Simplifying GC Syringes and Sample Introduction

Plunge deep into the world of syringes

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Sample Introduction

A complex process dependent on many variables.

Here are some of the keys to a successful injection:

- Minimal adsorption
- Narrow sample band
- Correct column installation
- Just enough (clean) sample
- Speedy injection
- No carryover
- No discrimination

“The Perfect Injection”
Anatomy of a GC ALS Syringe

**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.
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

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

Anatomy of a GC ALS Syringe
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**

- 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
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.
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
  - Solvent A Washed: 1
  - Solvent B Washed: 0
  - Sample Washed: 0
  - Sample Pumped: 0

- Plunger Speed (Variable)
  - Fast
  - Slow
  - Variable

- Draw
  - Solvent Wash: 300 µL/min
  - Sample Wash: 5000 µL/min

- Dispense
  - Injekt: 2500 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

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

Negative Effects

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

How To Avoid/Minimize

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

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

Liner capacity exceeded! Choose a liner of greater volume or modify method parameters.

Solvent Properties
- Water
- Boiling Point (°C): 100
- Density (g/cm³): 0.998
- Mol Wt. (amu): 18.02

Injection Liner
- 5183-4647 single-tapered 0°
- Liner Volume (µL): 650

Injection volume (µL): 1.00
Inlet Temperature (°C): 250
Inlet Pressure (gauge): 14.00

Estimated Volume: 1217 µL
% Capacity: 143%

Solvents
- Add
- Remove
- Defaults

Liners
- Add
- Remove
- Defaults

Units: kPa, psi, bar
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

Column overload = Fronting peaks

0.5 µL injection

Less injection volume = More Gaussian peaks
ALS Method Parameters

- **Injection**
  - Syringe Size: 10 µL
  - Injection Volume: 1 µL

- **Dwell Time**
  - Pre-injection: 0 min
  - Post-injection: 0 min

- **Sample Depth**
  - Enable: 0 nm

- **Washes and Pumps**
  - Preinj: 1
  - Postinj: 1
  - Volume (µL): Max

  - Solvent A Washes: 1
  - Solvent B Washes: 0
  - Sample Washes: 0
  - Sample Pumps: 1

- **Plunger Speed (Variable)**
  - Fast
  - Slow
  - Variable

- **Draw**
  - Solvent Wash: 800 µL/min
  - Sample Wash: 800 µL/min
  - Inject: 8000 µL/min

- **Dispense**
  - Viscosity Delay: 0 sec

- **Injection Type**
  - Standard:
    - L1 air gap: 0.2 µL
    - L2 volume: 1 µL
    - L1 air gap: 0.2 µL
    - L3 volume: 1 µL
    - L3 air gap: 0.2 µL
Washes and Pumps: Solvents

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

\[ \# \text{injections} \times \frac{(# \text{pre} + # \text{post}) \text{ washes}}{\text{injection}} \times \text{wash volume} = \text{wash solvent used} \]

> 2 mL

< 2 mL

4-mL fill volume

2.0 mL usable solvent volume

MINIMUM SOLVENT LEVEL

2-mL solvent remains

Injection
Syringe Size: 10 μL

Injection Volume: 1 μL x 1 = 1 μL

Multiple Injection Delay: 0 sec

Washes and Pumps

PreInj PostInj Volume (μL)
Solvent A Washes 3 3 5
Solvent B Washes 6 6 Max
Sample Washes 6 Max
Sample Pumps 1
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

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Toluene</th>
<th>Pentane</th>
<th>Methyl Ethyl Ketone</th>
<th>Methanol</th>
<th>Isopropanol (IPA)</th>
<th>Iso-Octane</th>
<th>Hexane</th>
<th>Heptane</th>
<th>Ethylene Dichloride</th>
<th>Ethyl Ether</th>
<th>Ethyl Acetate</th>
<th>1,4-Dioxane</th>
<th>Dichloromethane (DCM)</th>
<th>N,N-Dimethylformamide</th>
<th>Dimethyl Sulfoxide (DMSO)</th>
<th>1,1-Dimethylethanol</th>
<th>Chloroform</th>
<th>Cyclohexane</th>
<th>Acetone</th>
<th>Acetonitrile (ACN)</th>
<th>n-Butyl Alcohol</th>
<th>Acetone</th>
<th>1,4-Dioxane</th>
<th>Water</th>
<th>o-Xylene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Miscible</strong></td>
<td></td>
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<tr>
<td><strong>Immiscible</strong></td>
<td></td>
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</tbody>
</table>

- Miscibility Chart data includes various solvents and their miscibility statuses.
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
Choose high quality vials and caps

- Poorly constructed vial septa $\rightarrow$ siloxanes $\rightarrow$ bleed peaks
- Low quality vial $\rightarrow$ leach contaminants into sample
- Choose the right cap/septa for your solvent

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

* 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

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

Injection
Syringe Size: 10 μL

Injection Volume: 1 μL x 1 = 1 μL

Multiple Injection Delay: 0 sec

Washes and Pumps

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

Dwell Time
Pre-Injection: 0 min
Post-Injection: 0 min

Plunger Speed
- Variable

Draw
- Solvent Wash: 300 μL/min
- Sample Wash: 300 μL/min
- Inject: 6000 μL/min

Dispense
- Draw: 6000 μL/min
- Dispense: 6000 μL/min

Viscosity Delay: 5 sec

Sample Depth
- Enable: 0 mm

Tower Fan
- Tower fan on
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**
  - Solvent A Washed: 1 1 Max
  - Solvent B Washed: 0 0 Max
  - Sample Washed: 0 Max
  - Sample Pumped: 1

- **Dwell Time**
  - Pre-Injection: 0 min
  - Post-Injection: 0 min

- **Plunger Speed (Variable)**
  - Draw
    - Solvent Wash: 300 µL/min
    - Sample Wash: 5000 µL/min
    - Inject: 5000 µL/min
  - Dispense
    - 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 iso-octane
- 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

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
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)

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