Simplifying GC Syringes and Sample Introduction

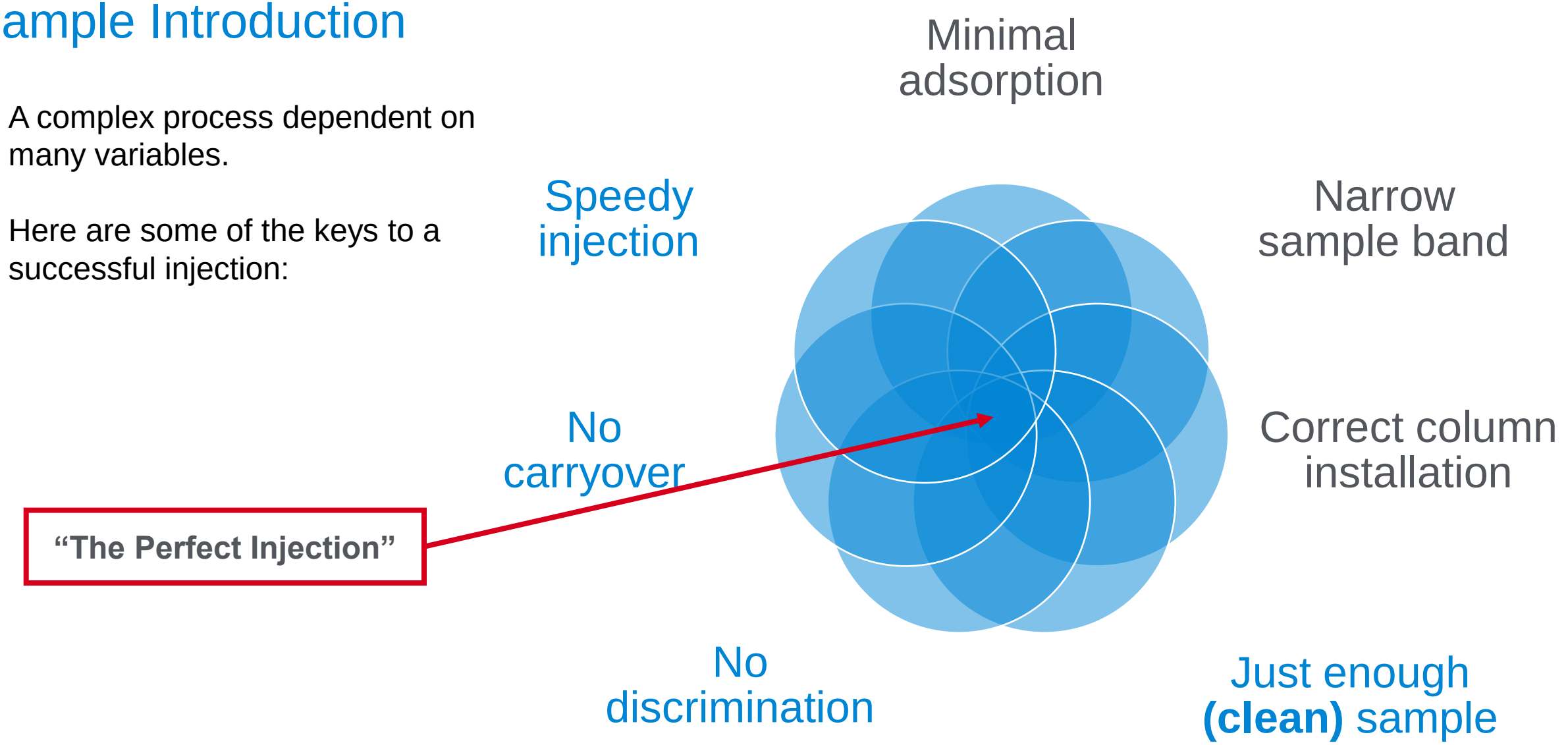
Plunge deep into the world of syringes

Agilent
Trusted Answers

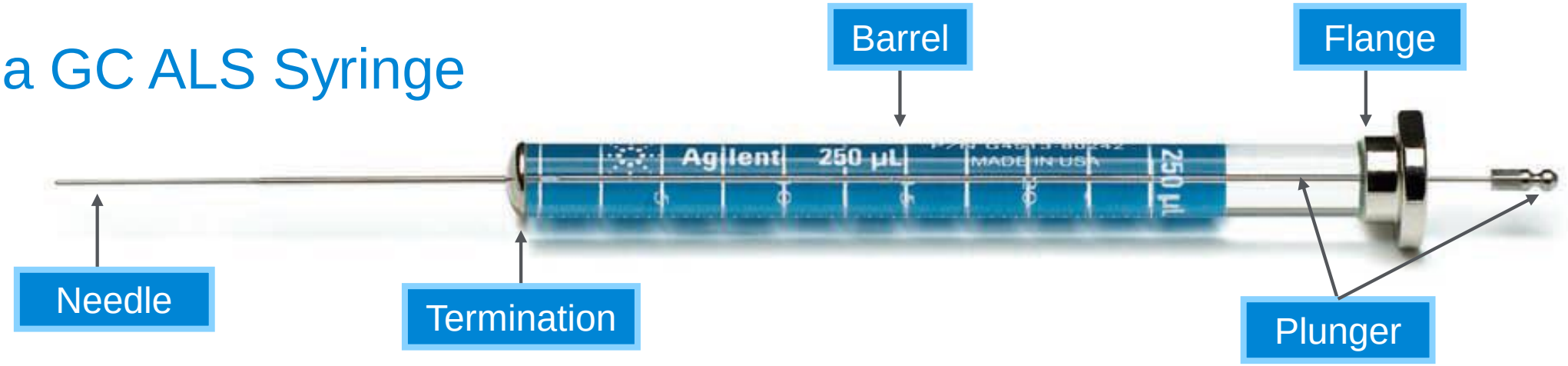
Sample Introduction

A complex process dependent on many variables.

Here are some of the keys to a successful injection:



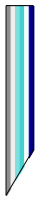
Anatomy of a GC ALS Syringe



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.



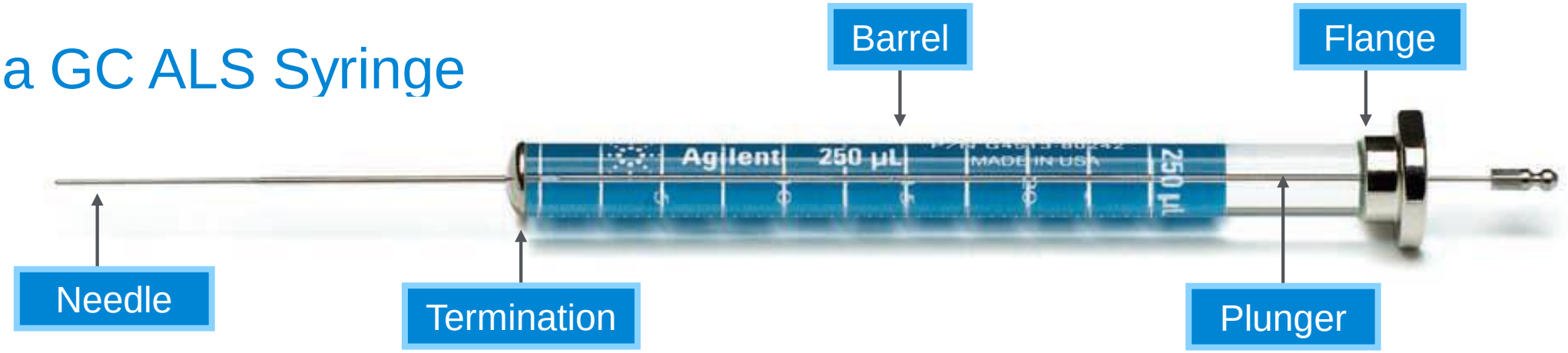
Bevel tip/PS 2

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



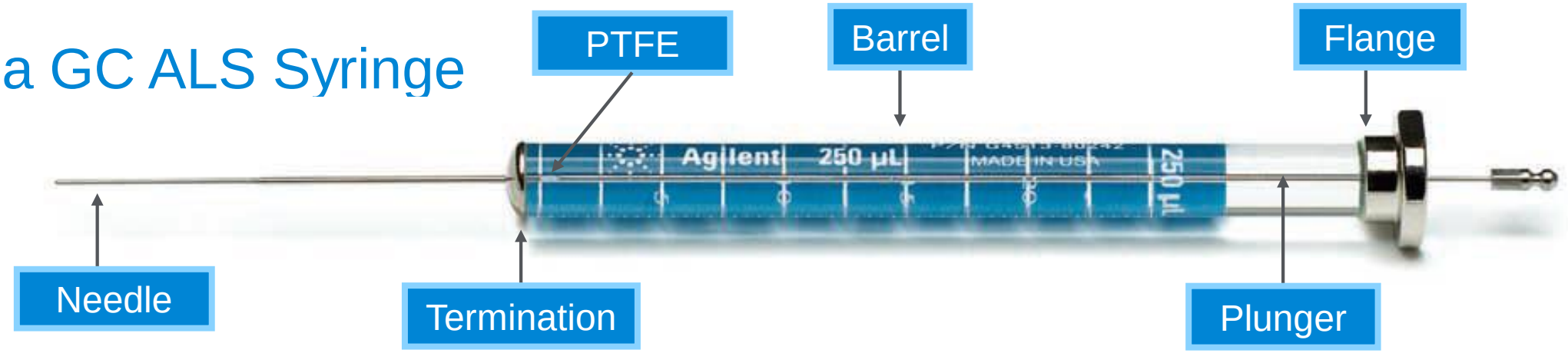
Fixed needle syringes (shown)

- 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
- Recommended for chlorinated solvents

Anatomy of a GC ALS Syringe

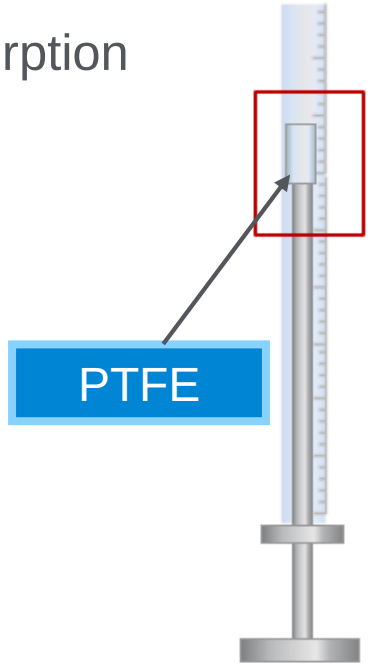


Standard plungers

- 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)

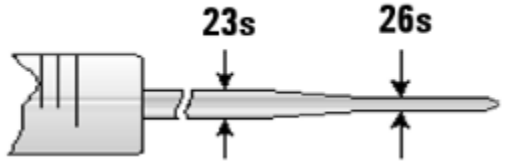
- Limit sample deposit adsorption
- Forms gas-tight seal
- Replaceable
- Requires maintenance to maintain PTFE seal
- Recommended for:
- “Dirty” samples
- Highly volatile samples
- Gas injections
- Chlorinated solvents



Syringe Selection Tips

best bet

- 10 µL 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



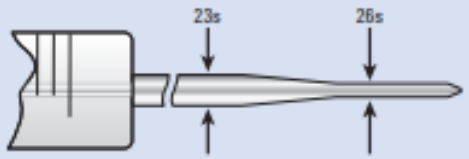
- Ensure proper syringe is configured in software

Gold vs. blue syringe

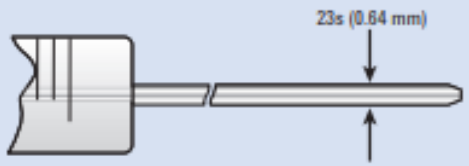
- **BLUE**
- **GOLD**

- Specifications for both are equivalent
  - completely interchangeable
  - Personal preference

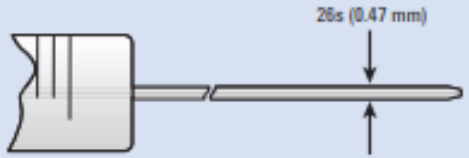
Needle Gauge



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



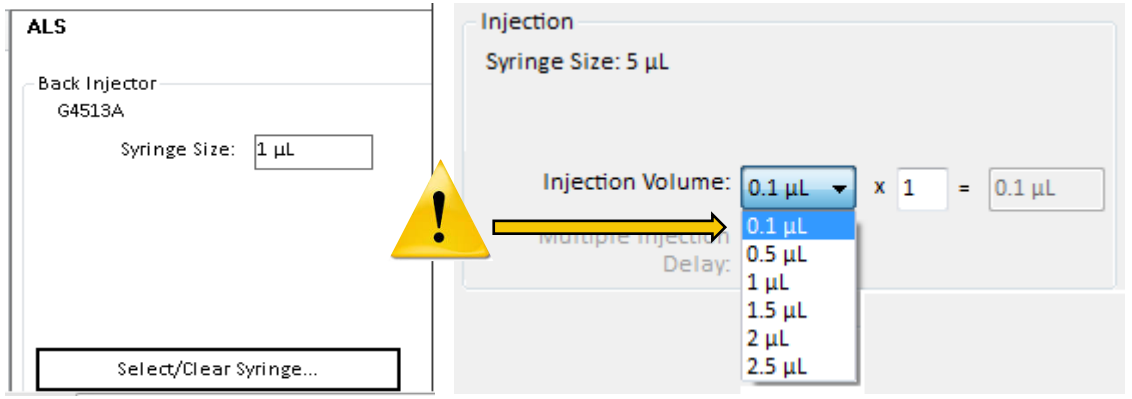
Single Gauge 26 or 26s (0.47 mm)
Packed column injector ports
Split/splitless injector ports

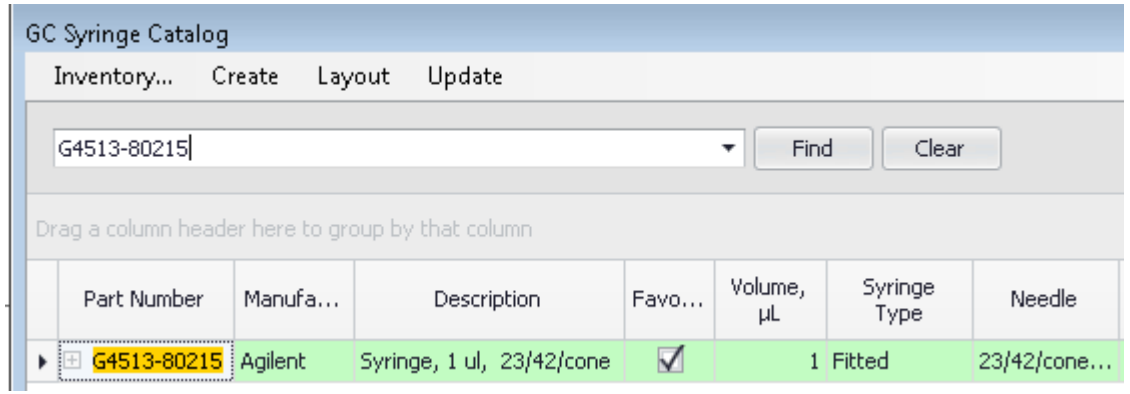
Note: Needles with an 'S' following the gauge are more durable, with a thicker needle wall and smaller ID bore.

Syringe Selection Tips: 5 µL

5 µL syringes are ideal for small volume injections BUT:

- Typically shorter lifetime (narrow plunger diameter → bends easily)
- 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





Micro/Nano Volume Syringes

Microvolume syringes

Blue line micro/nanovolume syringes are half-marked!

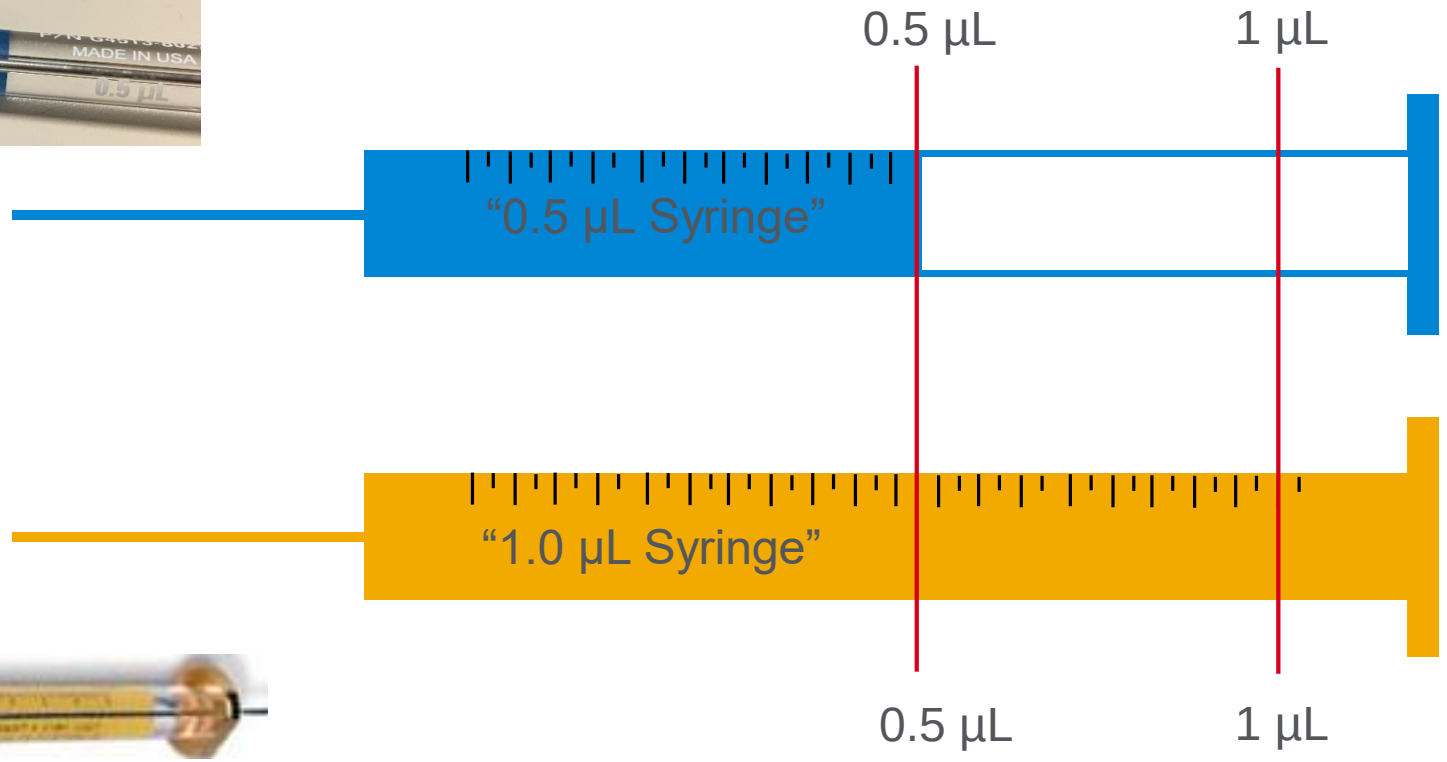
Need to configure ALS with 2x syringe volume
or risk getting half the response

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

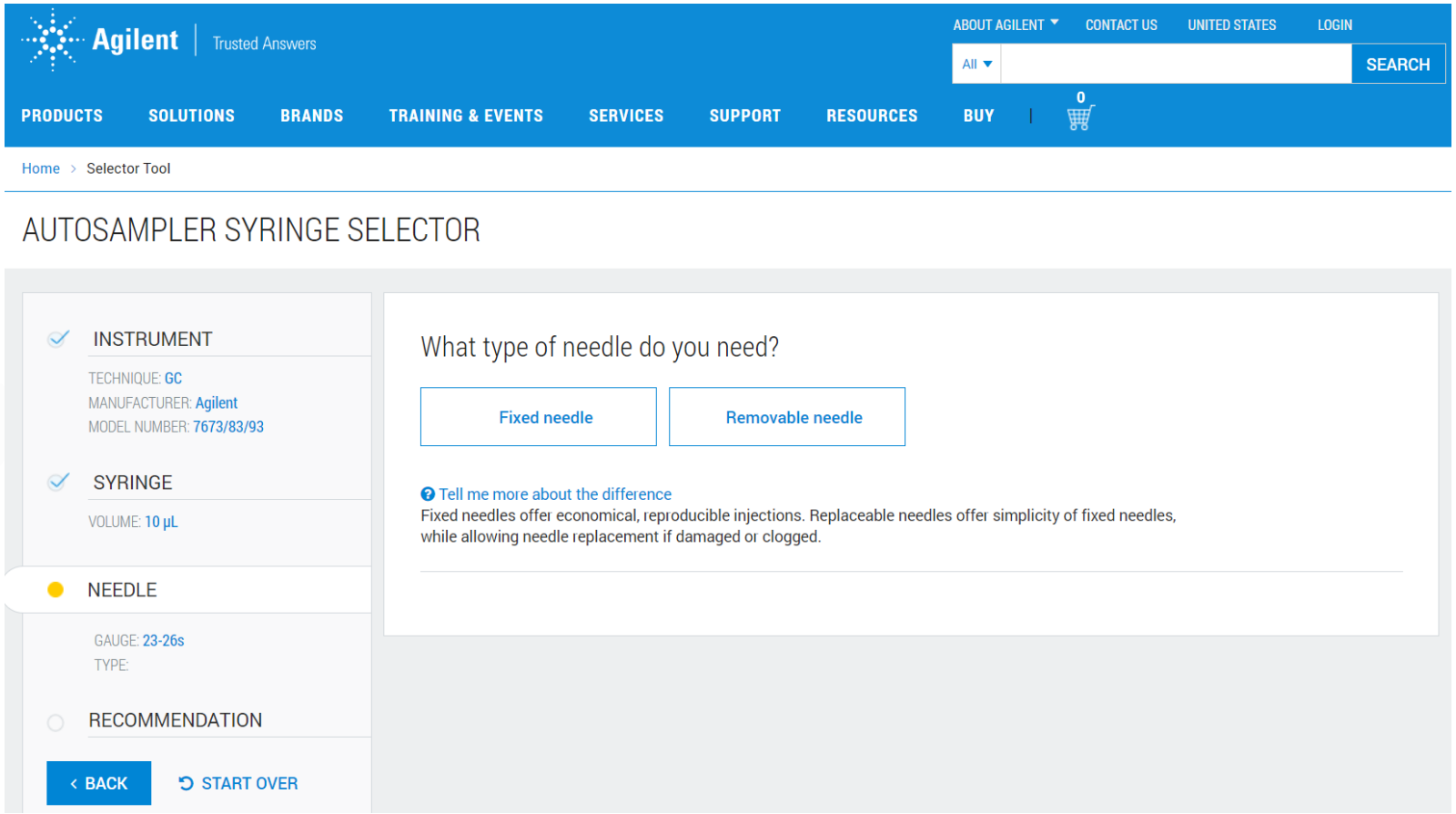
Volume (µL)	Description	Unit	Needle	Part No.
1	Cone-tipped		23/42/HP	5188-5246
2	Cone-tipped		23/42/HP	5188-5247



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

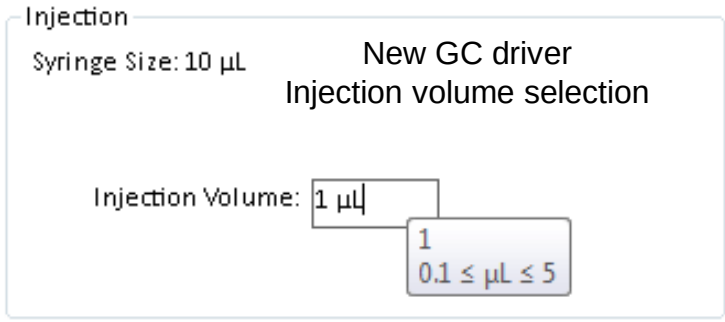
L3 volume: 1 µL

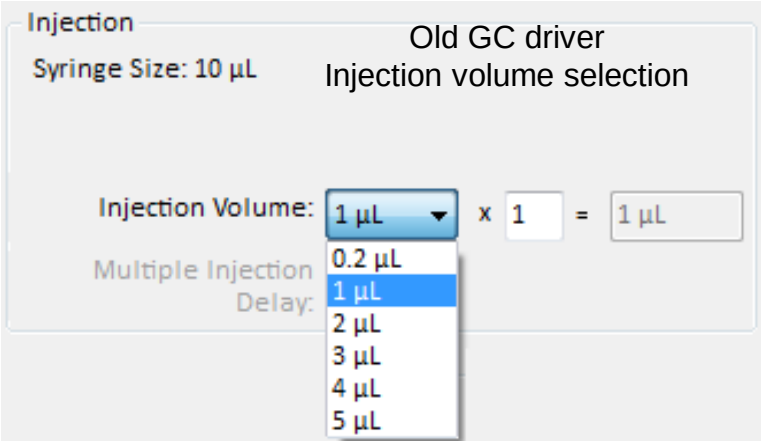
L3 air gap: 0.2 µL

Injection Volume / Rinse Volume

Syringe capacity:

- Avoid injection volumes below 10% of syringe capacity
  - Injection will work, but reproducibility may suffer
- ALS software automatically limits max injection volume to 50% of the configured syringe volume
  - 10 µL syringe → 5.0 µL injection size MAX
  - 5 µL syringe → 2.5 µL injection size MAX…etc





Max injection volume (50 %)

Max rinse volume (80 %)

Starting Points for Injection Volume

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

- Avoid Back-Flash!
  - 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
  - If you must, then calculate the expansion volume*
  - Rule of thumb: 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

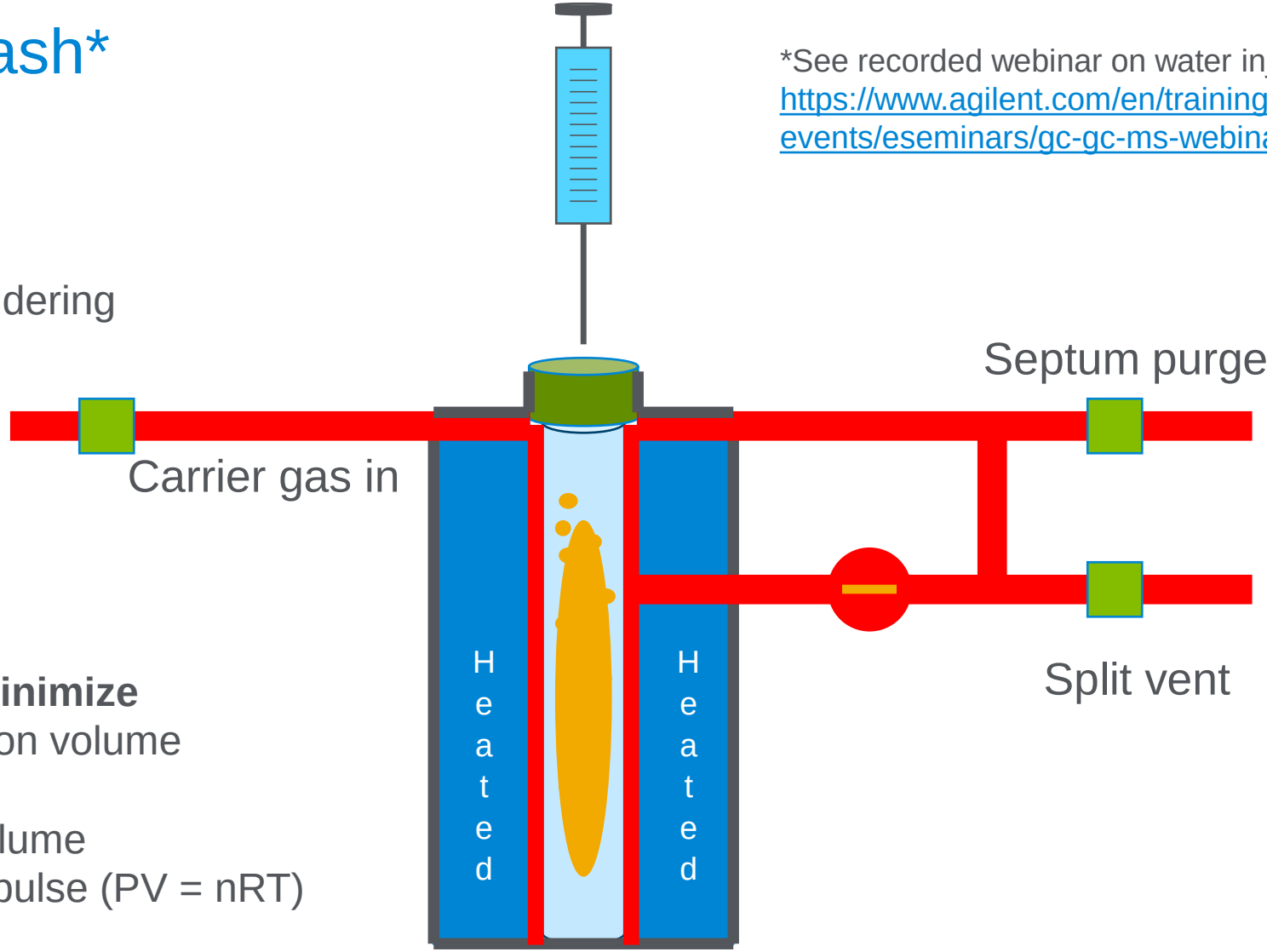
https://www.agilent.com/en/support/gas-chromatography/gccalculators

Sample Backflash*

Negative Effects

- Tailing
- Carry-over
- Peaks splitting/shouldering
- Low response
- Poor reproducibility

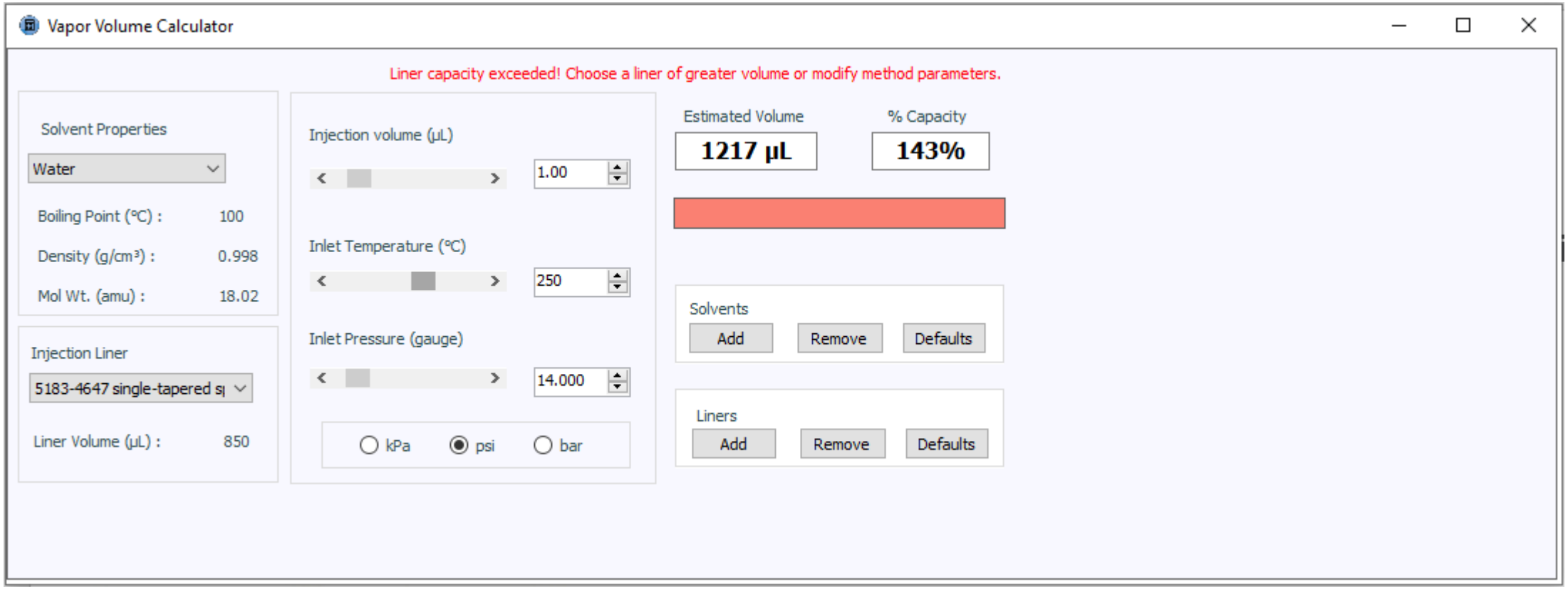
*See recorded webinar on water injections for more info: https://www.agilent.com/en/training-events/eseminars/gc-gc-ms-webinars



How To Avoid/Minimize

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

Vapor Volume Calculator

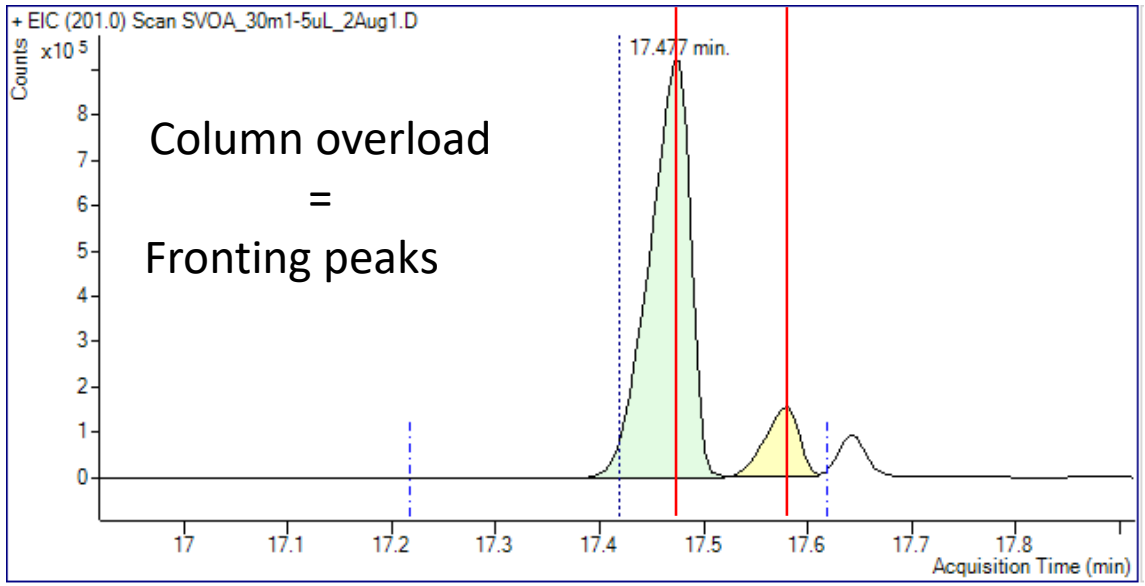


Chromatographic Signs that your Injection Volume is Too High

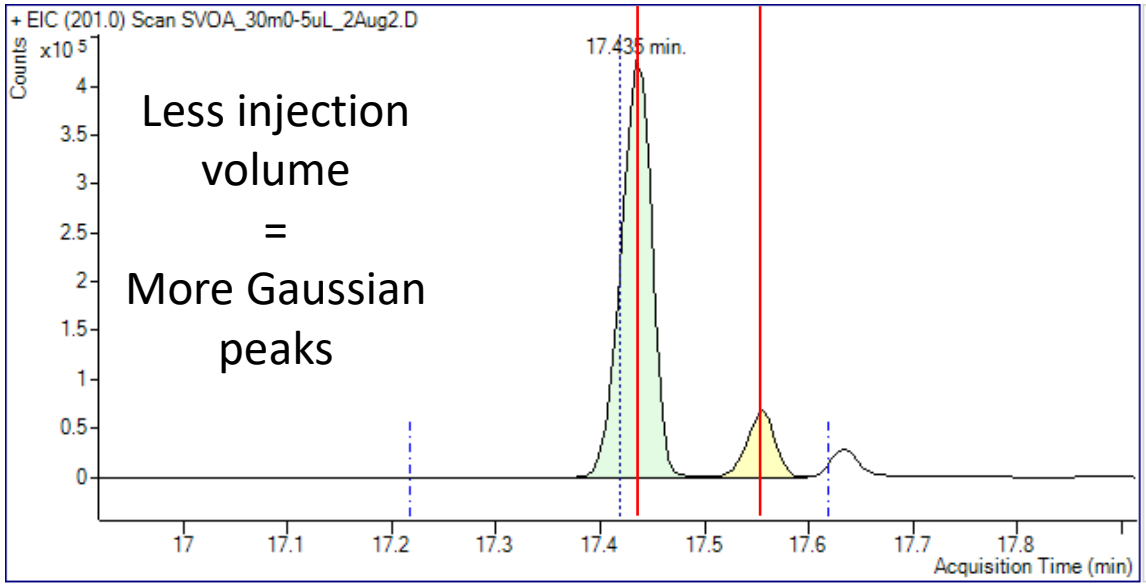
Overloading

- Watch for highly concentrated samples
- Keep injection volume small
- 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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Injection Volume: 1 µL

Washes and Pumps

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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

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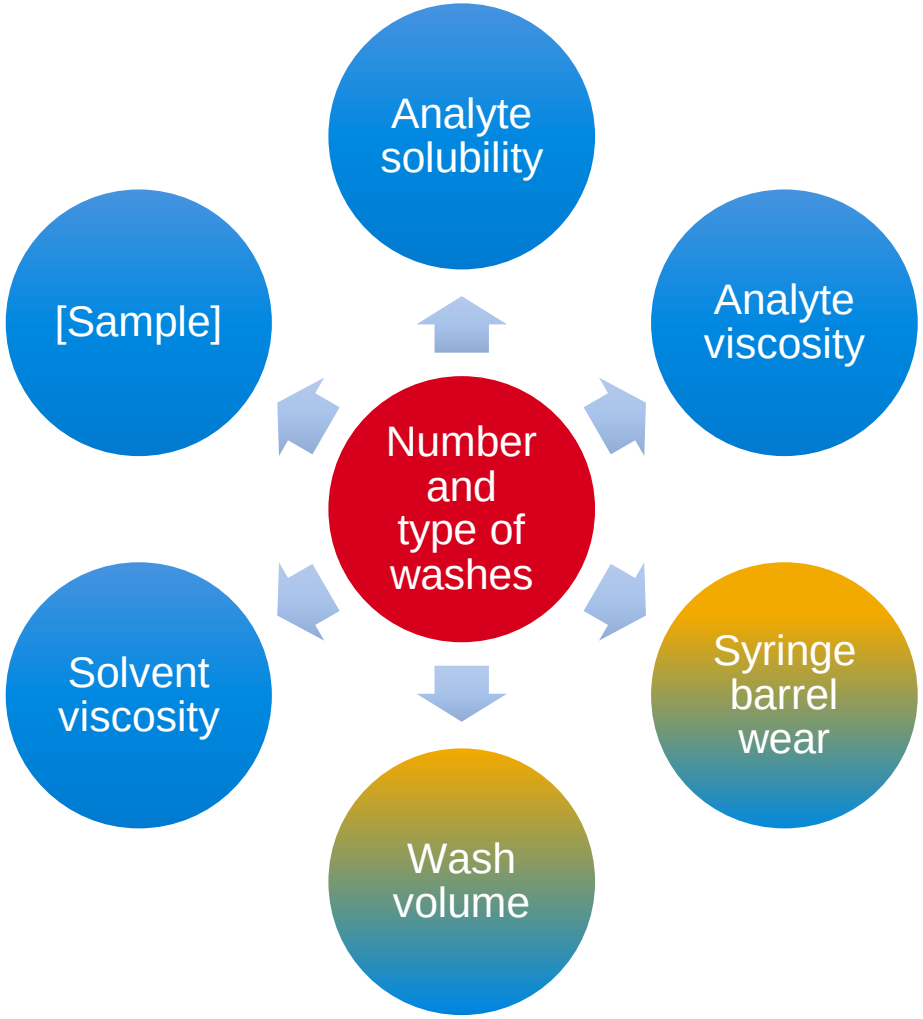
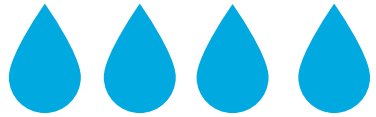
Washes and Pumps: Solvents

- 4 pre- and post washes reduces carryover to one part in 10,000



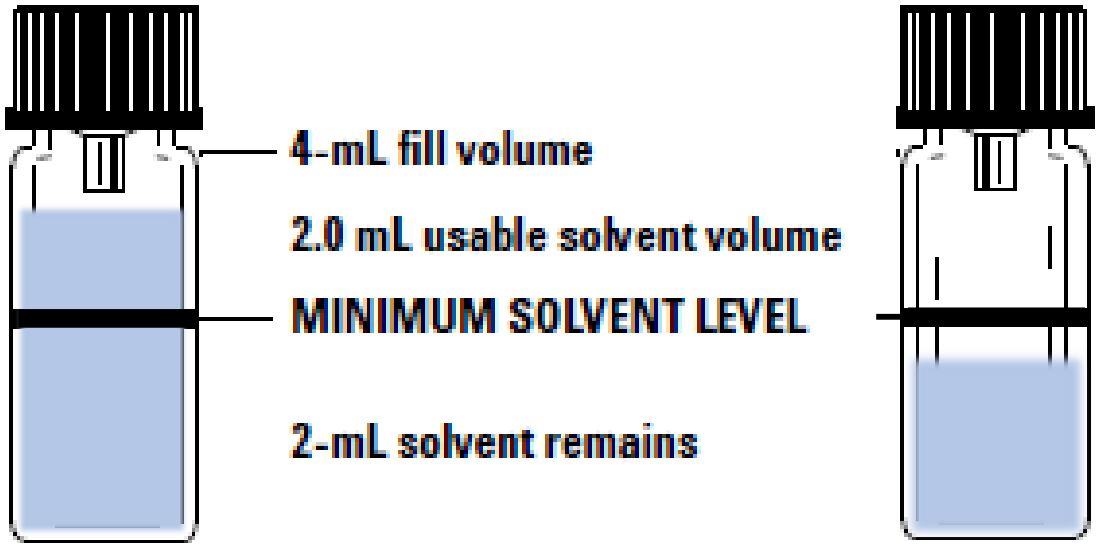


Washes and Pumps

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

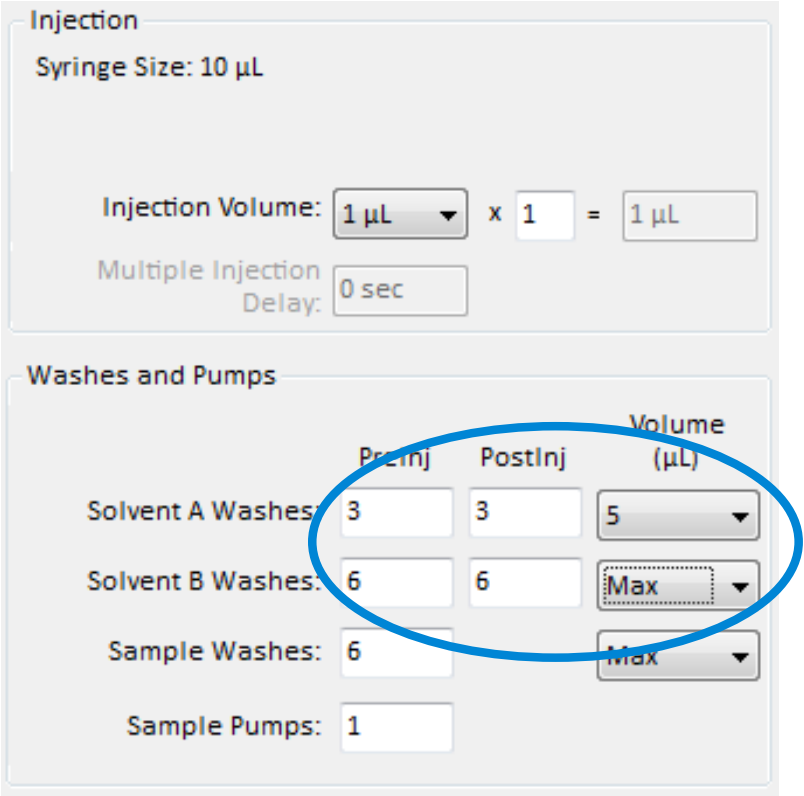
Wash Vial Volumes

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



> 2 mL ✓

< 2 mL ✗



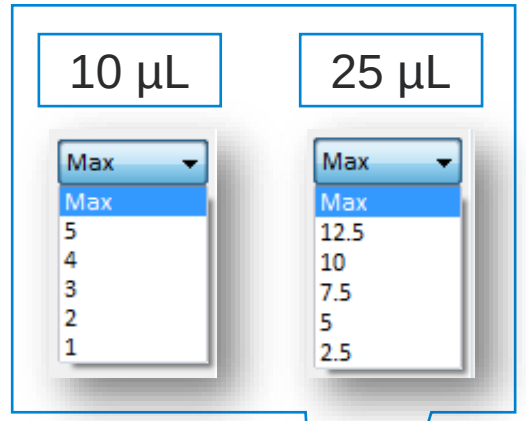
Washes and Pumps: Solvent Saver

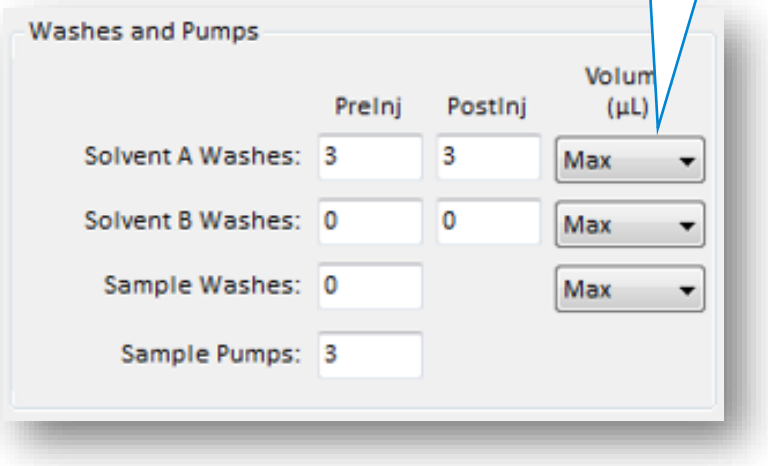
High wash application? Try solvent saver.

- 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

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

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

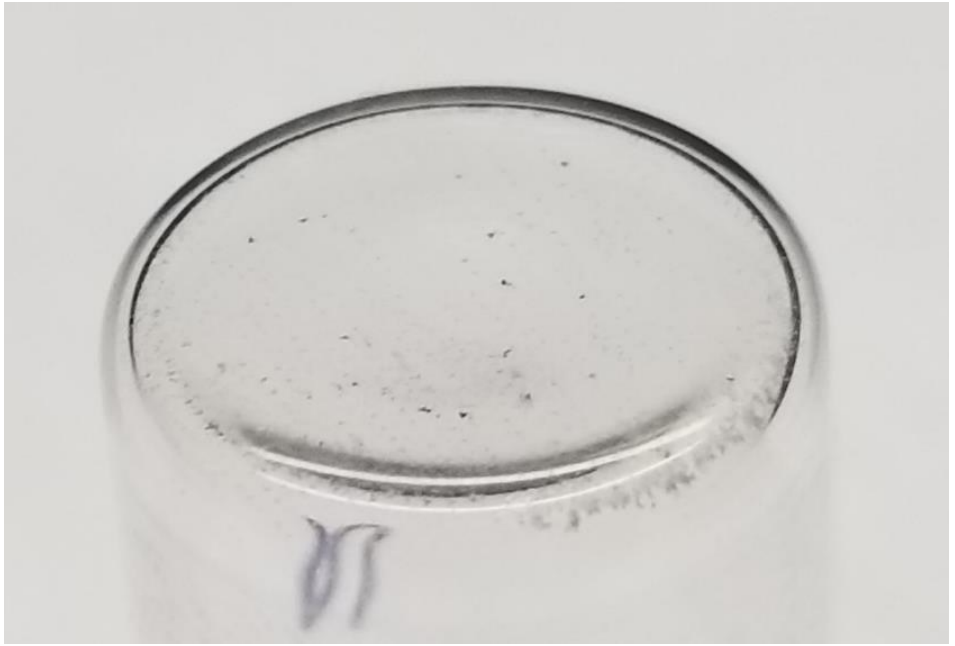




Washes and Pumps: Solvents

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

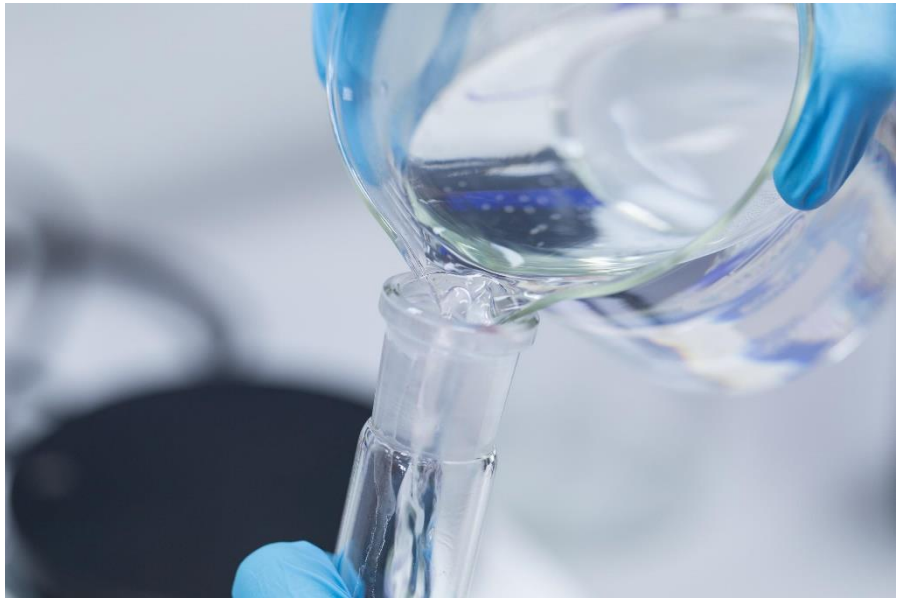
Choose a wash solvent(s) that make(s) sense for the analysis

- Is the analyte soluble in the solvent?
- Wash solvent = sample solvent when possible
- If using binary wash system make sure 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
  - 2nd wash vial will be cleaner than first
  - 2nd 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

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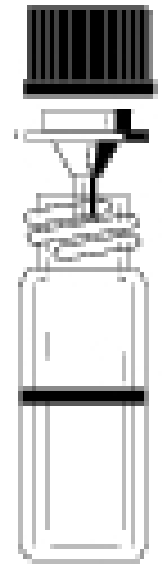
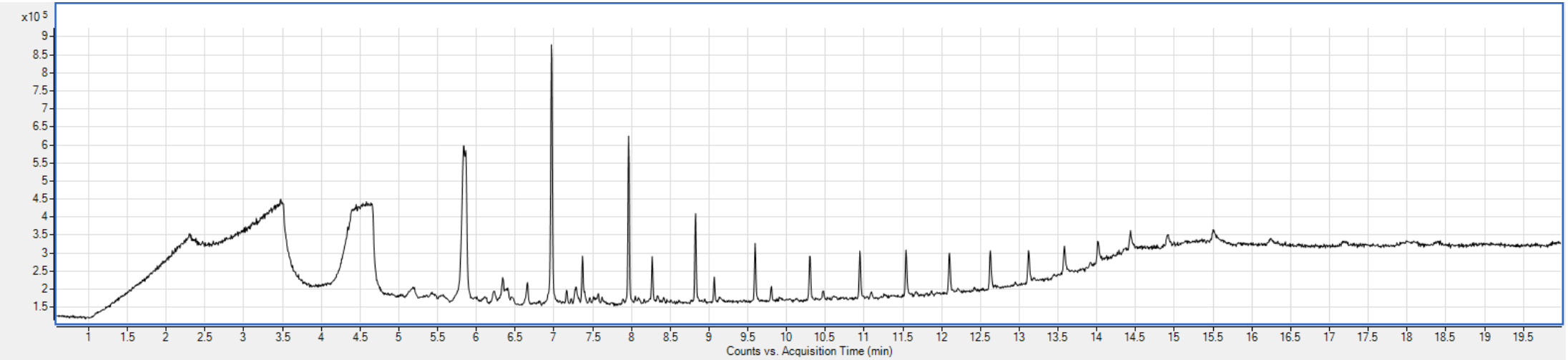
Washes and Pumps: Diffusion Caps

Diffusion caps are important

- Reduce volatile solvent diffusion
- Better alternative than using vial septa, which will 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

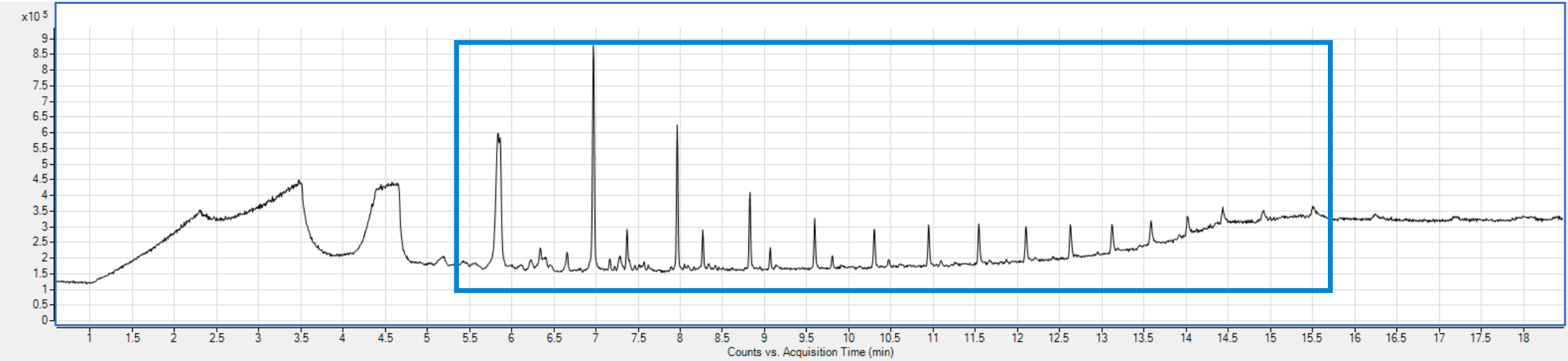
* Agilent silicone is platinum cured (versus peroxide cured), making it more inert and less likely to interact with samples.
** For up to 1 hour.

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

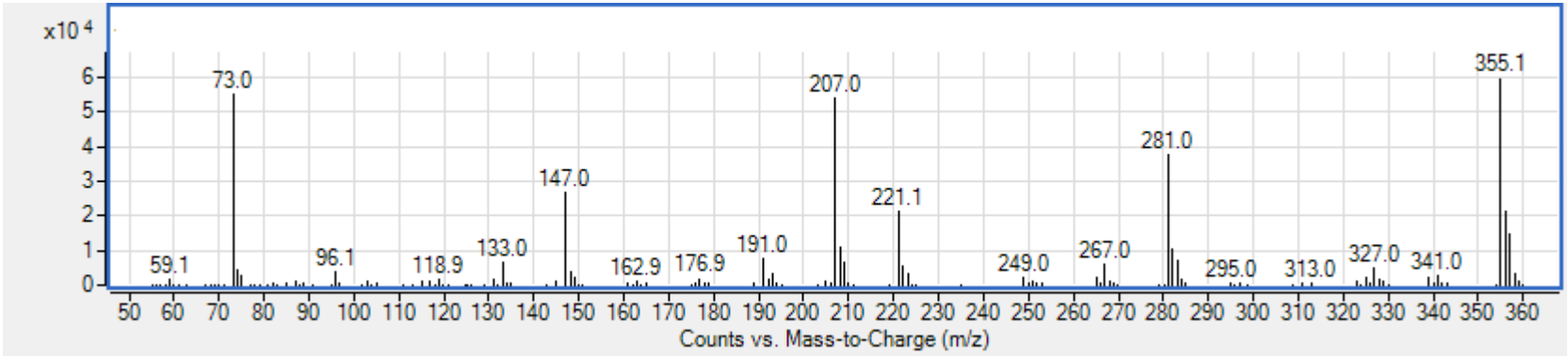
Septum maintenance: TIC of an inlet/vial septum



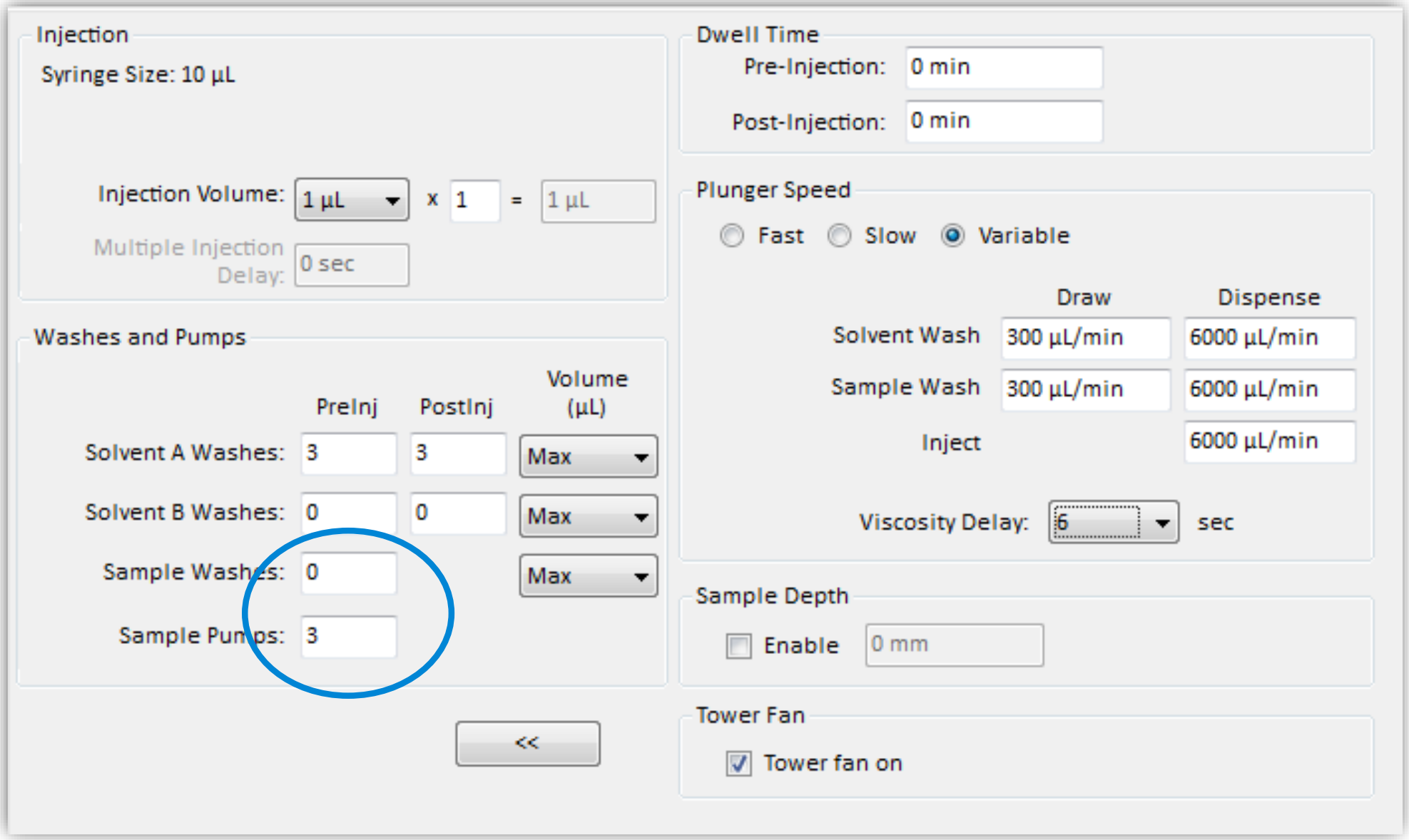
Common ions for siloxane molecules:
73
147
207
281
355

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:



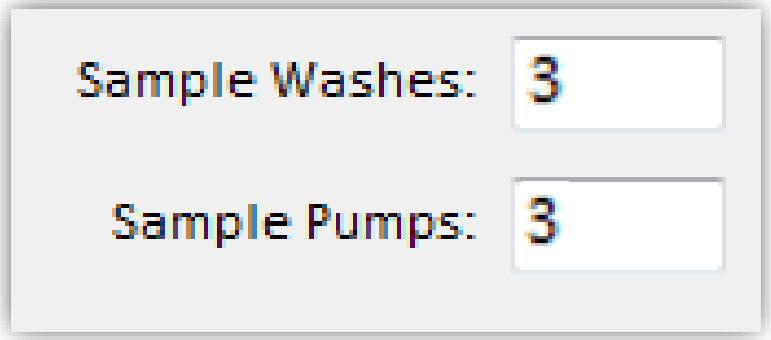
Sample Washes Vs Sample Pumps



Sample Washes Vs Sample Pumps

Sample washes

- Primes syringe barrel with sample, discards into waste bottle
- Improves reproducibility (reduces carry-over)
- ***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!***
  - 3–5 pumps is usually enough
  - ***Excessive pumping can reduce plunger lifetime!***

- Fill sample vial up to shoulder
- Leaving small headspace prevents cavitation, vacuum formation
- Improves reproducibility
- Do not over-tighten cap!
- Use microvial inserts to help assure good sampling depth for 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

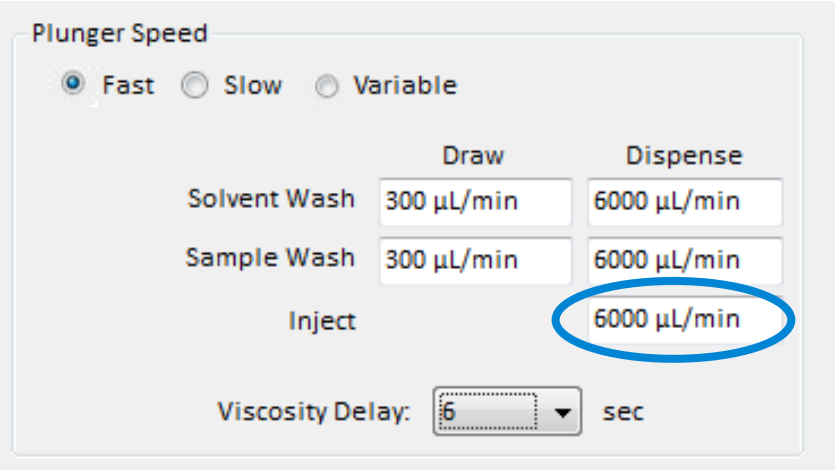
Sample Depth

- Recommend / default (3.6 mm from bottom of 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
  - Range is -2 mm to 30 mm

- Example uses:
  - Samples with 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

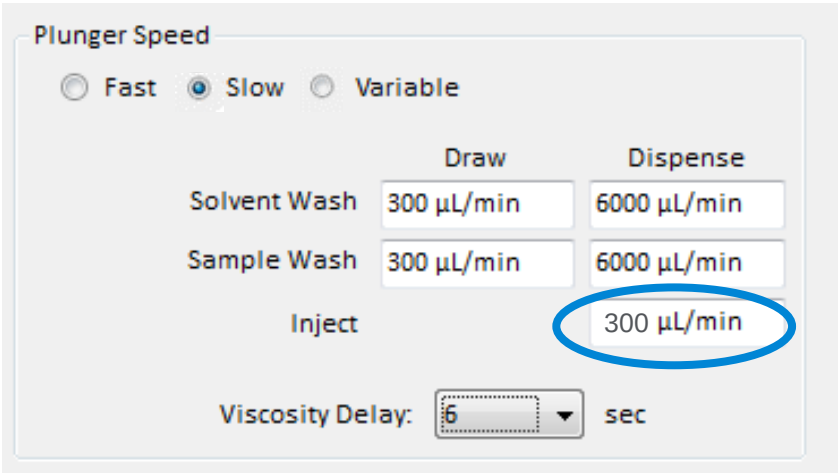
Plunger Speed

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

Slow

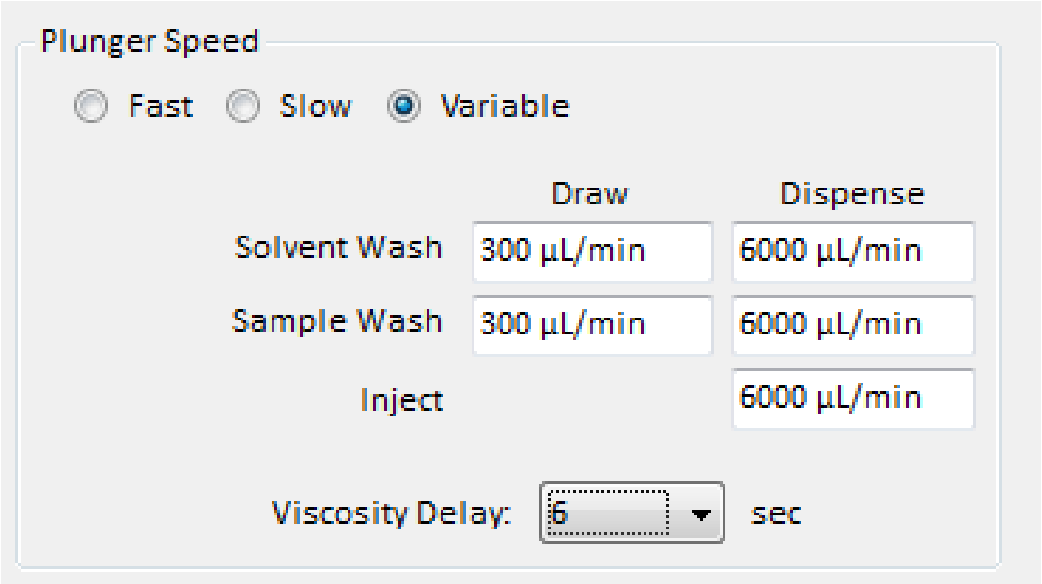


- Slow
  - Slows inject rate only (draw and dispense rates remain fast)
  - Use for COLD injection techniques (MMI/PTV/COC inlets)
  - Too slow → broad or split peaks for hot injection
    - Occurs when volatile compounds leave needle before plunger depressed

Viscosity Delay

Viscosity delay

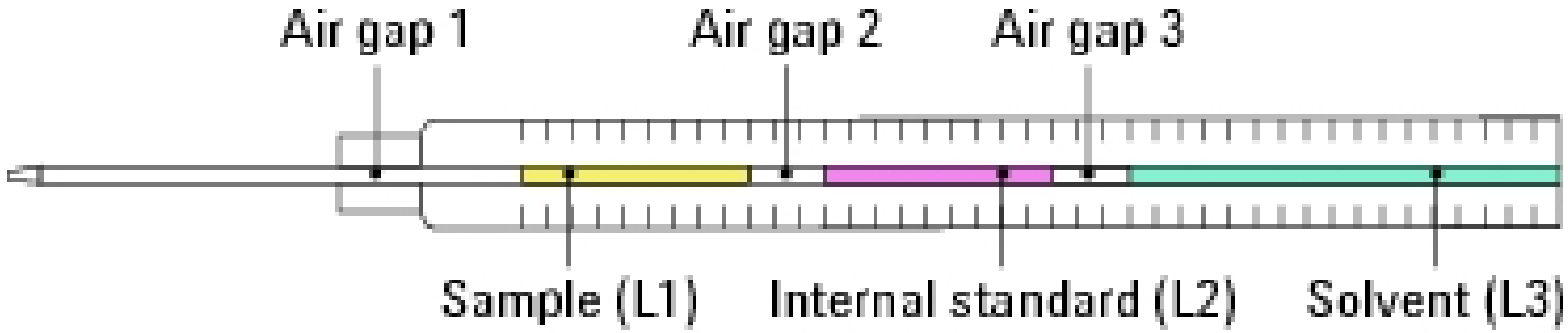
- Time (sec) plunger pauses between pump and injection
- Allows additional time for viscous samples to flow into syringe during pump
- Use for viscous solvents like isooctane
- Use for highly volatile solvents dichloromethane (to prevent cavitation/bubbles)
- A two-second viscosity delay can be beneficial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



Injection Types and Automated Sample Preparation (7693)

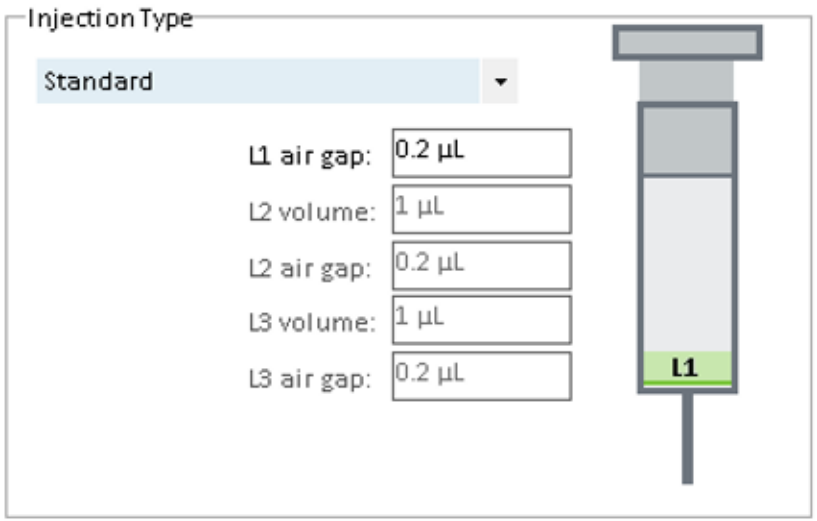
Injection types

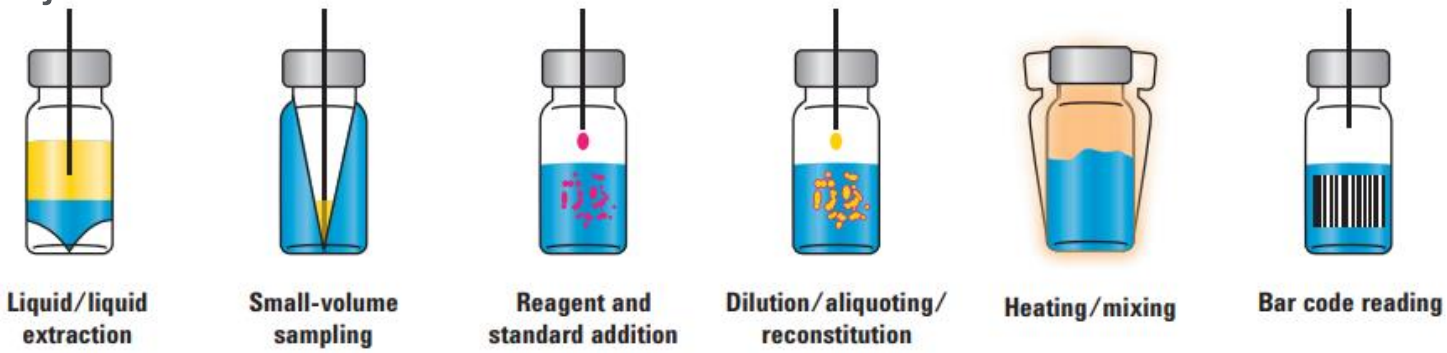
- Standard
- Sandwich injections
- Layered injections
- Multiple injections



Air gap

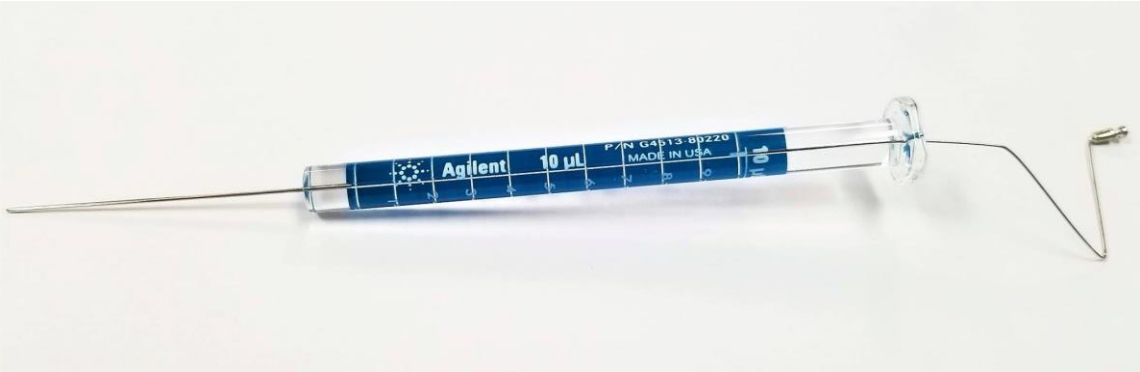
- 0.2 µL default
- Helps retain sample in syringe before injection

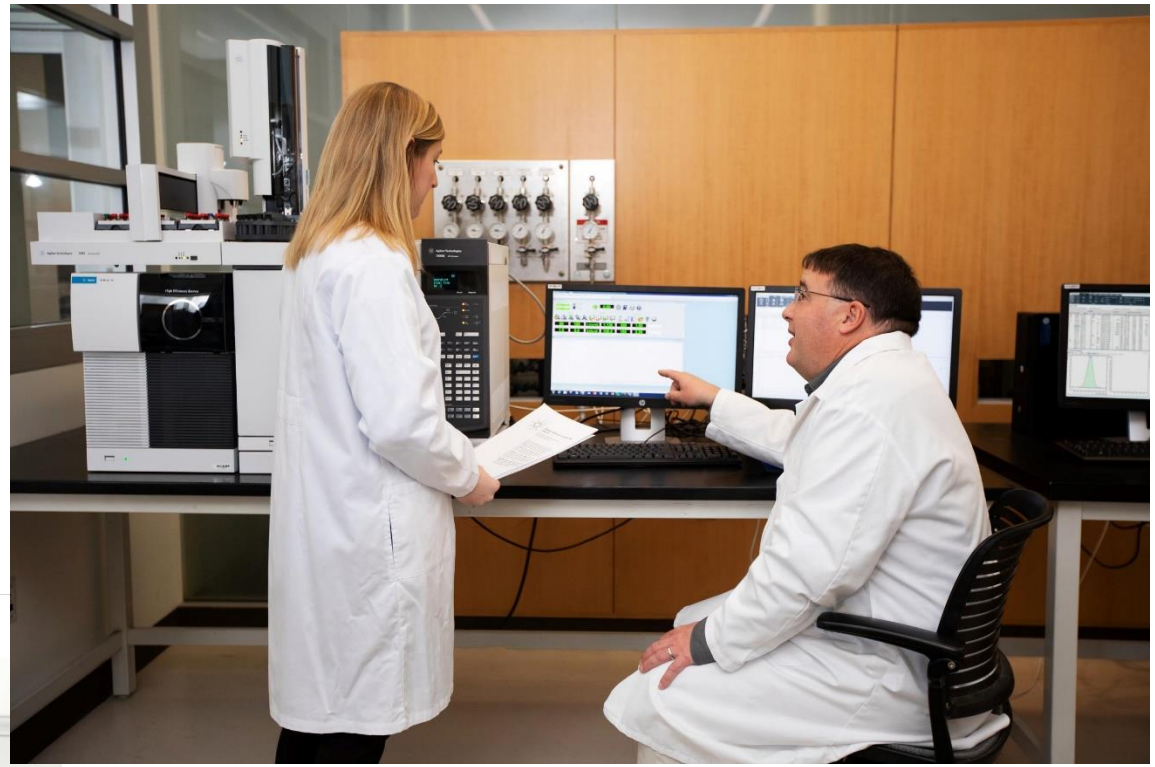


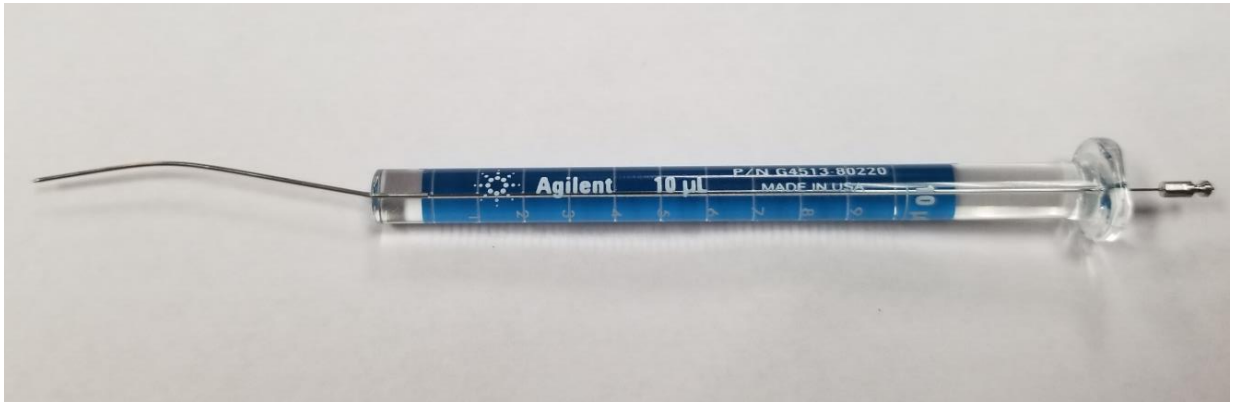


https://www.agilent.com/cs/library/applications/5991-7973EN.pdf

Troubleshooting







Troubleshooting

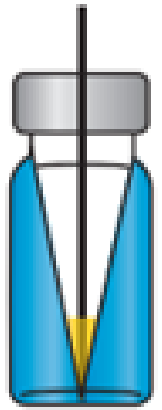
Problem: No peaks / Reduced Peaks

Possible cause(s):

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

Suggested action(s):

- 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

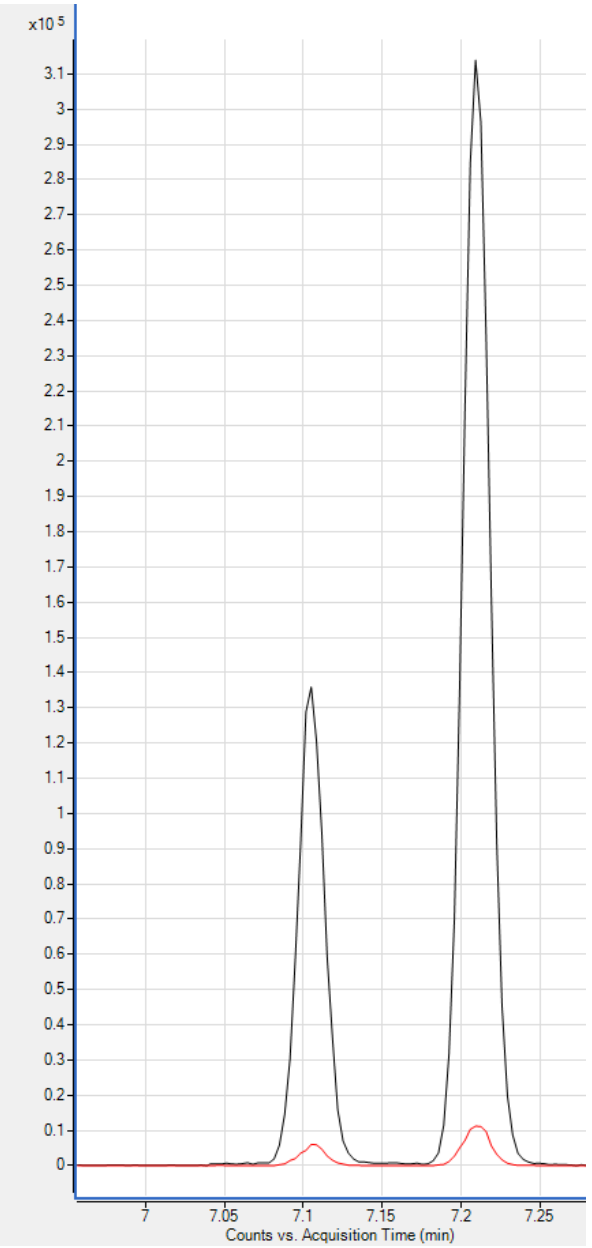
Possible cause(s):

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

Suggested action(s):

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

ALS needle guide- G4513-40525



Troubleshooting

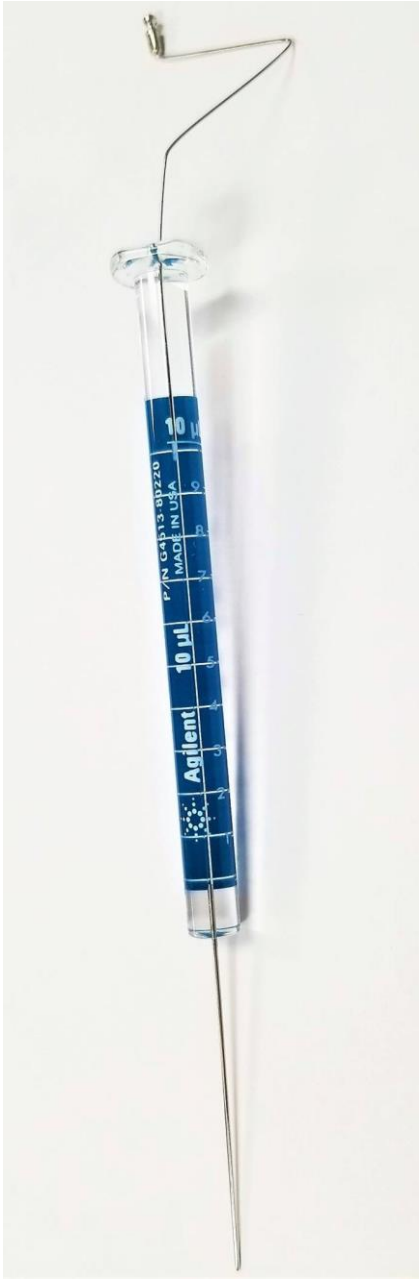
Problem: Bent Plunger or stuck syringe

Possible cause(s):

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

Suggested action(s):

- Clean up samples
- Switch to a syringe with PTFE-tipped plunger
- Avoid using 5 µL syringes where possible
- Clean syringe
- Never cycle the plunger in a dry syringe
- Do not “mix-and-match” plungers and barrels
- Immediately clean syringes after use



Best Practices – Plunger Binding

- Plunger binding is almost always sample matrix or solvent related (i.e. water or corrosives)
  - Plungers are perfectly matched to each barrel so it's 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 pre-wetting the syringe manually especially if the system has been sitting idle for some time 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 pre-cleaned syringe "at-the-ready" to save time
- Immediately clean syringes 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 cause(s):

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

Suggested action(s):

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

Troubleshooting

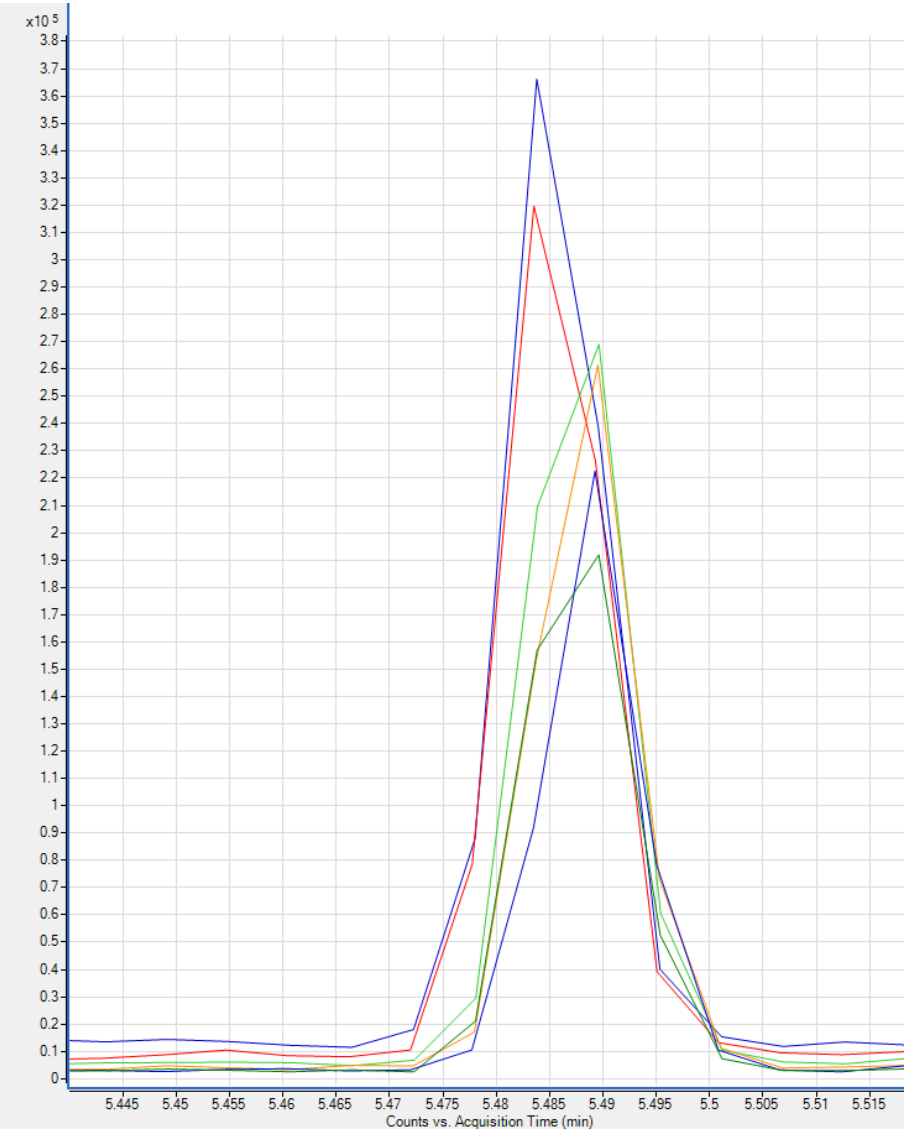
Problem: Poor reproducibility

Possible cause(s):

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

Suggested action(s):

- Clean or replace syringe
- “Restore” plunger tip (***PTFE only!***)
- Replace plunger (***PTFE only!***)
- 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



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 inlet quickly
- 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/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
- Option 5 for 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