Piercing Precision: Mastering Syringe Techniques in GC

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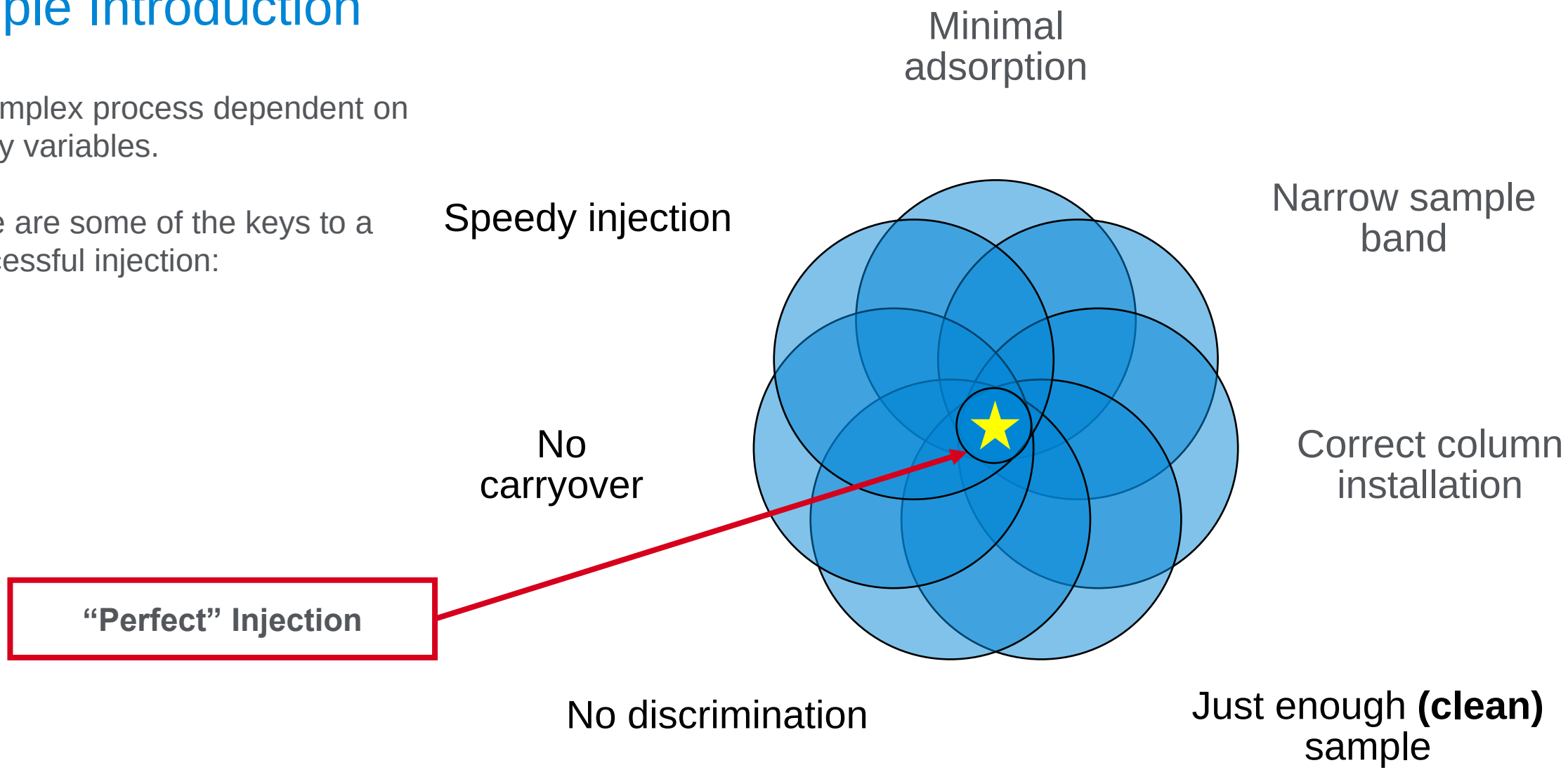
Agilent
Trusted Answers

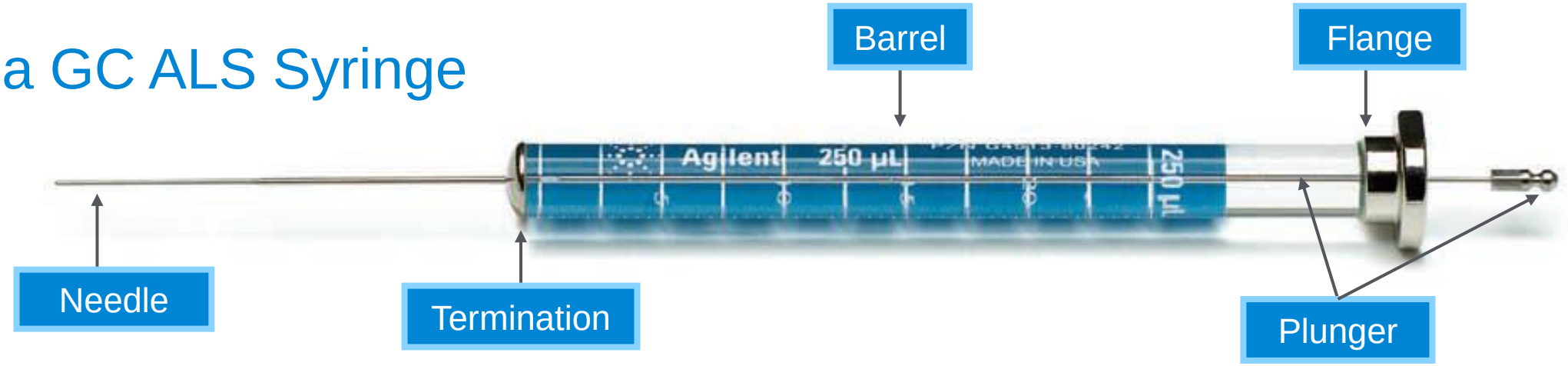
Sample Introduction

A complex process dependent on many variables.

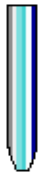
Here are some of the keys to a successful injection:



Anatomy of a GC ALS Syringe

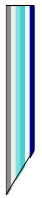


10	Tapered, fixed	23-26s/42/HP 5181-1267



Cone tip/PS AS/PS HP (shown)

Used in Agilent autosamplers for optimum performance and reliability by reducing septum coring.



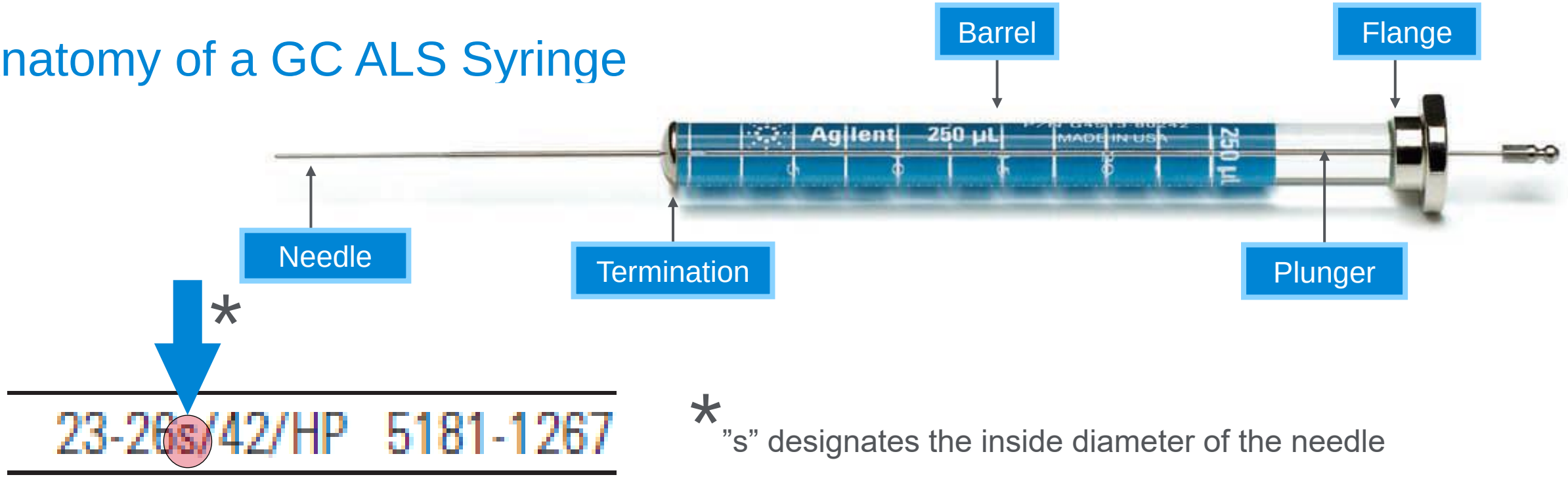
Bevel tip/PS 2

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



*"s" designates the inside diameter of the needle

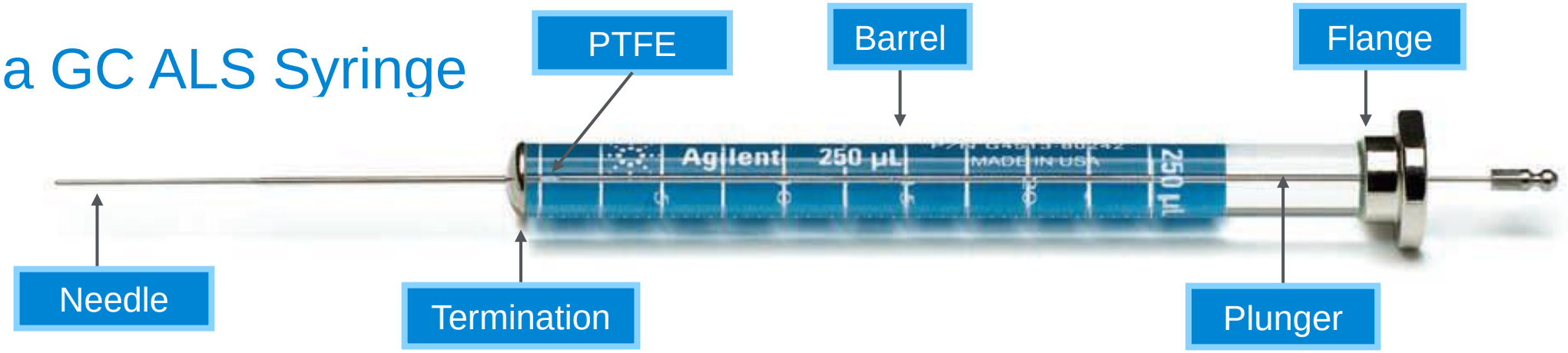
Fixed needle syringes (shown)

- Typically, abbreviated FN
- Needle "cemented" to barrel using epoxy
  - Cannot be replaced
- Typically used in autosamplers
- Preferred for applications requiring trace-level samples
- Can be heated up to 70 °C

Removable needle syringes

- Typically, abbreviated RN
- Allows use of various needle point styles
- Threaded connection with PTFE sealing ferrule that can be tightened to compensate for wear
- Can be heated up to 120 °C
- Can be prone to leakage

Anatomy of a GC ALS Syringe



Standard plungers

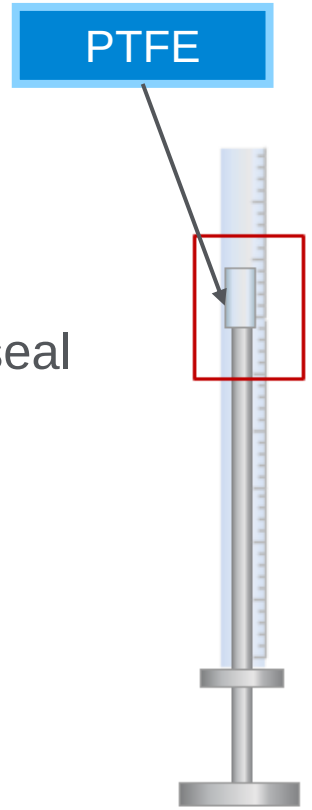
- Fit tightly within the syringe barrel
- Limit the loss of volatile sample
- Are individually fitted to the syringe
- Are not replaceable/not interchangeable
- Are recommended for analysis of liquid samples

PTFE-tipped (shown)

- Limit sample deposit adsorption
- Form gas-tight seal
- Replaceable/interchangeable
- Require maintenance to maintain PTFE seal
- Only available in 10 µL size or larger

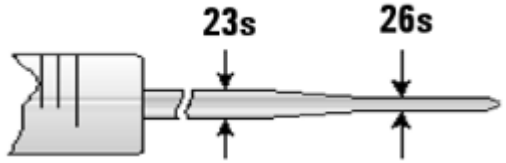
Recommended for:

- “Dirty” samples
- Highly volatile samples
- Gas injections
- Chlorinated solvents



Syringe Selection Tips

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



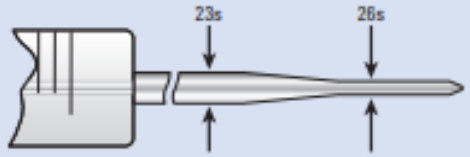
- Ensure the proper syringe is configured in the software

Gold vs. blue syringe

- **BLUE**
- **GOLD**

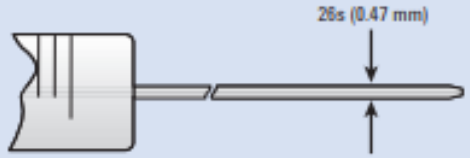
- Specifications for both are equivalent
  - Both fully compatible with Agilent ALS
  - Personal preference

Needle Gauge



Tapered Dual Gauge 23-26 or 23s-26s (0.64 – 0.47 mm)
Durability of a 23-gauge
Versatility of a 26-gauge for split/splitless and on-column injection

Single Gauge 23 or 23s (0.64 mm)
Merlin Microseal septa
Packed column injector ports
Split/splitless injector ports



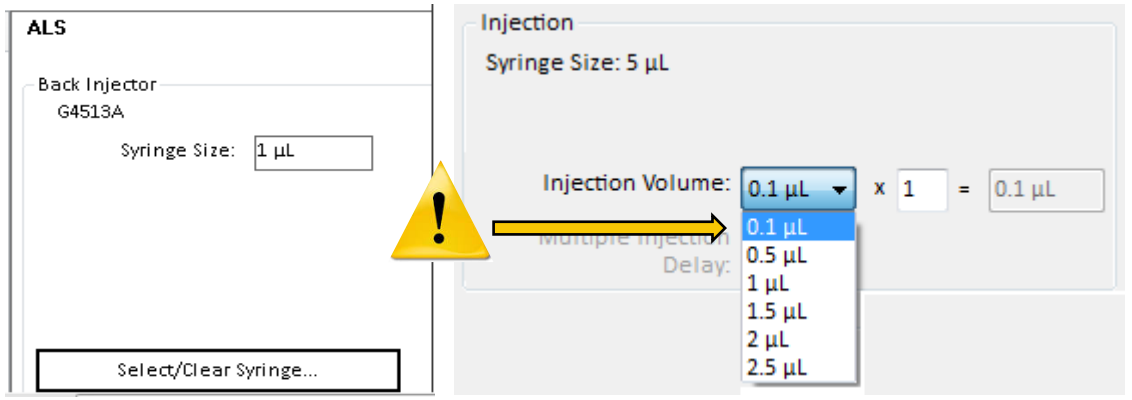
Single Gauge 26 or 26s (0.47 mm)
Packed column injector ports
Split/splitless injector ports

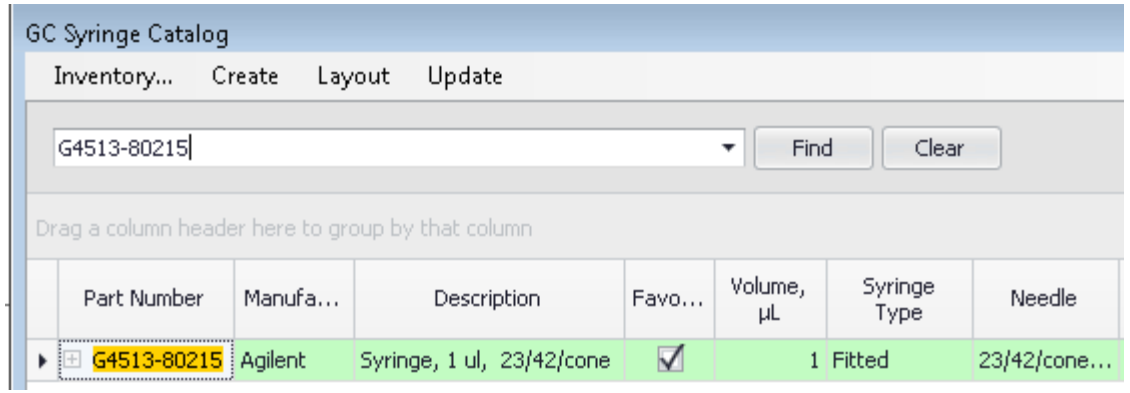
Note: Needles with an 'S' following the gauge are more durable, with a thicker needle wall and smaller ID bore.

Syringe Selection Tips: 5 µL

5 µL syringes are ideal for small volume injections, **but**:

- They typically have a shorter lifetime (narrow plunger diameter → bends easily)
- Do **not** use them in solvent saver mode (too much strain on the plunger)
- They are not available with PTFE tips (plunger is sensitive to PTFE friction)
  - Why not? PTFE can't be accurately machined at such a narrow diameter

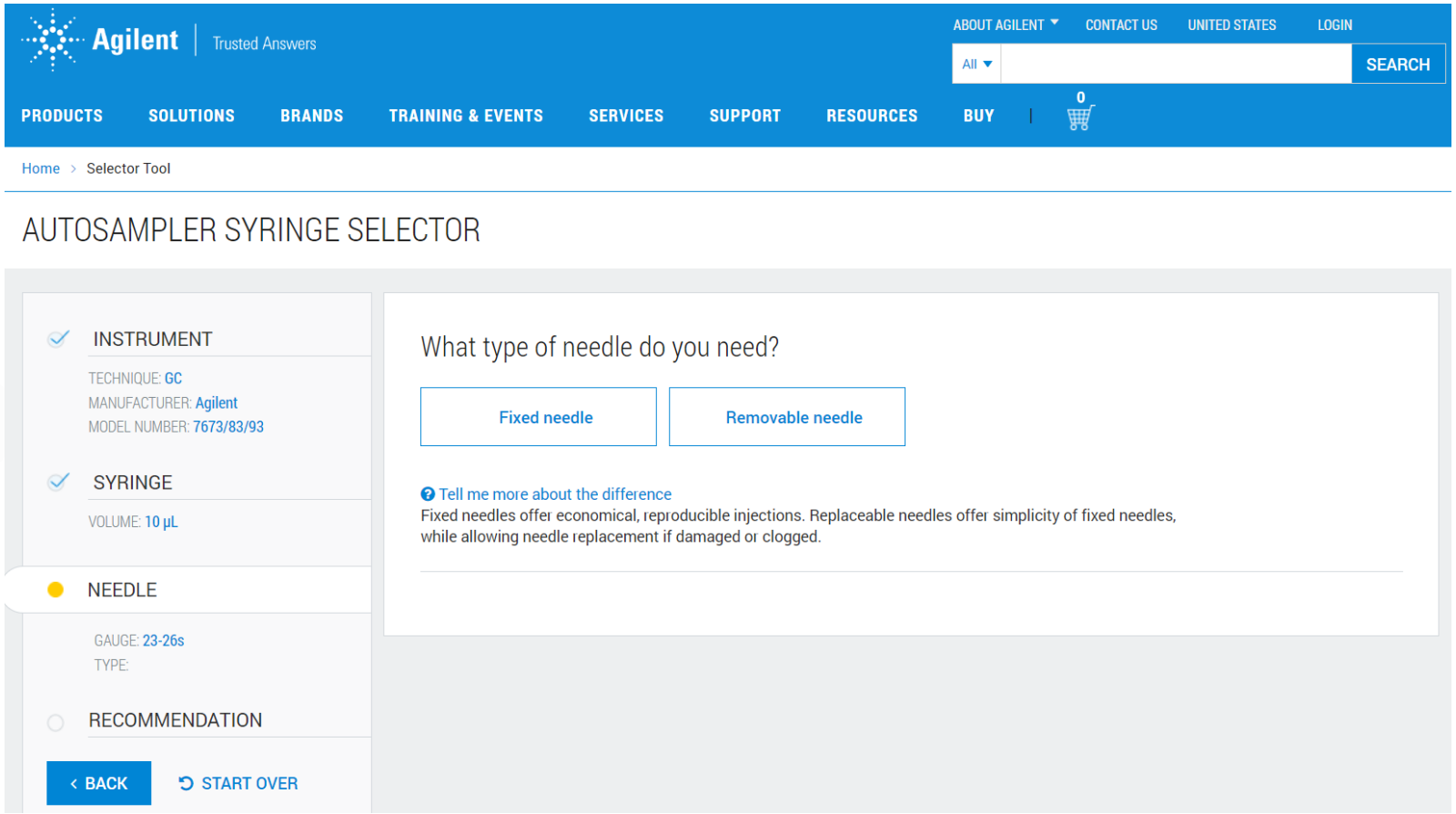




Need Help?

Check out our online syringe selector tool:

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



ALS Method Parameters

Injection

Syringe Size: 10 µL

Injection Volume: 1 µL

Washes and Pumps

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

<<

Dwell Time

Pre-Injection: 0 min

Post-Injection: 0 min

Sample Depth

Enable 0 mm

Plunger Speed (Variable)

Fast Slow Variable

	Draw	Dispense
Solvent Wash	300 µL/min	6000 µL/min
Sample Wash	300 µL/min	6000 µL/min
Inject		6000 µL/min

Viscosity Delay: 0 sec

Injection Type

Standard

L1 air gap: 0.2 µL

L2 volume: 1 µL

L2 air gap: 0.2 µL

L3 volume: 1 µL

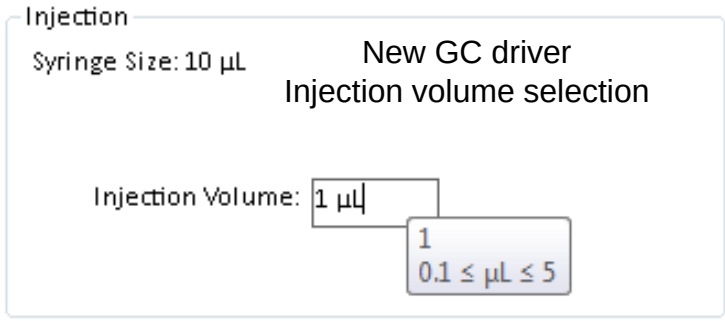
L3 air gap: 0.2 µL

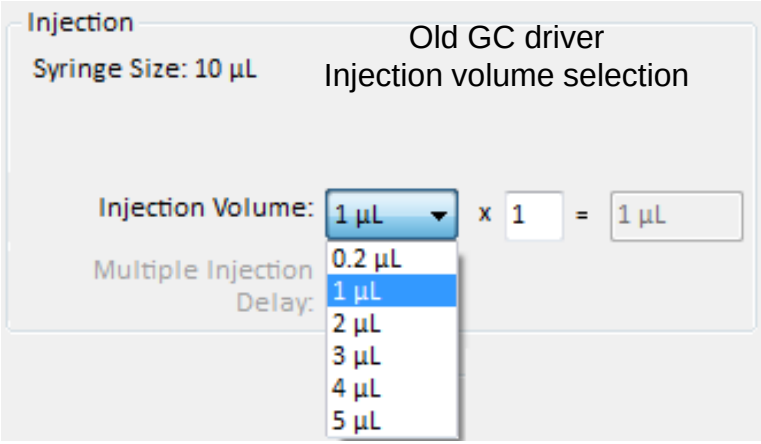
Injection Volume/Rinse Volume

Syringe capacity:

- Avoid injection volumes below 10% of the syringe capacity
  - The injection will work, but reproducibility may suffer

- ALS software automatically limits the maximum injection volume to 50% of the configured syringe volume
  - 10 µL syringe → 5.0 µL injection size
  - 5 µL syringe → 2.5 µL injection size





Maximum injection volume (50%)

Maximum rinse volume (80%)

Micro/Nanovolume Syringes

Microvolume syringes

Blue Line auto sampler syringes:

micro/nano-volume syringes are half-marked

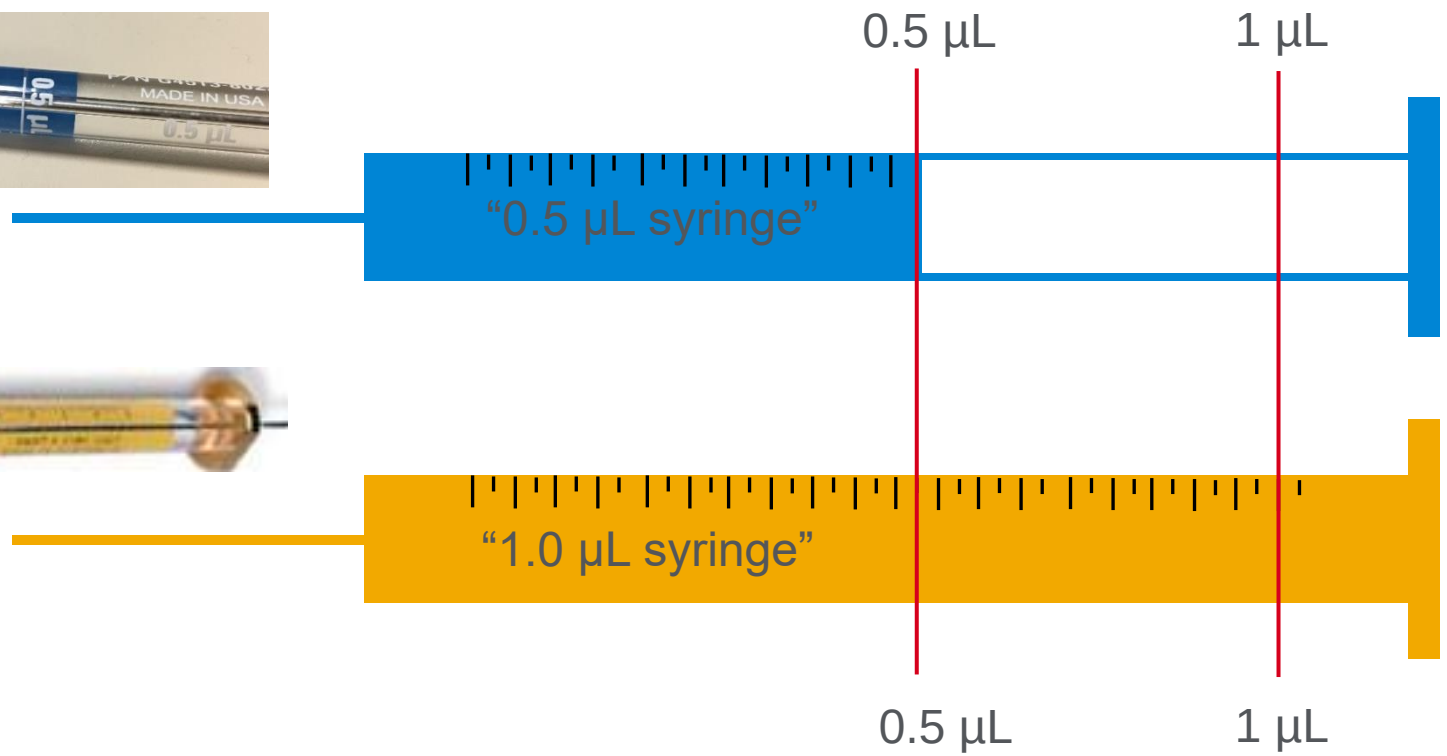
You need to configure your ALS with 2x syringe volume or risk getting half the response

Blue Line Autosampler Syringes with Fitted Plungers

Volume (µL)	Description	Unit	Needle	Part No.
0.5	Plunger in needle, fixed		23/42/cone tipped	G4513-80229
	Replacement needle/plunger			G4513-80240
1	Plunger in needle, fixed		23/42/cone tipped	G4513-80215
	Replacement needle/plunger			G4513-80239

Straight Needle, 23 and 26s Gauge Autosampler Syringes Gold

Volume (µL)	Description	Unit	Needle	Part No.
1	Cone-tipped		23/42/HP	5188-5246
2	Cone-tipped		23/42/HP	5188-5247



Starting Points for Injection Volume

Goal: Inject **as little sample as possible** to meet the detection limit and RSD reqs

- Avoid Backflash!
  - Use the vapor volume calculator*
- Injection volumes for most organic solvents should be within 1 to 2 µL or less
  - Split versus splitless
- Avoid injecting water – coefficient of expansion is too high
  - If you must, then calculate the expansion volume*
  - General rule: 0.5 µL maximum
- Higher injection volumes:
  - Dirty samples → more maintenance
  - Concentrated samples → overloading

Split ≤1 µL

Splitless ≤2 µL

More maintenance

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

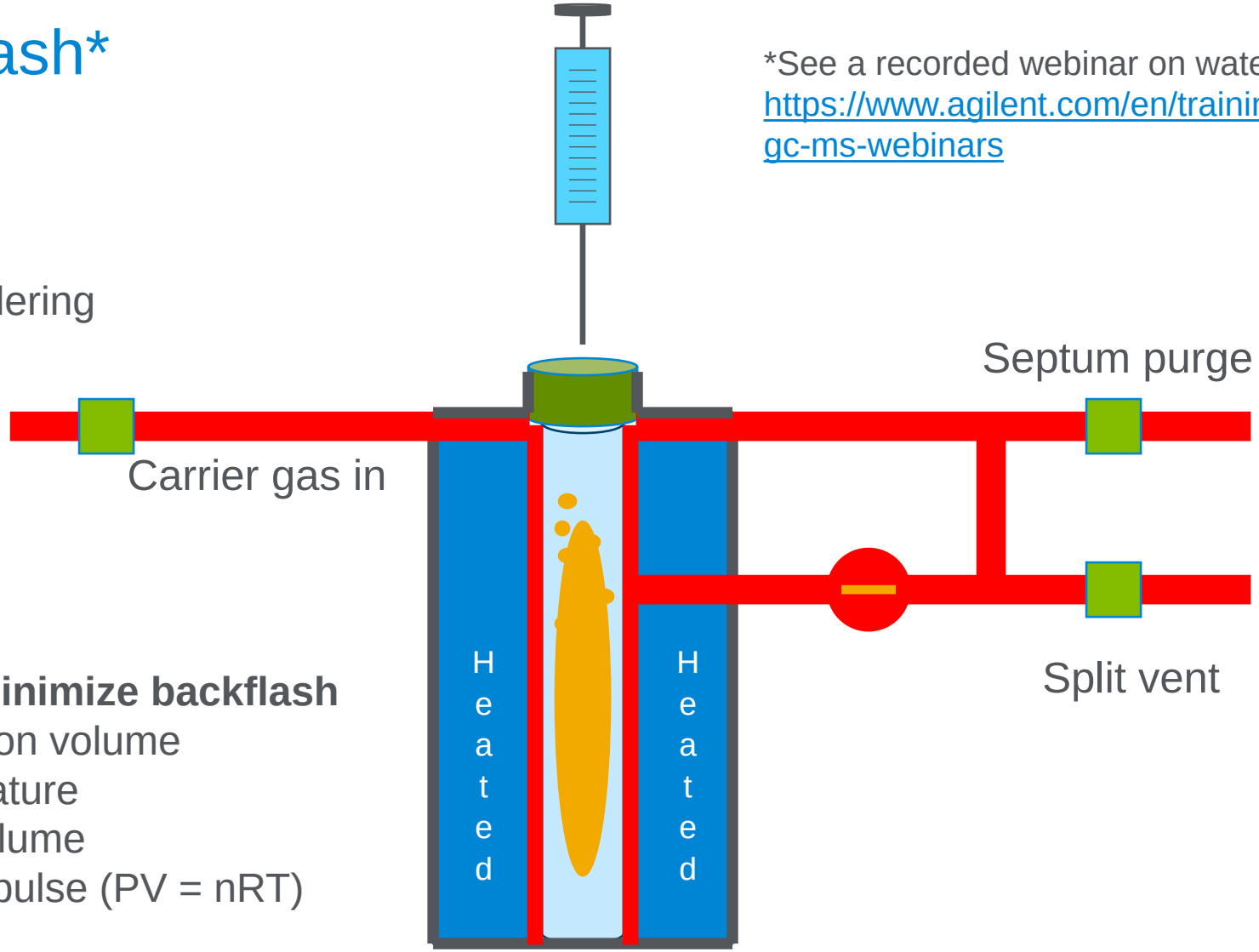
https://www.agilent.com/en/support/gas-chromatography/gccalculators

Sample Backflash*

Negative Effects

- Tailing
- Carryover
- Peak splitting/shouldering
- Low response
- Poor reproducibility

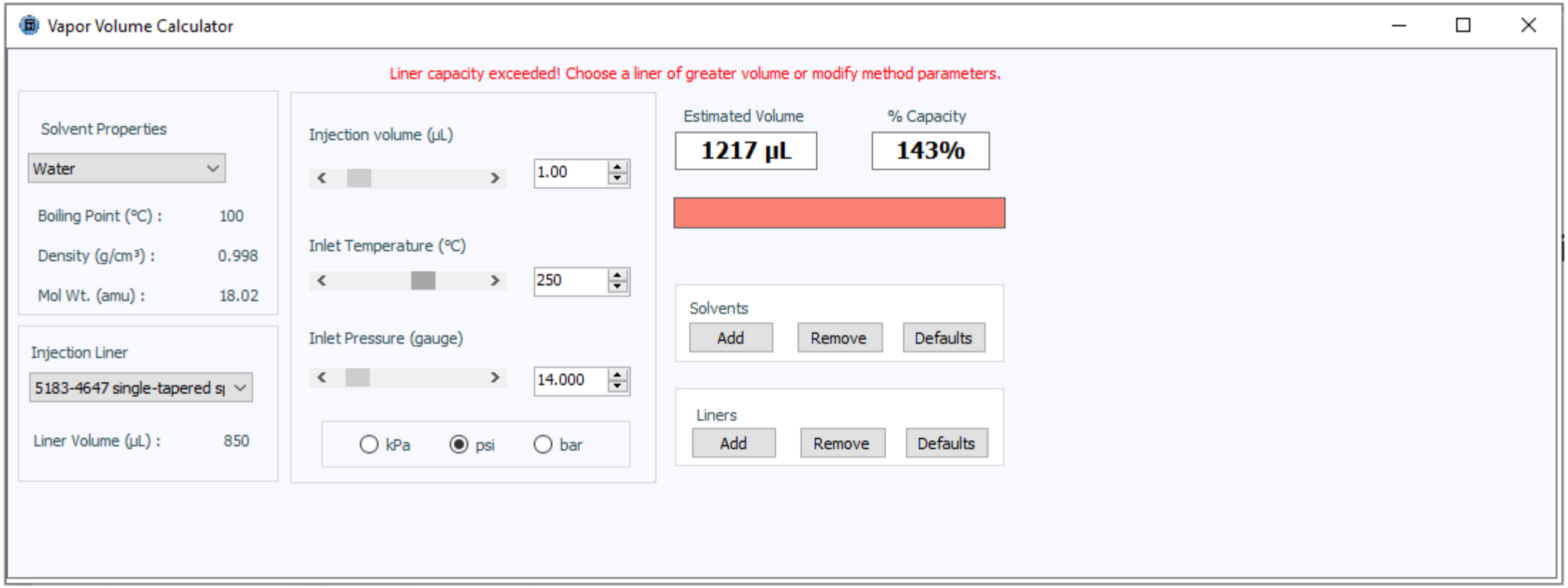
*See a recorded webinar on water injections for more info: https://www.agilent.com/en/training-events/eseminars/gc-gc-ms-webinars



How To Avoid/Minimize backflash

- Reduce injection volume
- Lower temperature
- Larger liner volume
- Use pressure pulse (PV = nRT)
- Tapered liner

Vapor Volume Calculator

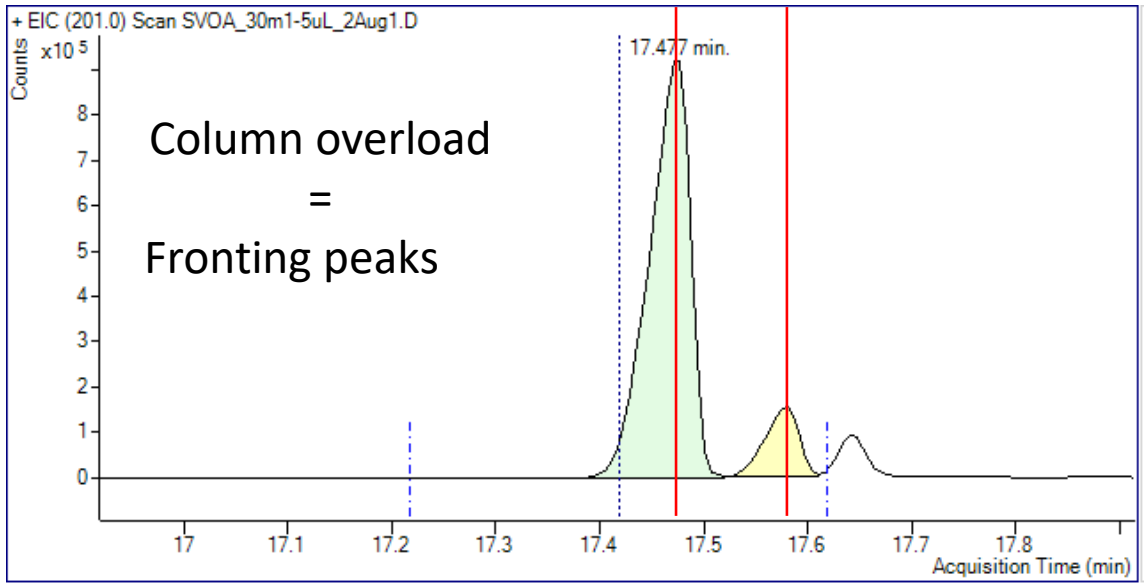


Chromatographic Signs That Your Injection Volume Is Too High

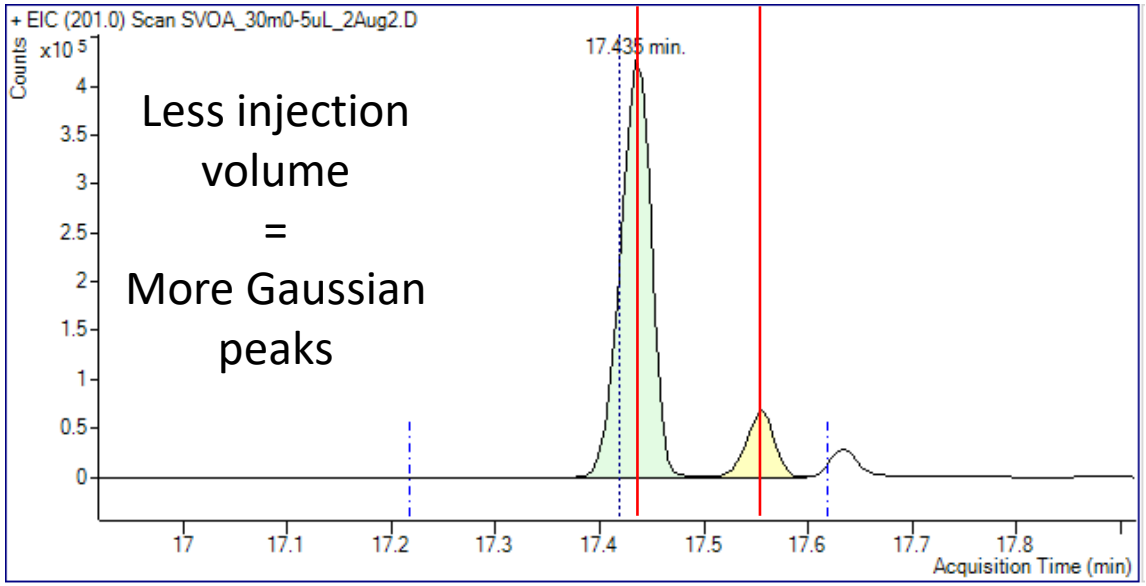
Overloading

- Watch for highly concentrated samples
- Keep the injection volume as small as possible
- An overload will result in peak “fronting” or “flagging”
- Adjust split ratio as needed
- Dilute

1 µL injection



0.5 µL injection



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Sample Depth

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Plunger Speed (Variable)

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L3 volume: 1 µL

L3 air gap: 0.2 µL

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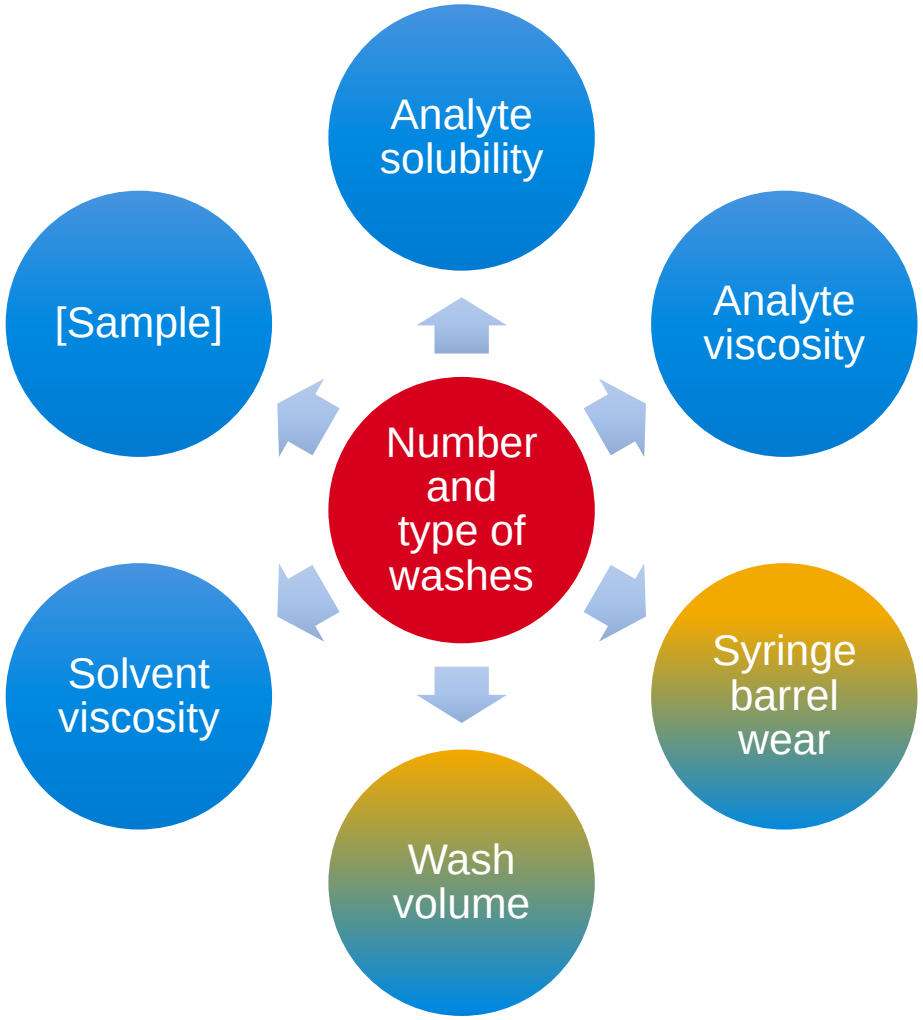
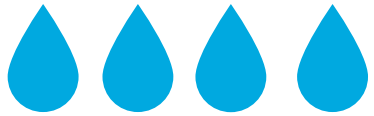
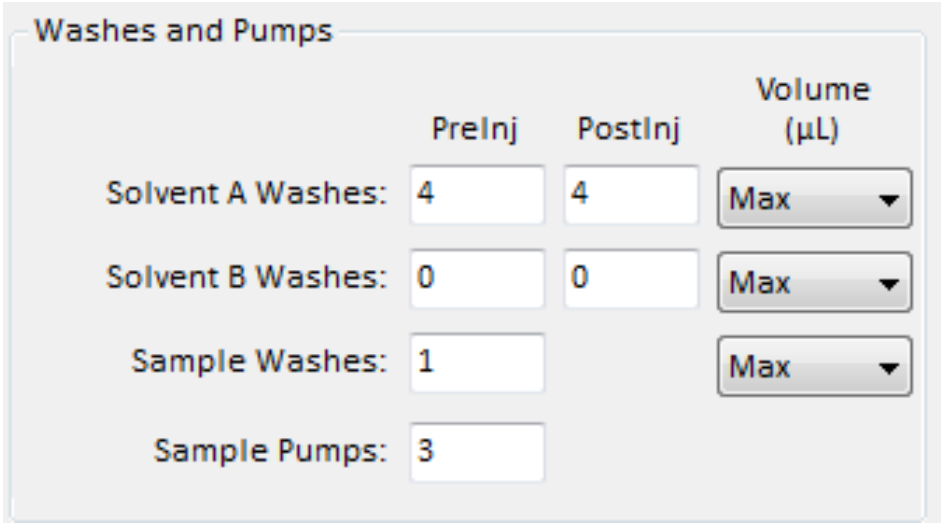
Washes and Pumps: Solvents

- Four pre- and post-washes reduce carryover to one part in 10,000

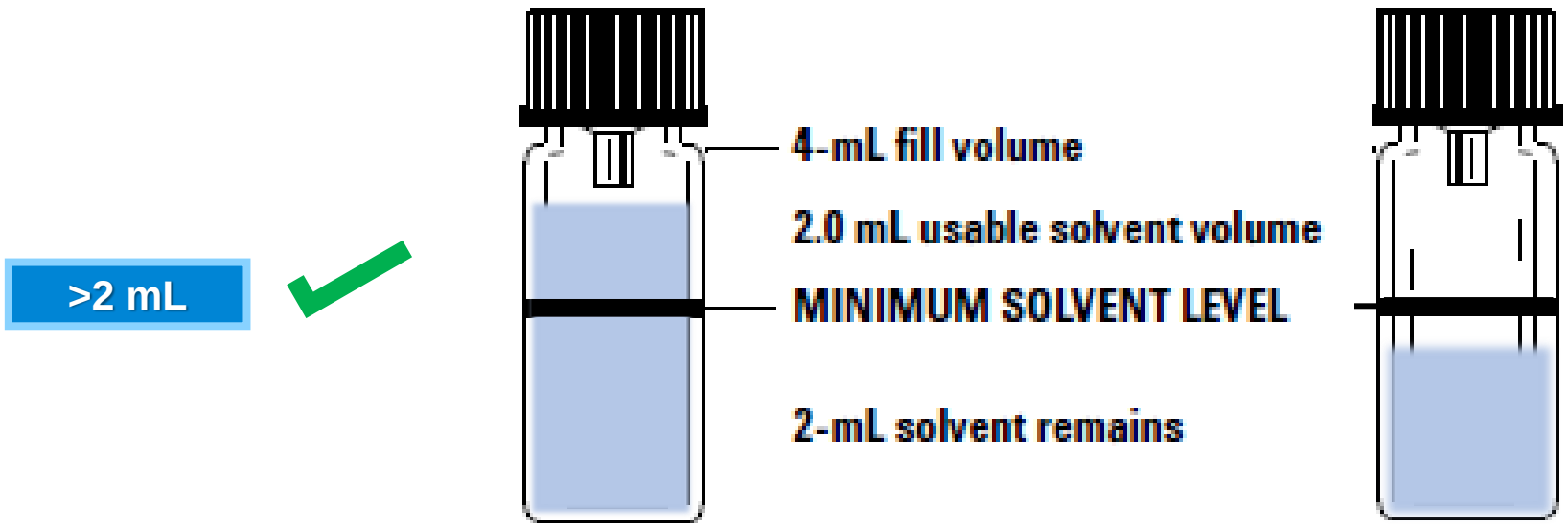


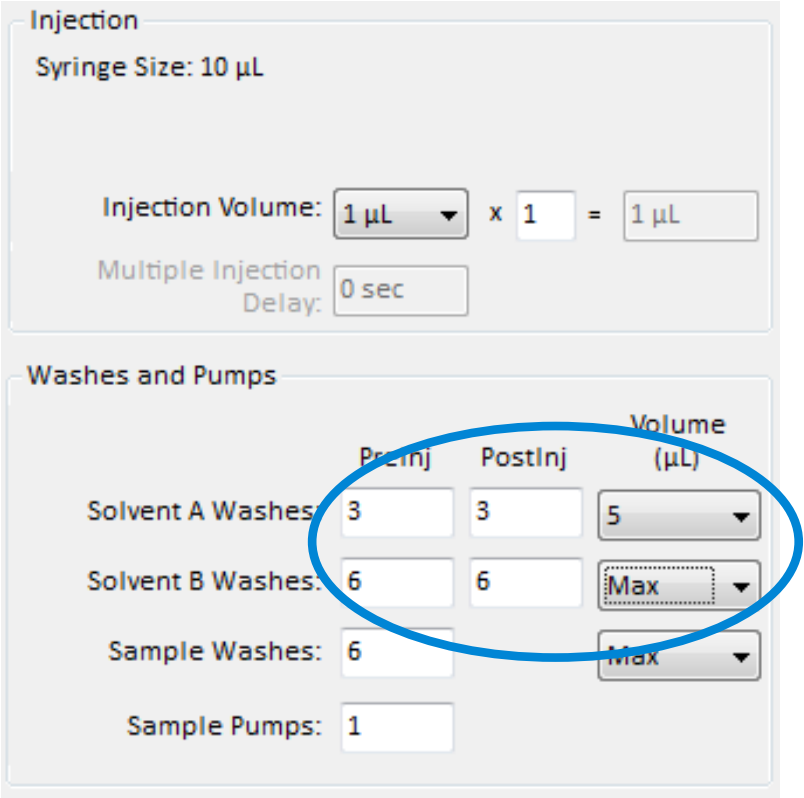




Wash Vial Volumes

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





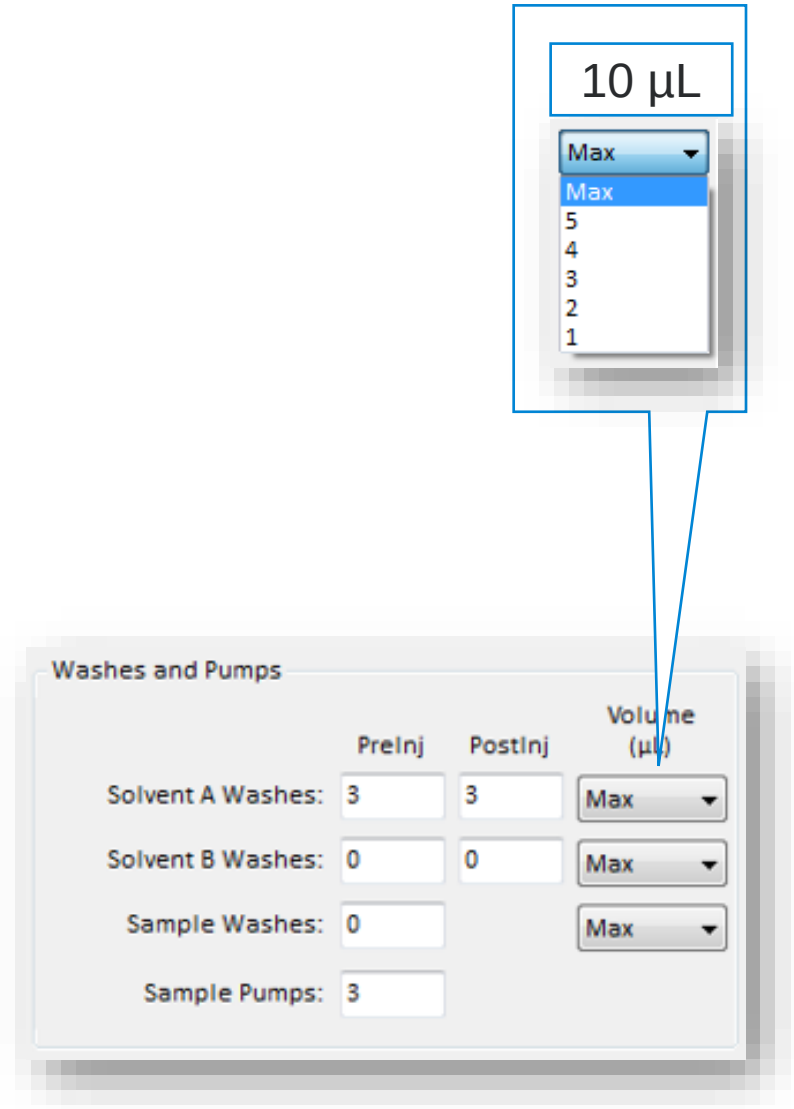
Washes and Pumps: Solvent Saver

High wash application? Try solvent saver.

- Steps:
  - Syringe draws in the solvent to a 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

- 10, 20, 30, 40, and 50% of syringe sizes (µL)
  - The wash volume will automatically be configured upon syringe size selection

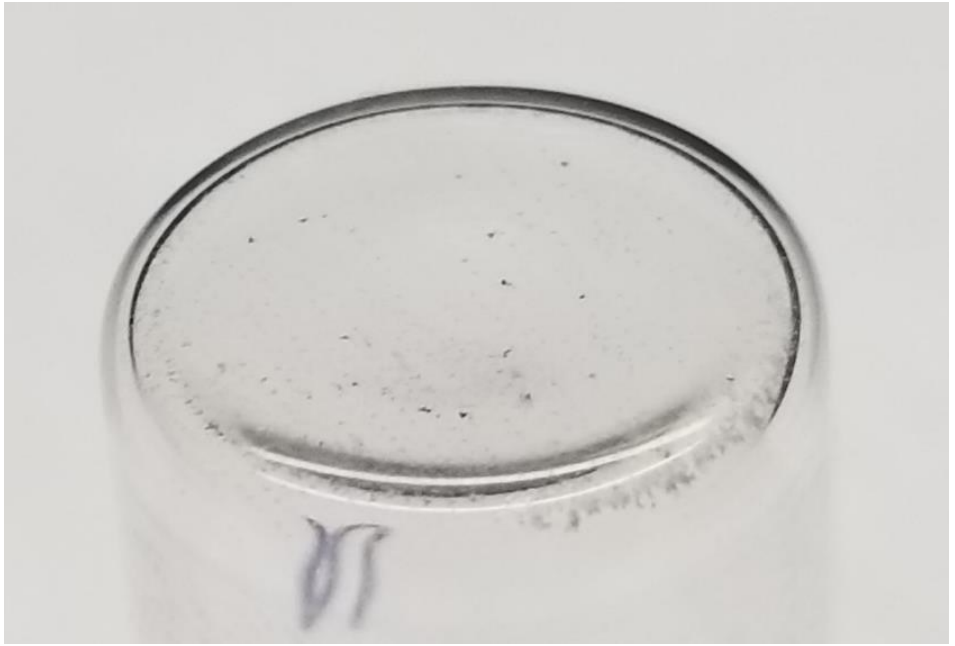
- Don’t let the wash vial run dry
- **You must** use a PTFE-tipped syringe
  - Fitted syringes lubricate insufficiently, causing premature failure



Washes and Pumps: Solvents

Frequently clean or replace the wash vials

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



Contaminated wash vial bottom

Contaminated wash solvent

Washes and Pumps: Solvents

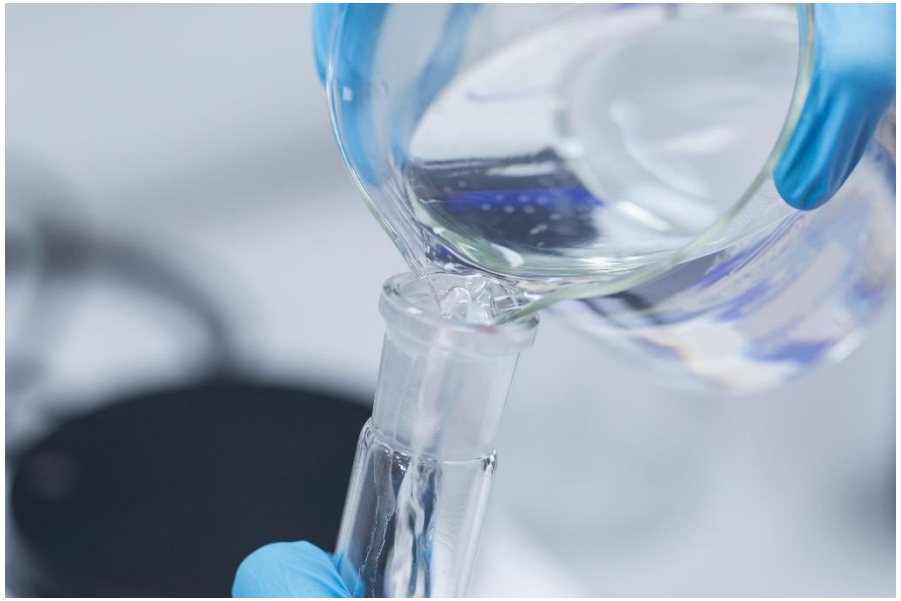
Choose a wash solvents that make sense for the analysis

- Is the analyte soluble in the solvent?
- Typically wash solvent = sample solvent
- If using a binary wash system, make sure that the solvents are miscible and rinse with the sample solvent last, just before the sample
- Do not use acidic or alkaline solvents with syringes

- Use both A and B wash vials
  - Second wash vial will be cleaner than first
  - Second wash vial should never be water (rust)

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.

Miscibility Chart



	o-Xylene	Water	Toluene	Tetrahydrofuran (THF)	Pentane	Methyl Ethyl Ketone	Methyl t-butyl Ether	Methanol	Isopropanol (IPA)	Iso-Octane	Hexane	Heptane	Ethylene Dichloride	Ethyl Ether	Ethyl Alcohol	Ethyl Acetate	1,4-Dioxane	Dimethyl Sulfoxide (DMSO)	N,N- Dimethylformamide	Dichloromethane (DCM)	Cyclohexane	Chloroform	n-Butyl Alcohol	Acetonitrile (ACN)	Acetone
Acetone																									
Acetonitrile (ACN)					■					■	■	■									■				
n-Butyl Alcohol		■																							
Chloroform		■																							
Cyclohexane		■						■										■	■					■	
Dichloromethane (DCM)		■																							
N,N- Dimethylformamide	■				■					■	■	■									■				
Dimethyl Sulfoxide (DMSO)	■				■					■	■	■		■							■				
1,4-Dioxane																									
Ethyl Acetate		■																							
Ethyl Alcohol																									
Ethyl Ether		■																■							
Ethylene Dichloride		■																							
Heptane		■						■										■	■					■	
Hexane		■						■										■	■					■	
Iso-Octane		■						■										■	■					■	
Isopropanol (IPA)																									
Methanol					■					■	■	■									■				
Methyl t-Butyl Ether		■																							
Methyl Ethyl Ketone		■																							
Pentane		■						■										■	■					■	
Tetrahydrofuran (THF)																									
Toluene		■																							
Water	■		■		■	■	■			■	■	■	■	■		■				■	■	■	■		
o-Xylene		■																■	■						

■ Immiscible

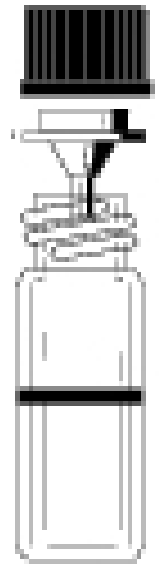
□ Miscible

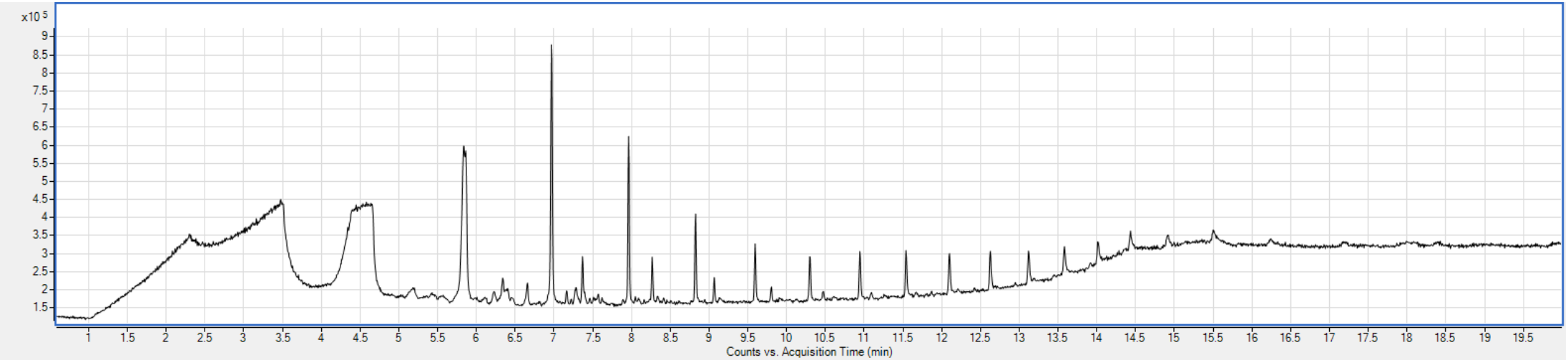
Washes and Pumps: Diffusion Caps

Diffusion caps are important

- They reduce volatile solvent diffusion
- Better alternative than using vial septa, which will core and contaminate the wash solvent vial → septum bleed peaks

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



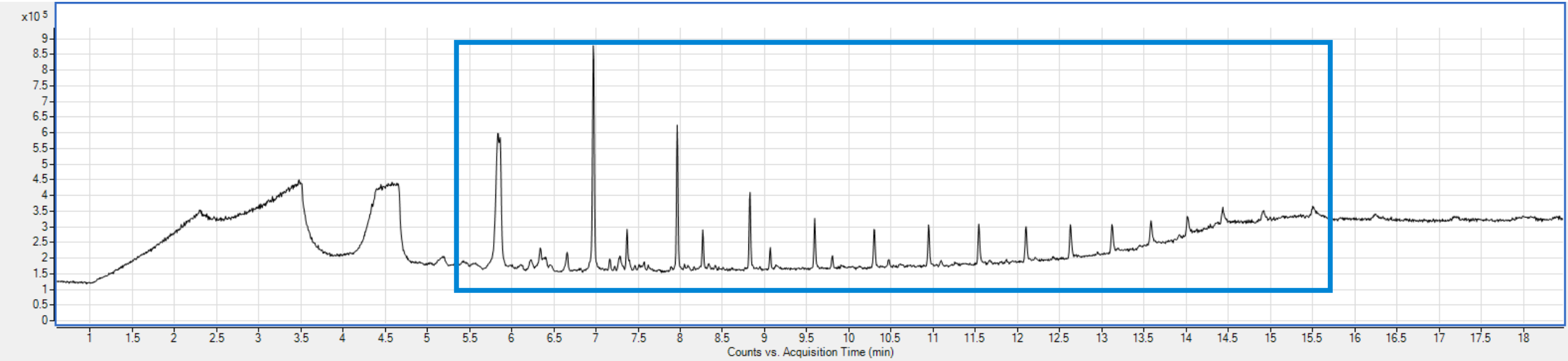


Septum/Solvent Compatibility

Septum Selection Guide

Septum Material	Compatible with	Incompatible with	Resealability	Max. Temperature
Rubber (Natural or Butyl)	ACN, acetone, DMF, alcohols, diethylamine, DMSO, phenols	Chlorinated solvents, aromatics, hydrocarbons, carbon disulfide	Excellent	< 100°C
PTFE/Natural or Butyl Rubber	PTFE resistance until punctured, then septa or liner will have compatibility of rubber		Good	< 100°C
Silicone/Silicone Rubber	Alcohol, acetone, ether, DMF, DMSO	ACN, THF, benzene chloroform, pyridine, toluene, hexane, heptane	Excellent	< 200°C
PTFE/Silicone, PTFE/Silicone/PTFE	PTFE resistance until punctured, then septa will have compatibility of silicone		Average	< 200°C
Viton	Chlorinated solvents, benzene, toluene, alcohols, hexane, heptane	DMF, DMSO, ACN, THF, pyridine, dioxane, methanol, acetone	Good	< 260°C

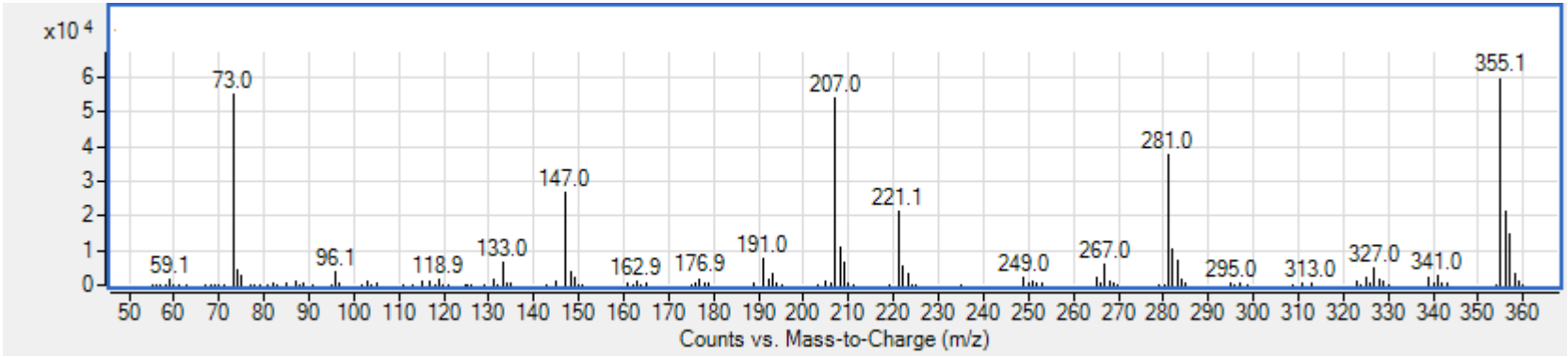
Septum Maintenance: TIC of an inlet/vial septum



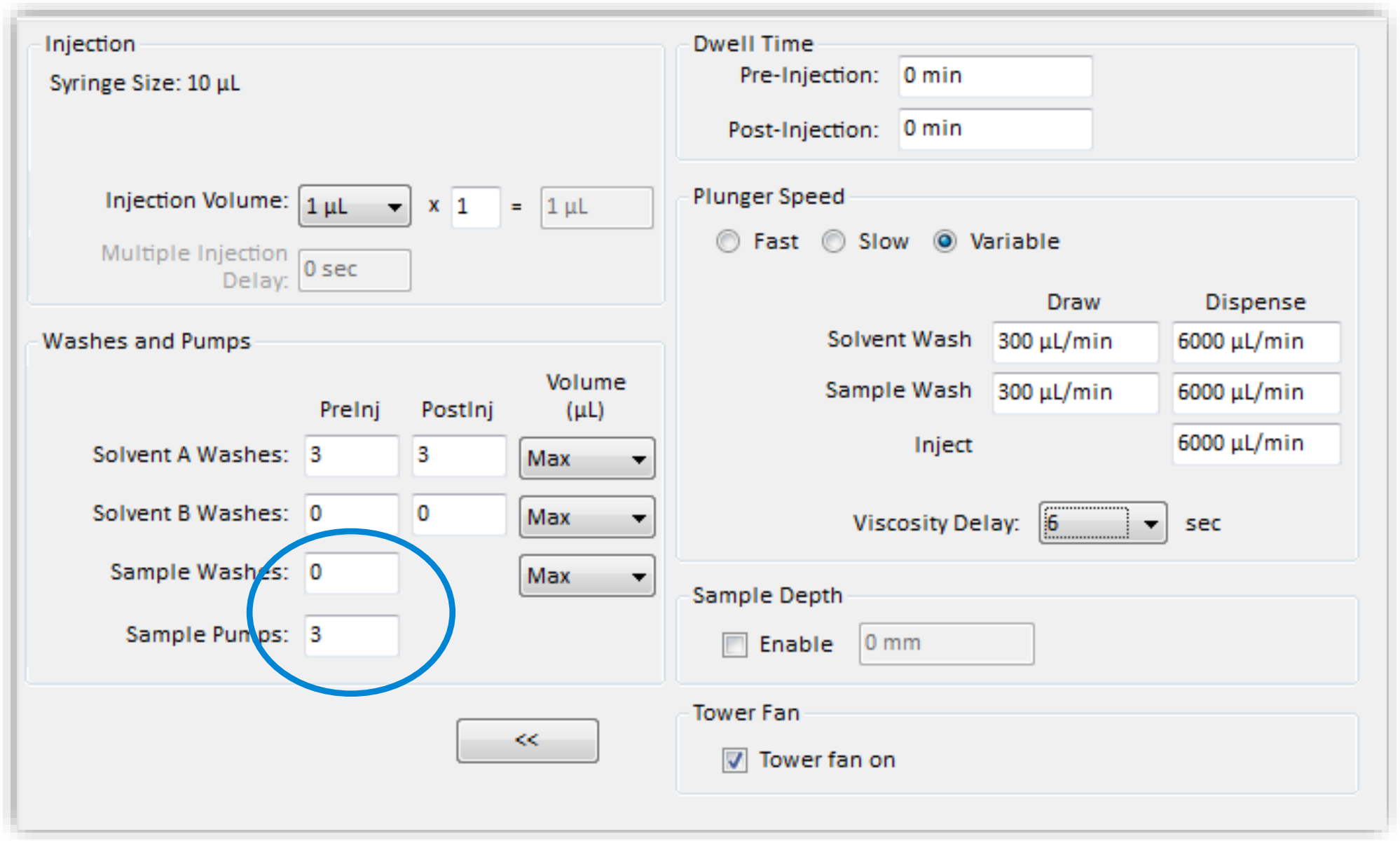
Common ions for Siloxane Molecules
73
147
207
281
355

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:



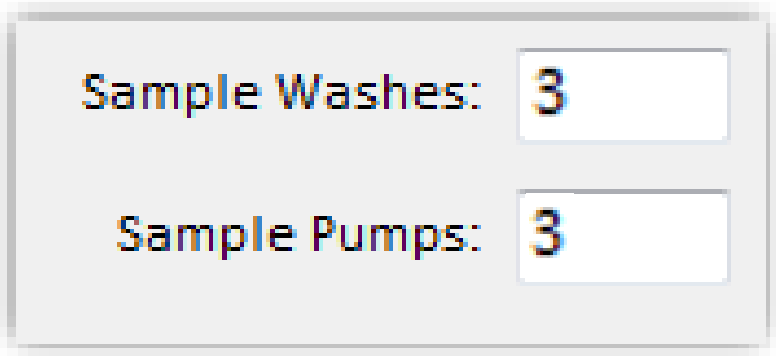
Sample Washes Versus Sample Pumps



Sample Washes Versus Sample Pumps

Sample washes

- Primes syringe barrel with sample, discards into waste bottle
- Improves reproducibility (reduces carryover)
- ***Be careful of reduced volume samples***



Sample pumps

- Draws sample into syringe, discards into same vial
- Eliminates air bubbles → improves reproducibility
- Exercise caution if using viscous samples or solvents
- ***Don't overdo it***
  - Three to five pumps is usually enough
  - ***Excessive pumping can reduce the plunger lifetime***

- Fill the sample vial up to the shoulder
- Leaving a small headspace prevents cavitation, vacuum formation
- Improves reproducibility
- Do not over-tighten the cap
- Use micro-vial inserts to help assure good sampling depth for the needle and to conserve sample

Advanced Method Parameters

Injection

Syringe Size: 10 µL

Injection Volume: 1 µL

Washes and Pumps

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

<<

Dwell Time

Pre-Injection: 0 min

Post-Injection: 0 min

Sample Depth

Enable 0 mm

Plunger Speed (Variable)

Fast Slow Variable

	Draw	Dispense
Solvent Wash	300 µL/min	6000 µL/min
Sample Wash	300 µL/min	6000 µL/min
Inject		6000 µL/min

Viscosity Delay: 0 sec

Injection Type

Standard

L1 air gap: 0.2 µL

L2 volume: 1 µL

L2 air gap: 0.2 µL

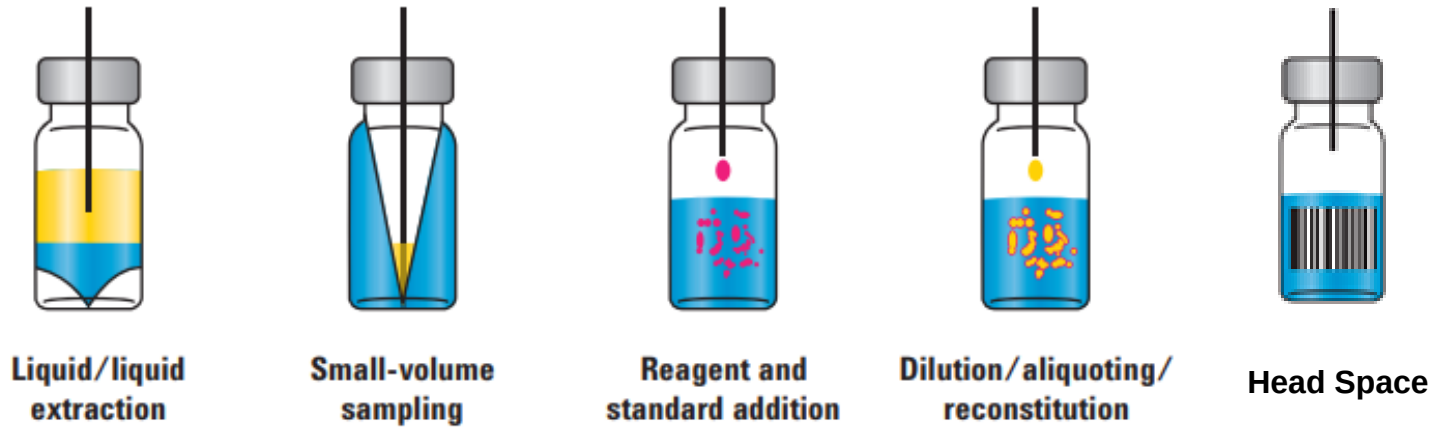
L3 volume: 1 µL

L3 air gap: 0.2 µL

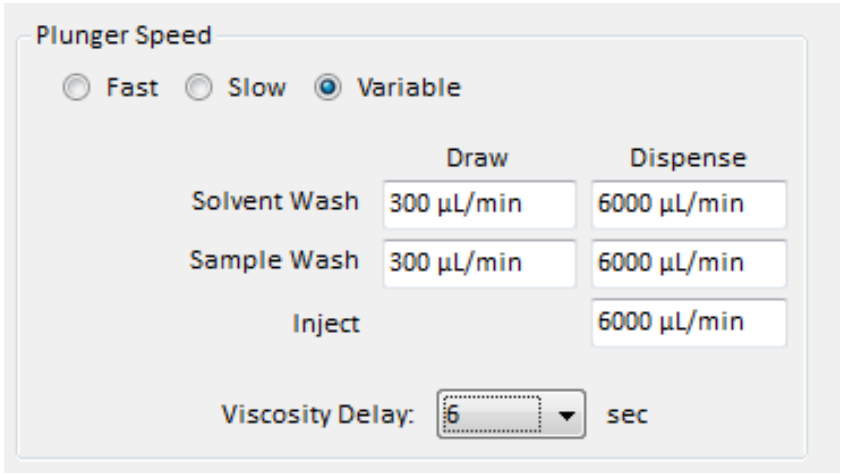
Sample Depth

- Recommended/default (3.6 mm from the bottom of the vial)
- Can change to sample from different heights in the vial
  - A setpoint of -2 mm will sample 1.6 mm from the vial bottom
  - The range is -2 mm to 30 mm

- Example uses:
  - Samples with sediment (although properly filtering the sample is best)
  - 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
    - Needle may bottom out
  - Ambient headspace analysis



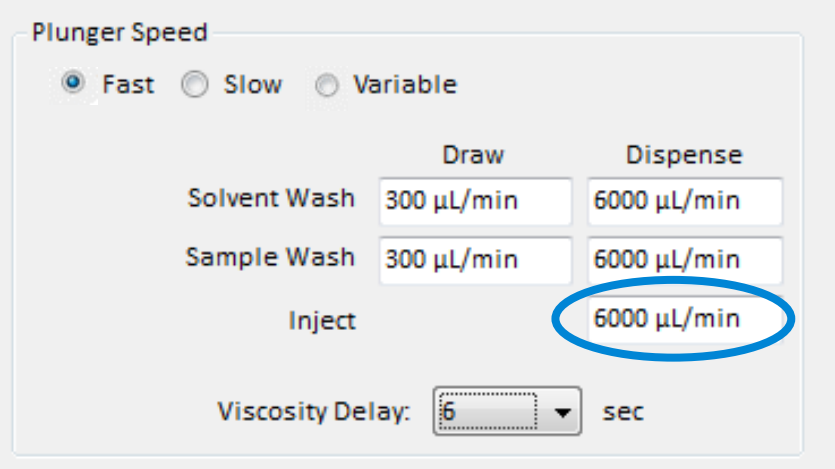
Plunger Speed and Viscosity Delay



Goal: Get sample in the inlet quickly

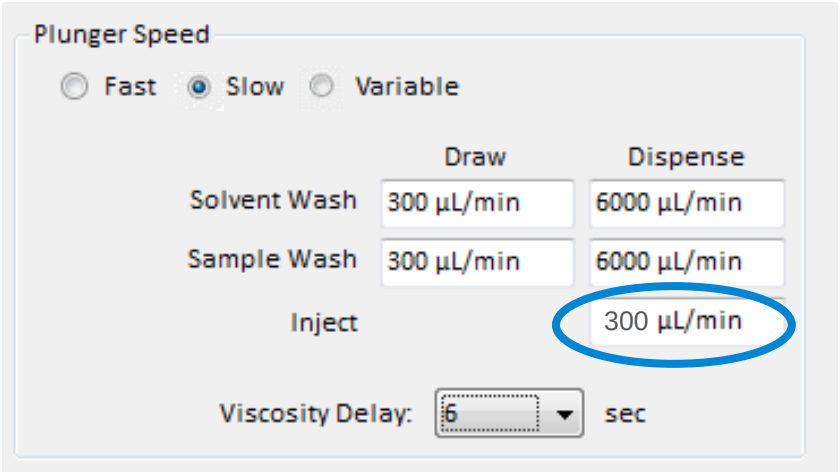
Plunger Speed

Fast/variable



- Speed setpoints depend on configured syringe size
- Fast (default)
  - 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
    - If using viscus solvents (butane diol, glycol eyc.) slow to 3000 µL/min
    - Use slower rate if experiencing barrel breakage

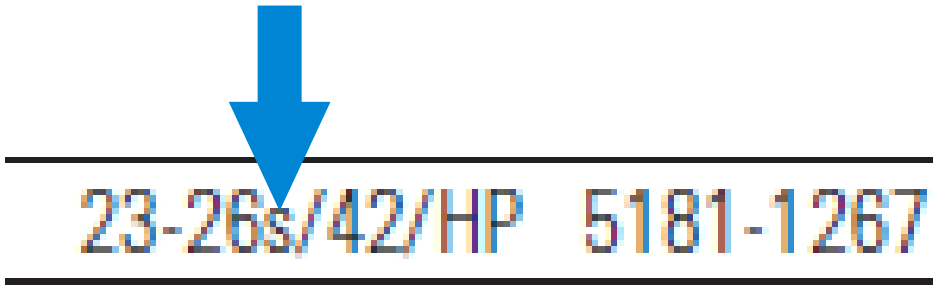
Slow



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

- Use “variable”: to use custom set points

New High Flow Syringes



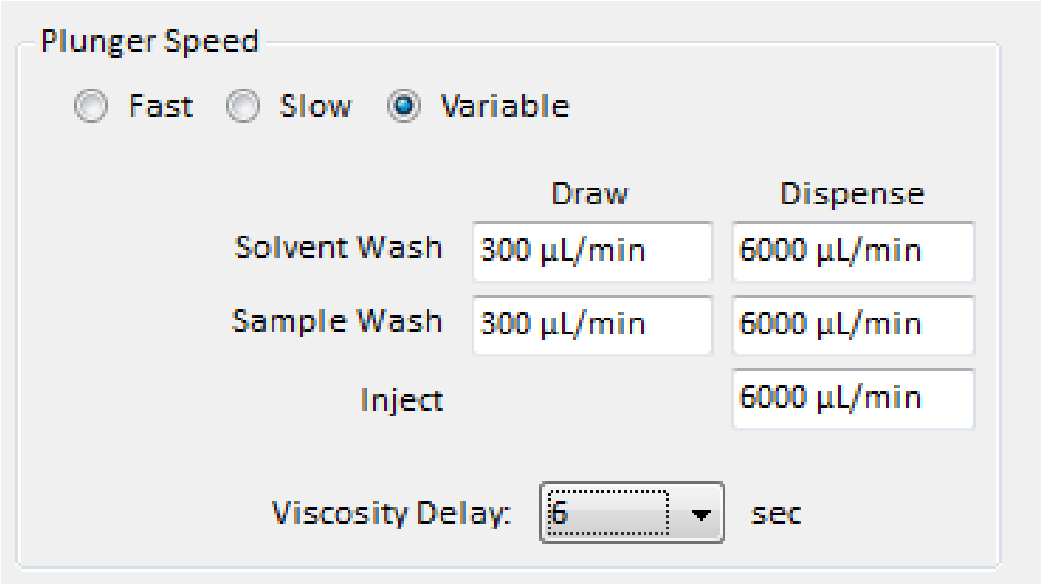
- Syringes with “s” designation have smaller ID needle (thicker walls) and thus haver stronger needles
- This can restrict flow and can cause high pressure during injection of viscous samples
- Viscous samples + high-speed injections (6000 µl/min) can be prone to breakage of the barrel with narrow ID syringe needles (“s” designation)
- New high flow “HF” syringes have an intermediate ID, resulting in higher flow than “s” syringes; they are also less prone to breakage for high viscosity + fast injections

New High Flow Syringes - needles

Designation Inside Diameter	Needles Wall Thickness	Strength	Comments	Solvent type / Injection Speed
Standard 0.25 mm		Highest flow Weakest needle	• Lower pressure during injection • Needle more prone to bending	Higher Viscosity / Higher speed
“s” – Slim 0.11 mm		Lowest Flow Strongest needle	• High pressure during fast injection • Possible barrel breakage • Needle less prone to bending	Lower Viscosity / Lower speed
“HF” – High Flow 0.15 mm		Intermediate flow Intermediate strength	• Intermediate pressure during fast injection • Needle less prone to bending	Intermediate Viscosity / Intermediate speed **5181-1267-HF (10 µL Fitted)** **5181-3360-HF (6 pk)** **5181-3354-HF (10 µL PTFE)** **5181-3361-HF (6 pk)**

Viscosity Delay

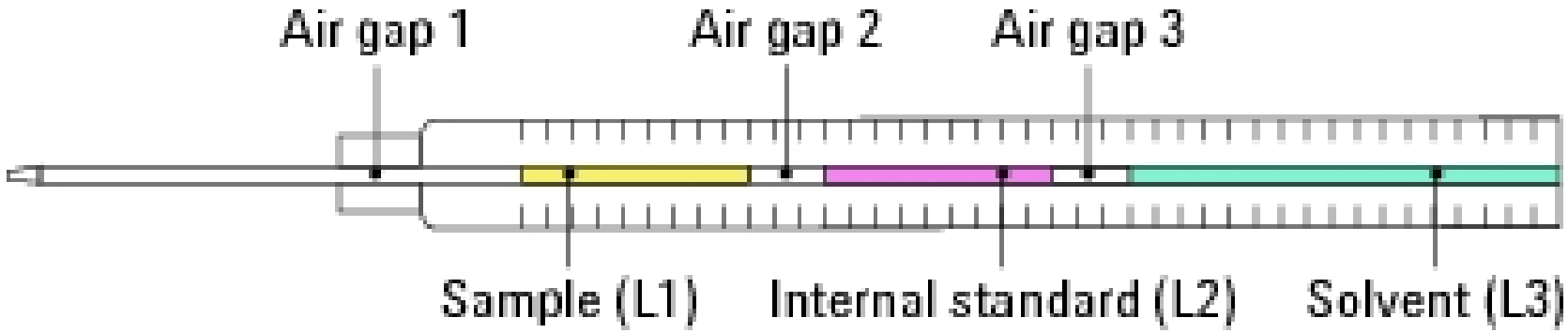
- This is the time (seconds) that the plunger pauses for between pump and injection
- Allows additional time for viscous samples to flow into the syringe during pump
- Use for viscous solvents like isooctane
- Use for highly volatile solvents such as dichloromethane (to prevent cavitation/bubbles)
- A two 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 (7693)

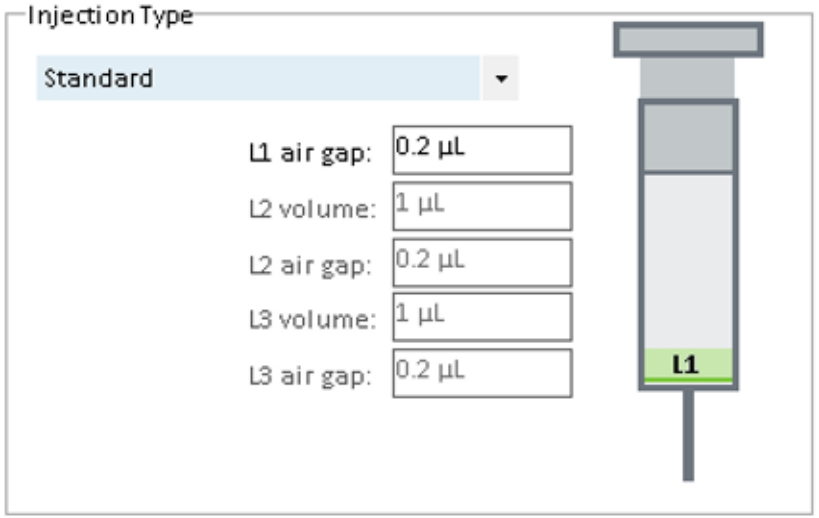
Injection types

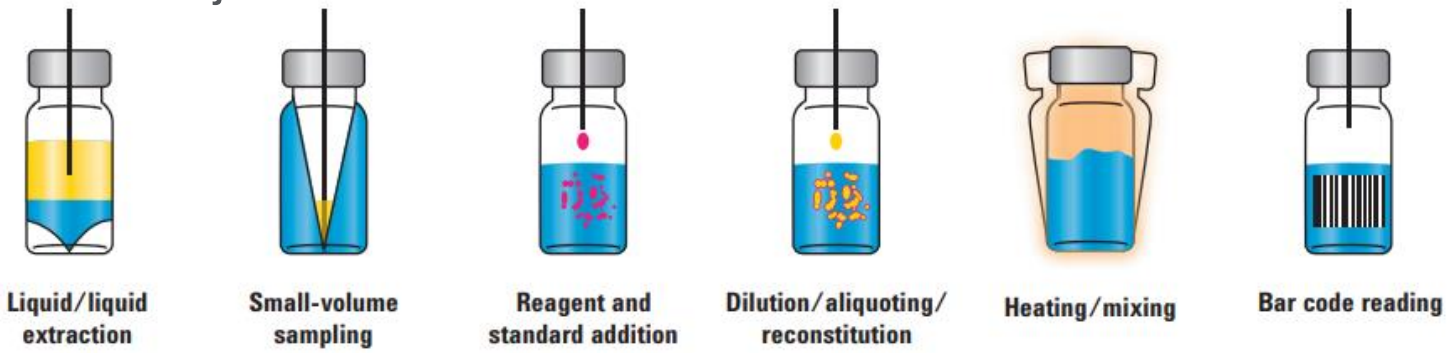
- Standard
- Sandwich injections
- Layered injections
- Multiple injections



Air gap

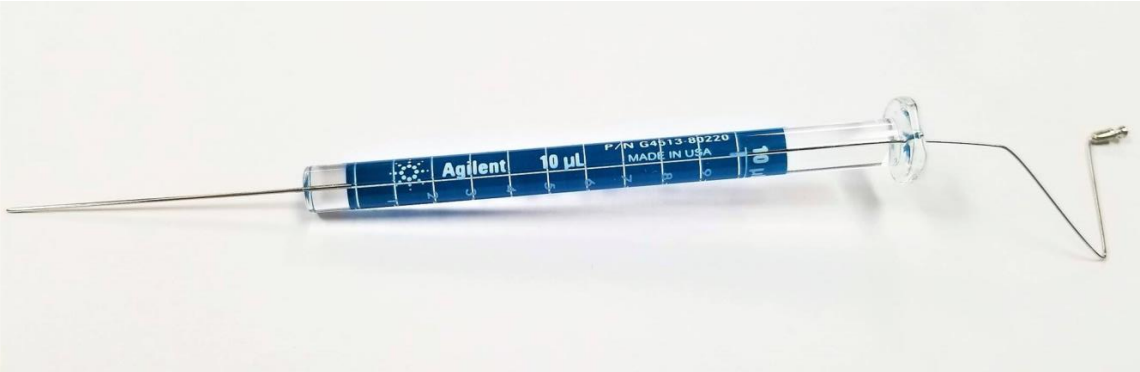
- 0.2 µL default (Air gap 1)
- Helps retain the sample in the syringe before injection

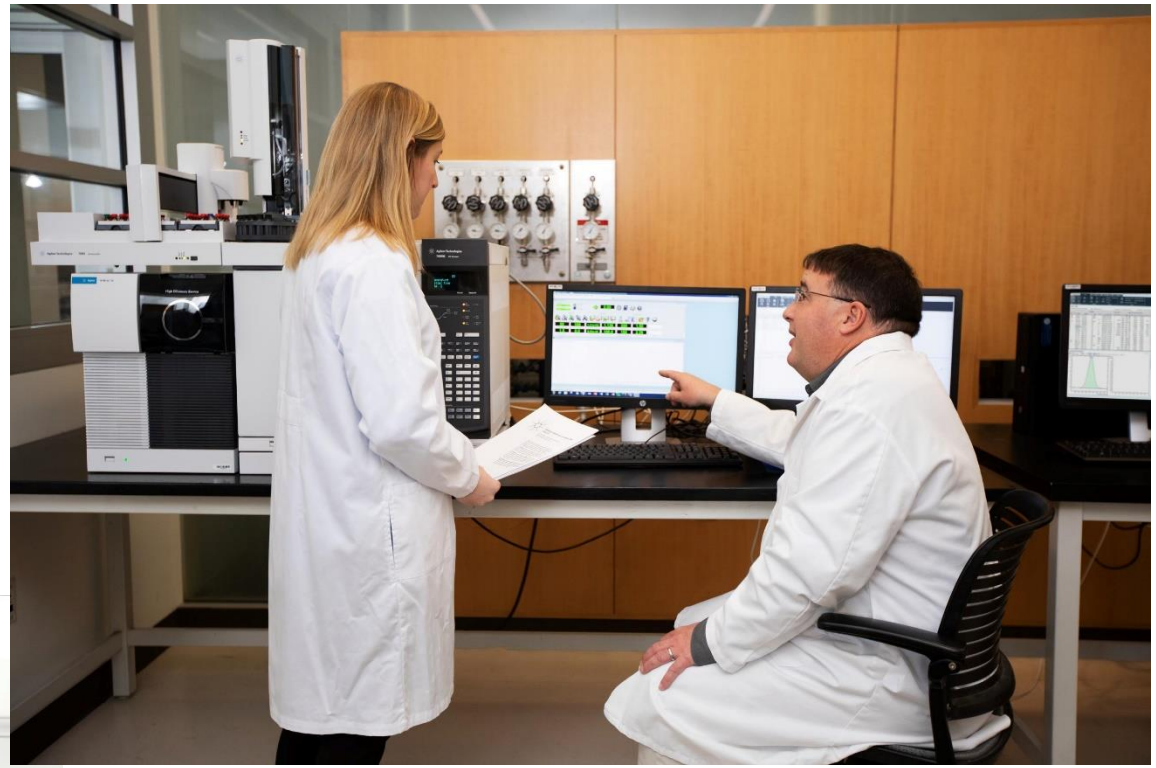


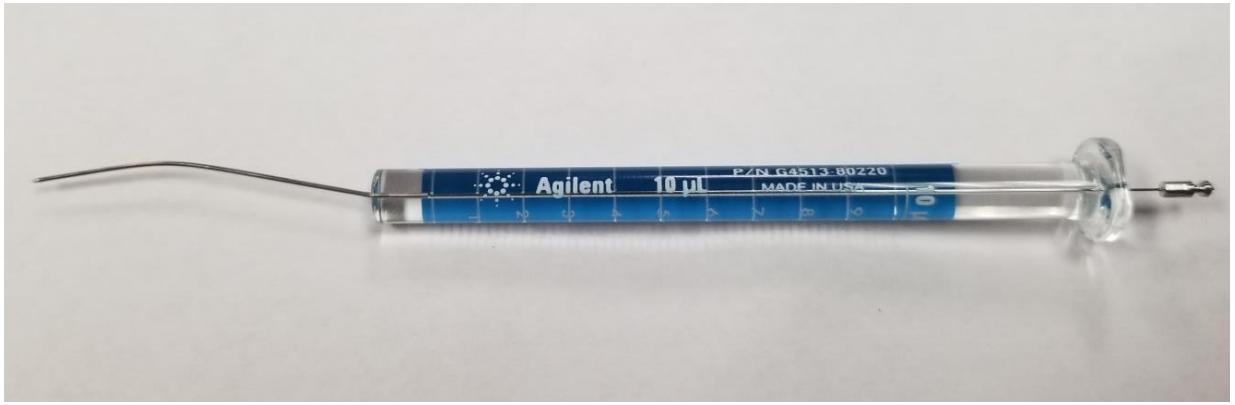


https://www.agilent.com/cs/library/applications/5991-7973EN.pdf

Troubleshooting







Troubleshooting

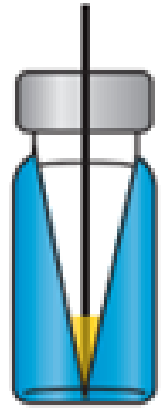
Problem: No peaks/Reduced Peak Areas

Possible causes:

- Plugged needle (most common)
- Syringe plunger malfunction
- Not enough sample
- Sample is too viscous (V-delay)

Suggested actions:

- Clean or replace syringe
- Check sample level, use low-volume vial insert
- Check sample depth setting in method
- Increase viscosity delay time



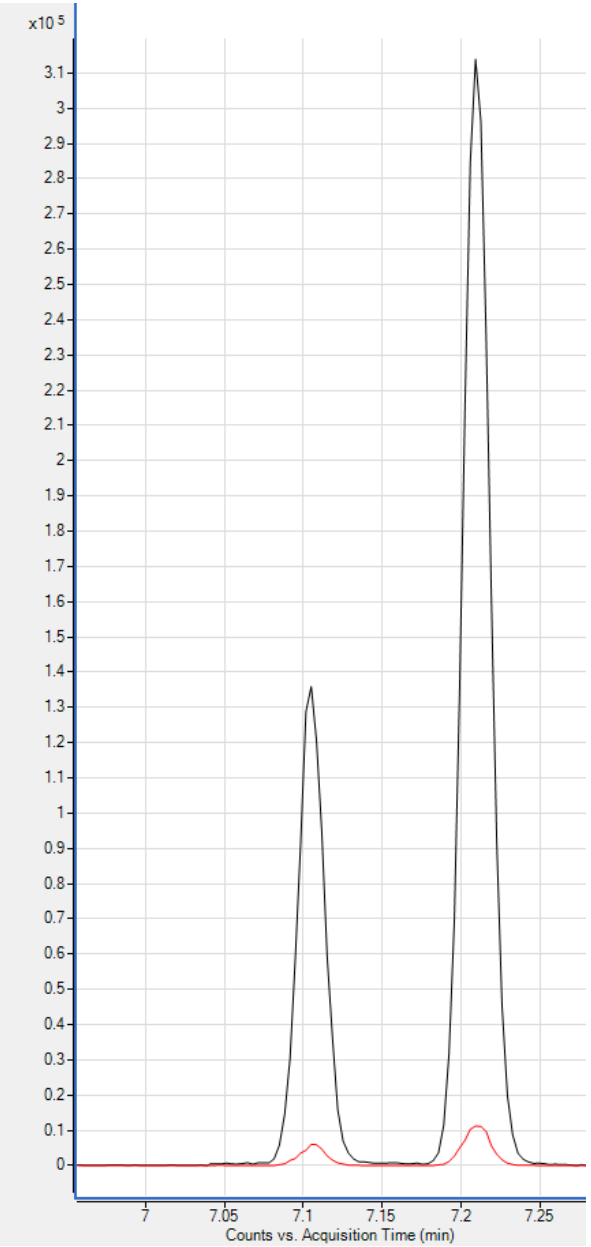
Troubleshooting

Problem: Sample carryover

Possible causes:

- Insufficient number of washes
- The solvent wash vial is empty
- The solvent wash vial is contaminated
- Wrong wash solvent
- Dirty ALS needle guide
- Dirty septum nut

ALS needle guide- G4513-40525



Suggested actions:

- Increase the number or type of washes
- Rinse with various polarity solvents
- Clean or replace the syringe
- Ensure samples and solvents are miscible
- Occasionally replace the needle guide (or “needle foot”)
- Check the septum nut for sample residue

Troubleshooting

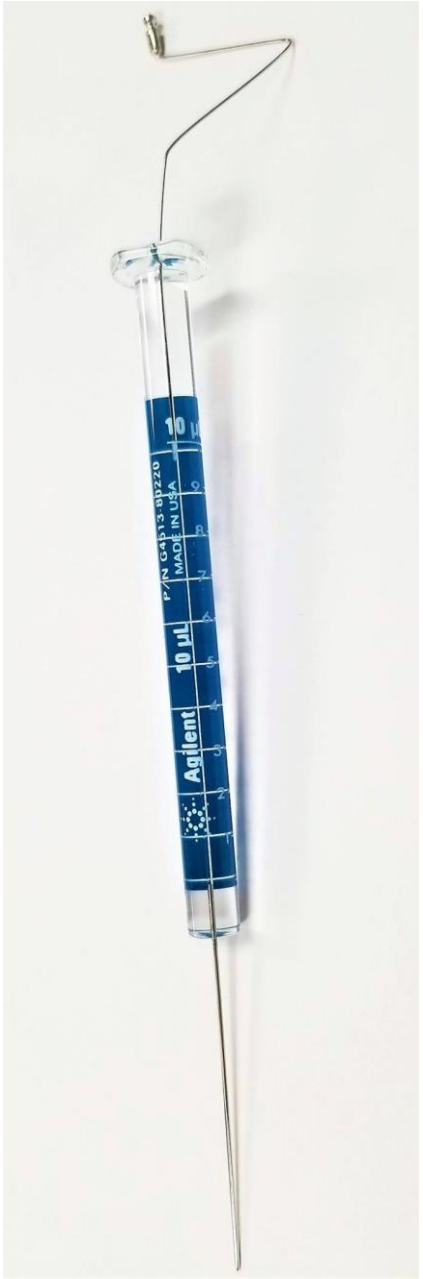
Problem: Bent plunger or stuck syringe

Possible causes:

- Typically, from sample matrix residuals
- Corrosive solvent
- Non matched plunger

Suggested actions:

- Clean up the samples
- Switch to a syringe with a PTFE-tipped plunger
- Avoid using 5 µL syringes, where possible
- Clean the syringe (especially just before sequence)
- Never cycle the plunger in a dry syringe
- Do not “mix-and-match” plungers and barrels
- Immediately clean syringes after use and just prior to use
- Consider manually rinsing the syringe before each sequence to prewet the plunger



Best Practices – Plunger Binding

- Plunger binding is almost always sample matrix or solvent-related (water or corrosives)
  - Plungers are perfectly matched to each barrel which makes for a very tight fit (plungers are not interchangeable) – this is true for all non-PTFE syringes
- Don’t let the plunger dry out especially with a matrix sample – use pre-injection rinses or manual rinses
- Consider prewetting the syringe manually, especially if the system has been sitting idle or if you have a known dirty or sticky sample matrix
- Use a binary solvent system in the ALS to rinse the syringe (differing polarity, but still miscible)
- Periodically remove and manually wash/rinse the syringe with various solvents (between sequences)
- Swap back and forth between two syringes so you always have a precleaned syringe “at the ready” to save time
- Immediately clean your syringes manually after each use/sequence – especially for dirty matrix samples
- For really dirty samples use syringes with a PTFE plunger (which is replaceable)
  >10 µL only

Troubleshooting

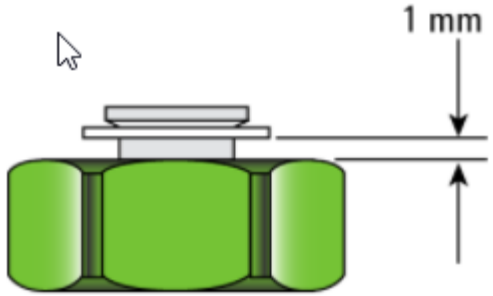
Problem: Bent needle

Possible causes:

- Improper needle alignment
- Narrow gauge needles (26 guage) bend more easily than larger gauge (23 guage) needles
- Needles more often bend when inserted into the sample vial, not the inlet
- On column inlets – wrong needle gauge; needle not fitting in column
  - Use correct needle support

Suggested actions:

- Use syringes with 23 to 26 gauge tapered needles; best of both worlds
- Realign the autosampler
- Check the septum nut is not over-tight



https://community.agilent.com/knowledge/gc-portal/kmp/gc-articles/kp384.replacing-the-inlet-septum-for-split-spitless-and-multimode-inlets

Troubleshooting

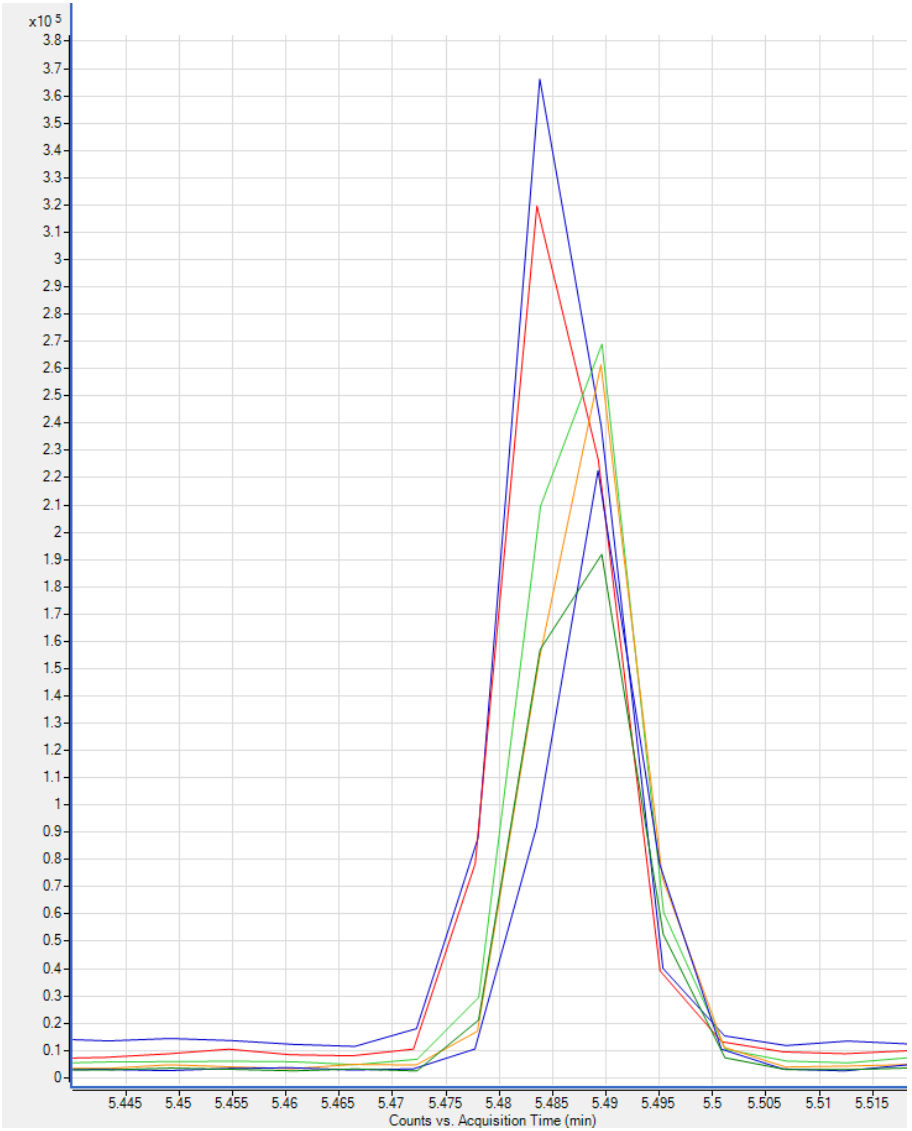
Problem: Poor reproducibility

Possible causes:

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

Suggested actions:

- Clean or replace the syringe
- “Restore” the plunger tip (***PTFE only***)
- Replace the plunger altogether (***PTFE only***)
- Rinse and refill the solvent wash vial
- Do not allow the sample to crystallize inside the syringe between injections
- Make sure the solvents used are miscible and compatible with the syringe



Sample Introduction: Important Takeaways

- Successful GC injection is a complex process
- PTFE-tipped 10 µL syringe are more forgiving
  - Handle the syringe carefully
  - Avoid pumping the plunger when “dry”
  - Plungers are replaceable
- Don’t let the wash solvent run low/dry/become contaminated
  - How long is your sequence? (i.e. do you have enough wash solvent?)
  - Do not top of solvent wash vials; replace with new/clean vials
- Get the sample into the inlet quickly
  - Be aware of solvent viscosity
    - reduce injection speed
    - Use HF syringes as needed
- Balance number of syringe strokes against carry-over
- Be aware of advanced parameters for special applications
- If you’re not sure, reach out and ask for help

Contact Agilent Chemistries and Supplies Technical Support

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

- Option 1 for GC or GC/MS columns and supplies
- Option 2 for LC or LC/MS columns and supplies
- Option 3 for sample preparation, filtration and QuEChERS
- Option 4 for spectroscopy supplies
- Option 5 for chemical standards (formerly ULTRA)

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

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.