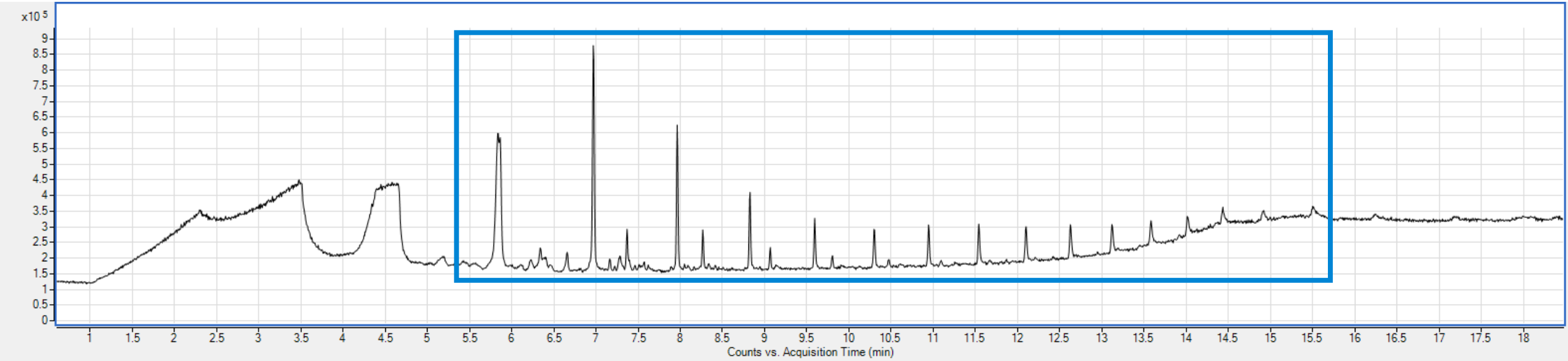
- After many injections, pieces of rubber from the septum may break off and fall into the inlet liner.
  - This is called septa coring
  - Replace the inlet septa and liner frequently to prevent septa contamination
  - Use a cone tipped syringe to reduce the chance of tearing the septum

Septum core placed in a clean liner, and a blank injection performed.

- Inlet: 320 °C, split mode, 10:1 split ratio
- Oven: 35 °C to 300 °C at 20 °C per minute
- Detector: Single quadrupole EI Scan, 35 to 500 amu



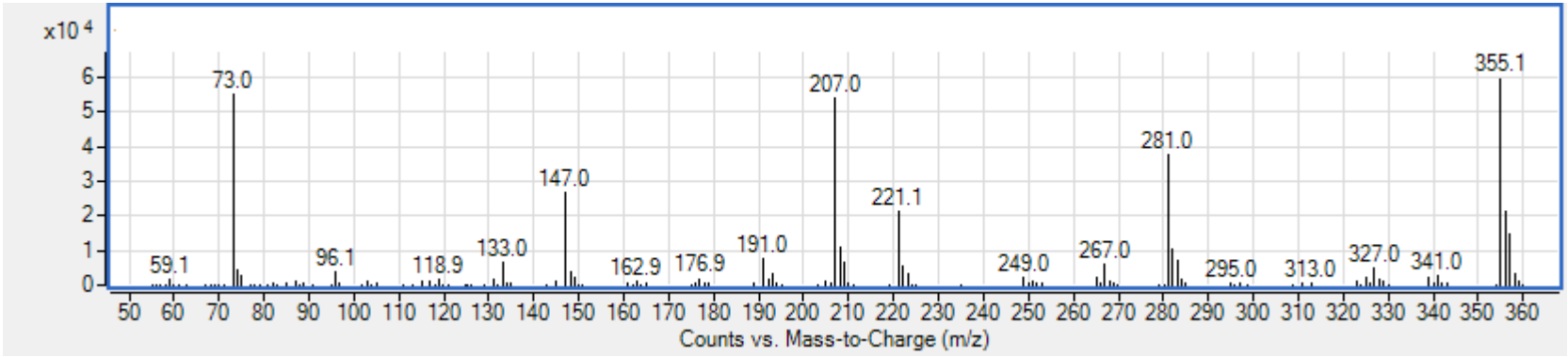
Septum maintenance: TIC of an inlet septum



Common ions for siloxane molecules:
73
147
207
281
355

Septa contamination in wash vials or inlet liners can be diagnosed by looking for siloxane polymers in your total ion chromatogram. Each peak in the chromatogram corresponds to a cyclized (ring structure) siloxane molecule. These molecules fragment with very similar patterns.

Example spectrum:



Sample Introduction: Important Takeaways

- Successful GC injection is a complex process

- Start with a PTFE-tipped 10 µL syringe
  - Handle the syringe carefully
  - Avoid pumping plunger when “dry”

- Don’t let the wash solvent run low/dry/become contaminated
  - How long is your sequence?
  - How is your wash vial hygiene?

- Get the sample into the liner quickly
- Be aware of advanced parameters for special applications
- If you’re not sure, reach out and ask for help