PAL SYSTEM
Ingenious sample handling

Tips and Hints for PAL3 LC-Systems

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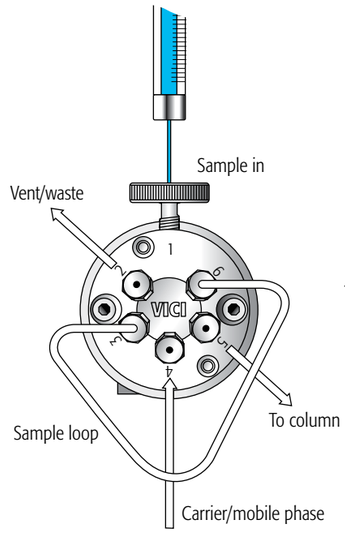
1. Valve Type and Characteristics

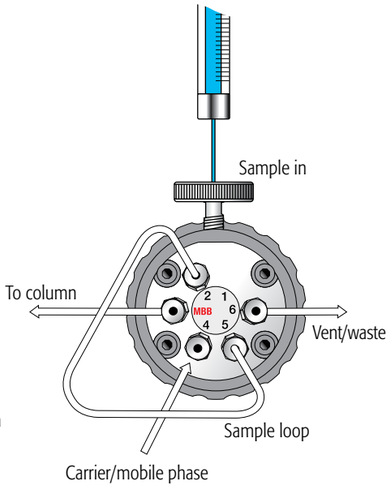
Valve PNo.	Rotor/Stator Spare Part No	Valve Characteristics	Application
PAL3-C2V-3006D-CTC-K	C2-30R6 / C2V-3C06	VICI Cheminert-Ventil 6-Port 0.75mm, 5000psi/340bar, inkl. Needle Guide, Needle Seal G19	Semi prep-analytical flow rates
PAL3-C2V-2006D-CTC-K	C2-20R6 / C2V-2C06	VICI Cheminert-Valve 6-port 0.40 mm, 5000 psi / 340 bar	Analytical flow rate
PAL3-C82VX-1676D-CTC-K	C72-16R6 / C72V-1C76	Cheminert Injection Valve, 6-port, 0.25mm, 15000psi, 1034bar	UHPLC valve for narrow bore columns
PAL3-C82VX-1676DCTC1-K	C72-16R6-CTC1 C72V-1C76-CTC1	VICI Cheminert-Valve 6-port 0.40 mm (port 1 - port 2) and 0.25 mm, 16000 psi / 1100 bar	UHPLC valve for narrow bore columns, for samples potentially containing particles or matrix contaminants
PAL3-C82VU-6676D-CTC-K	C72-66R6 / C72V-6C76	VICI Cheminert-Valve 6-port 0.15 mm, 18500 psi / 1275 bar	UHPLC valve for narrow bore and capilary columns

Listing is not complete. Only the most common valve types are listed. Other configurations are available.

2. Injection Valve Plumbing Diagram

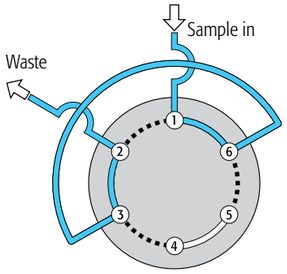


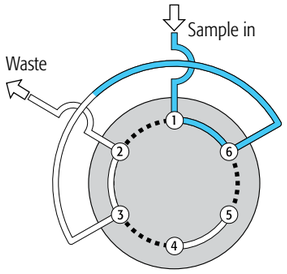
VICI/Valco Cheminert Type Valve



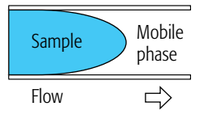
Rheodyne Type Valve

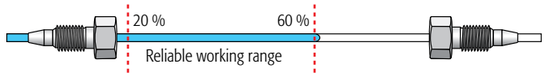
3. General Rules for Loop Filling





Graphic A





Full loop injection:

Overfill Loop 3 to 5 times.

Example 1: Small loop size: Loop 20 µl. Volume needed to fill loop 60 to 100 µl.

Example 2: Large loop size: Loop 200 µl. Use at least 300 µl to fill the loop

Partial loop filling:

Reliable working range: 20 to 60 % of loop content.

Example: Small loop size: Loop 20 µl. 4 to 12 µl sample volume.

Loops with larger volume can be filled within a range of 20 to max. 80 % of loop content (200 µl or larger).

Disregarding the rules will result in poor repeatability.

The reason for these rules is the principle of hydrodynamic flow patterns in the solvent front reaching the loop inlet and outlet. See graphic A, left

Injection speed:

Example for a 20 µl loop: Injection speed is 5 to 10 µl/s.
Higher speed will cause turbulence in the loop, resulting in poor repeatability.

Injection speed is a PAL method parameter and must be adjusted for the type of solvent (viscosity and boiling point), loop size, or rather loop internal diameter, and the valve bore size.

See the recommended method parameters listed in the PAL Firmware software overview.

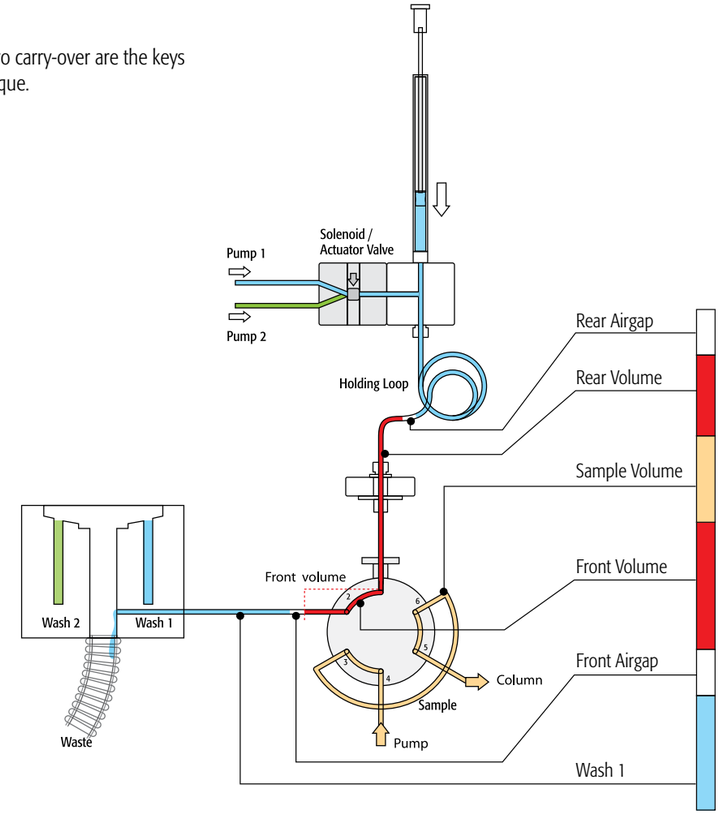
Examples:

Loop volume [µl]	Injection volume [µl]	Injection speed [µl/s]
20	80	10
20	10	1
2	8	5
2	1	1

The injection technique (Partial Loop, Overfill) is not a setting for injection procedure, but results from the ratio between loop volume and the Injection volume which is defined in the work list of the CDS.

4. Injection Technique Using the LC/MS Tool & Low Volume Injection Parameter Values:

Fast cycle time, high reproducibility and near zero carry-over are the keys for the LC/MS Tool injection and washing technique.



PAL Sample Control Method: LC Injection LC/MS Standard General

Parameter	Full Loop	Partial Loop
Sample Volume [µl]	80 (8)*	10 (1)*
Rear Air Gap [µl]	3	3 (1)*
Front Air Gap [µl]	3	3 (1)*
Rear Volume [µl]	5	5
Front Volume [µl]	5	5
Fill Speed [µl/s]	5	5
Inject Speed [µl/s]	5	5 (1)*
Pre Inject Delay [ms]	500	500
Post Inject Delay [ms]	2000	2000
Pullup Delay [ms]	2000	2000
Post Clean with Solvent 1	1	1
Post Clean with Solvent 2	1	1
Valve Clean with Solvent 1 [µl]	80	80
Valve Clean with Solvent 2 [µl]	80	80
Stator Wash	Off	Off
Clean Valve Flow Rate [µl/s]	20	20

* Numbers in brackets are valid for 2 µl loop size only, all other numbers are valid for both loop sizes.

5. Tubing Internal Diameter versus Flow Rate

The tubing internal diameter must be adjusted to flow rate, valve type, and application to avoid high backpressure or chromatographic irregularities.

Tubing ID:
Points to consider are:

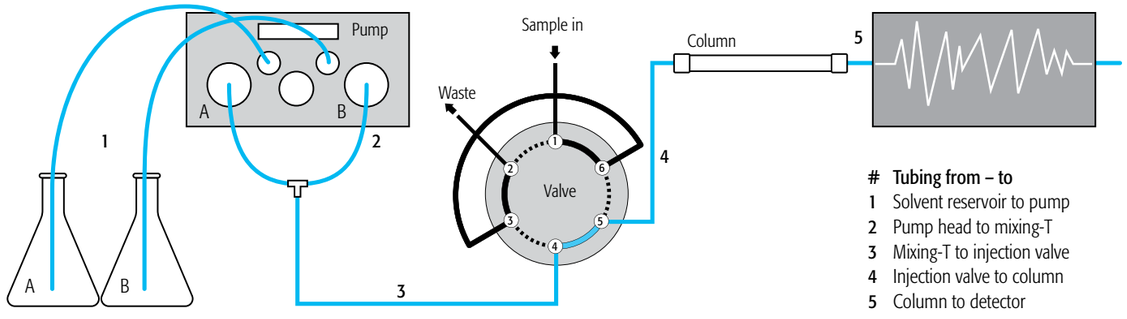
- Delay volume of entire HPLC System
- Time needed for gradient to go active at column inlet
- Adjust tubing diameters and length:
  - Solvent reservoir to pump: Cavitation?
  - Valve to column: ID as small as possible, considering backpressure
  - Column to detector: ID as small as possible
  - (if possible, smaller than tube valve to column, consider backpressure)

Tubing Internal Diameter versus Tubing Volume
Examples are calculated for a tube length of 100 mm.

Tubing [inch]	Tubing [mm]	Tubing volume [ml]
0.040	1.00	78.55
0.020	0.50	19.64
0.010	0.25	4.91
0.005	0.13	1.33
0.0025	0.064	0.32

Valve Type Bore size [mm]	Flow Rate Range from - to	Tubing ID [inch] / [mm]	Tubing from - to # see diagram below
0.75	5 to 100 ml/min Preparative application	0.25 / 6.35 0.040 / 1.0	1: Reservoir 2-5: Same ID for entire HPLC system plumbing to avoid backpressure
0.40	0.5 to 5 ml/min Standard HPLC Column ID 4 mm	0.125 / 3.18 0.020 / 0.50 0.010 / 0.25	1: Reservoir 2-3: Pump to Valve 4-5: Valve to Detector
0.25	10 to 500 µl/min Standard HPLC Column ID 1 to 2 mm	0.040 / 1.0 0.010 / 0.25 0.005 / 0.13	1: Reservoir 2-3: Pump to Valve 4-5: Valve to Detector
0.15	100 nl to 100 µl/min Micro flow application	0.020 / 0.50 0.020 / 0.50 0.005 / 0.13 0.005 / 0.13	1: Reservoir 2: Pump Head to Mixing–T 3: Mixing–T to Valve 4-5: Valve to Detector
0.10	10 nl to 10 µl/min Nano flow application	0.020 / 0.50 Fused silica 50 µm Fused silica 50 µm Fused silica 25 µm	1: Reservoir 2: Pump Head to Mixing–T 3-5: Mixing–T to Detector flow rates > 1 µl/min 3-5: Mixing–T to Detector flow rates < 1 µl/min

Data for Tubing IDs are recommendations only. Variations depend on the application, mobile phase, flow rate, column ID and sample load.



#	Tubing from – to
1	Solvent reservoir to pump
2	Pump head to mixing-T
3	Mixing-T to injection valve
4	Injection valve to column
5	Column to detector

6. Needle Guide, Needle Seals and Valve Needle Guide

6.1 Available Needle Guide Types:

PAL3-LowNdlGdeRepl

Needle Guide without needle transport magnet for PAL3 Tools D7, D8, LC/MS, Dilutor. Without nut for SPME-Fibcond valve. Needle Guide Type installed in the Dilutor Tool and the LC/MS Tool. The absence of magnets prevents possible corrosion issues.

PAL3-LowNdlGdeReplMG

Default Needle Guide used for GC

PAL3-LowNdlGdeCntMG

Default Needle Guide used for µSPE. It provides most reliable transport performance for 2 ml vials and µSPE cartridges.

Needle guides are held in place using a single screw and can therefore be easily exchanged.

PAL3-MagnetAdap-20mL

Can be attached to the Needle Guide for transporting 10 or 20 ml vials.

6.2 Available Needle Seal Types for Gauge 22 Needles

PAL3-NdlSeal-2

PAL Seal gauge 22 for Valco and Rheodyne valves Compatible with Needle Guide
PN: PAL3-LCInj-G22-2

6.3 Available Valve Needle Guide for Gauge 22 Needles

PAL3-LCInj-G22

Standard Valve Needle Guide for Rheodyne/ Vici valves, Gauge 22
Compatible with Needle Seal PN: PAL3-NdlSeal and PAL3-NdlSeal-2

PAL3-LCInj20mL-G22

Needle Guide for Rheodyne/Vici valves, Gauge 22
Designed for tools with adapter for 20 ml magnet or foil cutter
Compatible with Needle Seal PN: PAL3-NdlSeal and PAL3-NdlSeal-2

6.4 Syringes with Gauge 19 Needles

Please note that special needle seals and special valve needle guides are required for gauge 19 needles.

PAL3-NdlSeal-19

Needle seal gauge 19 for Valco valve
Compatible with needle guide PN: PAL3-LCInj-V-G19

PAL3-LCInj-V-G19

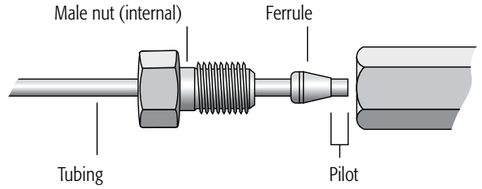
Standard Needle Guide for Vici valves,
Gauge 19

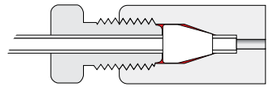
6.5 Teaching Accuracy of Valve Needle Guides

Accurate position teaching of the Valve Needle Guide is critical for a reliable instrument performance. After the change of the injection valve a check of the teaching is recommended.

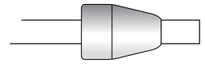
7. Nuts and Ferrules

- Do not use a nut or a ferrule from a vendor other than specified for the product
- While tightening the nut, keep tubing tightly positioned to ensure correct pilot distance.
- Do not over-tighten the nut / ferrule.
- Do not reuse an installed nut / ferrule for any other connection.
- Eliminate trapped air by installing nut / ferrule into wetted ports only.

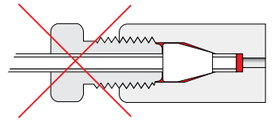




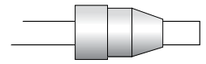
Tubing seats correctly at the bottom



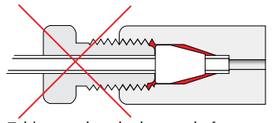
VICI Valco



Tubing doesn't reach the bottom, introducing dead volume



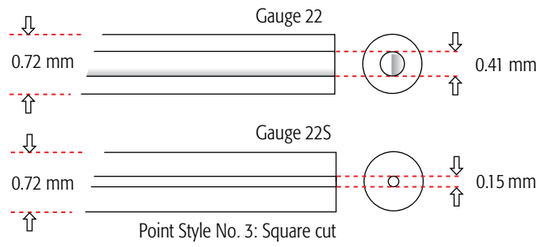
Rheodyne



Tubing reaches the bottom before ferrule seats

8. Syringes and Needles

Syringe Needles / Standard Needle for HPLC Technique:



Needle Gauge 19: OD 1.04 mm. Mandatory for prep valve with bore size 0.75 mm. (Needle with Gauge 22 fits into valve bore.)

Needle gauge versus fill speed and Needle volume

Needle gauge	Fill speed[1)]	Time to fill a syringe	Needle volume[2)]
Gauge 22S	5 to 10 µl/s	10 to 20 s	0.9 µl
Gauge 22	200 µl/s	0.5 s	6.73 µl

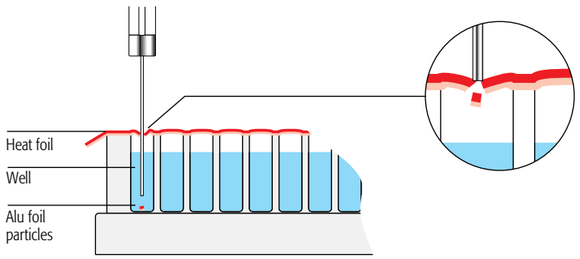
Example: 100 µl syringe

1) Maximum fill speed before cavitations are observed.
Fill speed example with solvent water / methanol (1:1)
2) Needle volume for 51 mm standard needle

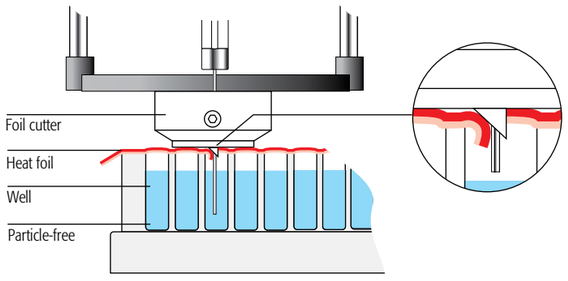
Information on PAL Smart Syringes

9. Microtiter / Deepwell Plates and the Piercing of Foils

For well plates with sealing foils and mats please use foil cutter.



Foil cutter: Article No.: PAL3-FoilCutter
For the piercing of sealing foils and mats on well plates.



10. Repeatability & Carry-Over Troubleshooting

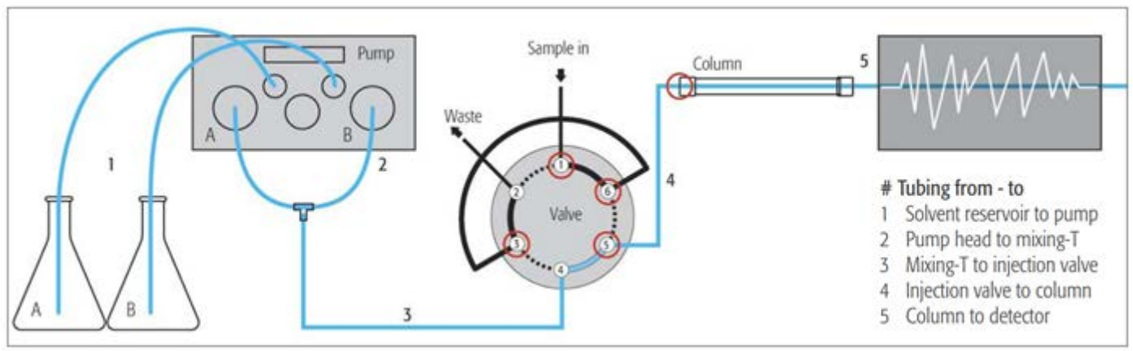
What is carry-over?

Carry-over is the appearance of an analyte signal in blank sample after the analysis of samples with a high(er) analyte concentration. It is often quantified as a certain percentage of the Upper or Lower Level of Quantitation (ULOQ, LLOQ). The carry-over is compound and method dependent.

Physical carry-over:
Dead volumes caused by bad connections between tubing and fittings.
Scratches on rotor/stator of valves.
Generally badly flushed volumes (cavities).

Sorptive carry-over:
Chemical adsorption of molecules to surfaces of tubings, loops, injection needles, or valves.
Sample adsorption to the column's stationary phase or inner surfaces.
Solvent contaminants concentrated on and released from the column during a gradient run.

Please note that the PALSystem is only one part of a complete chromatography system. Therefore, it is necessary to include all critical parts of the chromatographic system in the trouble shooting. Dead volumes caused by bad connections between tubing and fittings are a critical source for carry- over (marked in red in the picture below):



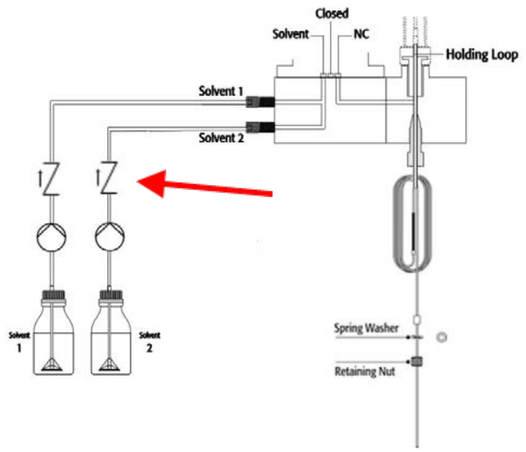
10.1 Carry-over and Repeatability Troubleshooting, Important Points to Check

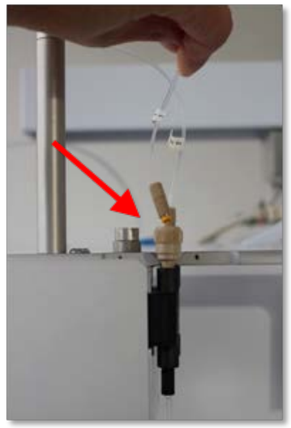
	Influence on carry over	Influence on repeatability	Remarks
Wash solvents			
Does the wash solvent match?	!	!	What is the composition of the analytical sample solvent? Is the matrix soluble in the wash solvents? What is the composition of the mobile phase? Avoid using 100 % water as wash solvent. Avoid using immiscible wash solvents Example of wash solvent combination: Wash 1: 95 % water, 5 % acetonitrile, 0.1 % formic acid Wash 2: 100 % acetonitrile, 0.1 % formic acid Wash steps for biological samples: Wash 1: aqueous Wash 2: organic Reset the system to aqueous
Valve system			
Is the valve intact?	!	!	- Remove Rotor seal and check visually - Check if the grooves of the Rotor Seal are flat or showing sign of wear

	Influence on carry over	Influence on repeatability	Remarks
Is the valve clogged?	!	!	Remove Stator and Rotor and check for clogged ports - Check if the grooves of the Rotor Seal are showing sign of wear - Replace Rotor Seal if necessary - Replace complete valve if the stator is showing sign of wear or clogging cannot be removed
Valve needle seal	!	!	Are any leaks observed? Check by manually injecting wash solvent with a syringe or Prime LC-Tool with ≥10 filling strokes. Replace the needle seal.
Is the valve waste line open?	!	!	Check by manually dispensing wash solvent with a 100µL syringe into the injection port. Make sure the inner diameter of the waste line is >0.5mm
Is the valve configured correctly			Test Active/Load position and Stand-By/Inject position, by manual injection of liquid without loop installed on valve. See following section Injection valve check
Is the backpressure at normal level?	!	!	
Is the Valve Type (dimensions) appropriate for the flow rate?	!	!	
Sample loop installed on injection valve		!	Deformed sample loop or transfer lines => Replace sample loop or transfer line
LC/MS Tool / Syringe			
Is the tubing free of gas bubbles and filled with liquid?	!	!	Prime the LC/MS Tool from the Terminal, Options, Prime LC-Tool.
Verify if the check valves are installed on the pumps	!	!	See following section Installation of check valves on LC/MS-Tool pumps
Verify if check valves are clogged	!	!	Remove the wash line from the Check-Valve side ▪ Remove the Check Valve ▪ Aspirate syringe from Check-Valve Kit with wash solvent ▪ Attach the Check-Valve to the syringe in flow direction (use the adapter from the kit) ▪ Dispense liquid through the check valve (be careful: liquid can spill) ▪ Repeat for second Check-Valve
Syringe	!		Is the plunger tight? Is the syringe intact? Check manually the resistance of the plunger.
Solvent frits	!	!	Check if solvent frits are restriction free. Replace or clean in ultrasonic bath if necessary.
Wash pump	!	!	Wash Pump does not deliver required amount of liquid. See following section LC/MS tool pump test procedure. If flow rate is too low, call qualified Service Engineer.
Nuts and ferrules			
Are only matched nuts and ferrules used?			Are the tubings cut square and are they open without any restriction? Replace if necessary. See also section "Nuts and Ferrules"
Are all connections made correctly without dead volume or leak?			See also section "Nuts and Ferrules"
Loop injection			
			Full loop injection: Is the loop overfilled 3 to 5 times? Partial loop filling: Is the rule of 20 to 60% of the loop content applied? Consider also the valve volume. The filling percentage of the loop is defined by the ration between the injection volume in the sample list and the valve loop volume.
PAL method parameters			
Sample Vial Depth	!	!	Needle penetration depth into the vial measured from top of the vial cap. Or needle penetration depth into the plate. The set value is ignored with enabled bottom sensing feature. Verify if the needle tip is immersed in the sample during liquid aspiration or activate Bottom Sensing

	Influence on carry over	Influence on repeatability	Remarks
Sample Aspirate Flow Rate	!	!	Sample aspiration speed. Critical for good accuracy and precision. Example: 5 µl/s for 1uL aspiration, 10 µl/s for 80 µl aspiration. Set 5 µl/s or a lower value for all volumes when liquid monitoring is enabled.
Pullup delay			Verify if there is a minimum pullup delay of 1second. Higher viscosity sample needs longer pullup delay time.
Inject Sample Flow Rate	!	!	Sample injection speed. Critical for good accuracy and precision. Example: 1 µl/s for 1µl injection, 5 µl/s for 20 µl injection.
Valve Clean Solvent 1	!	!	Cleaning of the LC/MS Tool and the injection valve with wash solvent 1
Valve Clean Solvent 2	!	!	Cleaning of the LC/MS Tool and the injection valve with wash solvent 2
Post clean solvent 2	!	!	Cleaning of the LC/MS Tool and its needle (outside) into the wash station with wash solvent 2
Post clean solvent 1	!	!	Cleaning of the LC/MS Tool and its needle (outside) into the wash station with wash solvent 1
Stator wash	!		Stator wash is a post cleaning option which rinses sections in the injection valve that are not in contact with wash solvent during the regular post cleaning. To enable stator wash two steps are necessary: 1) Set Stator wash to ON 2) Define the time after the injection when the stator wash has to be executed: Stator wash delay This wash process has to be timed with the chromatography because it will load wash 1 onto the analytical column. Consequently, it is recommended to execute this wash step after all peaks of relevance have reached the detector. The amount of wash 1 which is loaded onto the column is defined by the size of the loop installed on the injection valve.
HPLC coulmn			
The analytical column is dirty or has reached the end of lifetime	!	!	Replace the column
Is the guard column clean/ replaced?			
Sample concentration			
Sample concentration is too high for the detector or analytical column	!	!	Reduce sample concentration (dilute)
HPLC system parameters			
Is the selected equilibration time long enough?		!	General rule is to flush the column/trap 5 to 10 times of column volumes with the starting condition of the gradient.
Column carry over	!		Verify for incomplete desorption of material from a column during a gradient separation.
Detection & integration			
Is the peak detection and integration verified?			Peak tailing? Baseline assignment? Is the peak detection and integration verified? S/N ratio? Area rejected? Peak slope detection?
Is the signal within the dynamic linear range of the detector?			Linearity?

11. Installation of Check Valves on LC/MS Tool Pumps





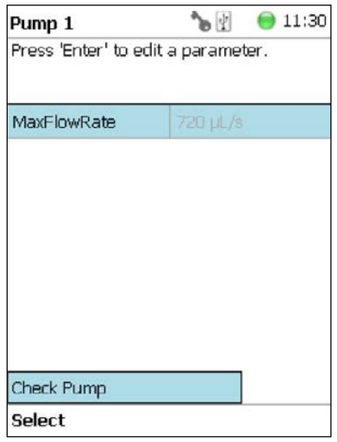
12. LC/MS Tool Pump Test Procedure

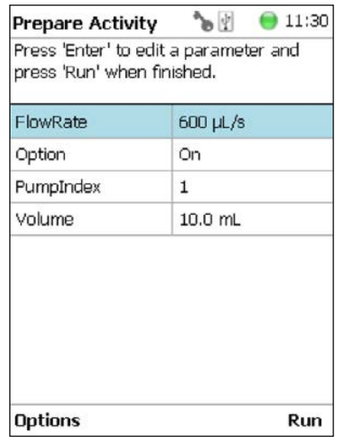
12.1 Test LC/MS-P Pump Module Pump Only

Remove the wash line from the side-port of the Tool
Hold the wash line inside a graduated cylinder
Options/Setup/Pump Modules/Pump n (Pump Module) ➔ Check Pump
Choose a default flow rate of 600 µl/s and 7 ml
Measure if the volume in the cylinder is 7 ml +/- 20 %

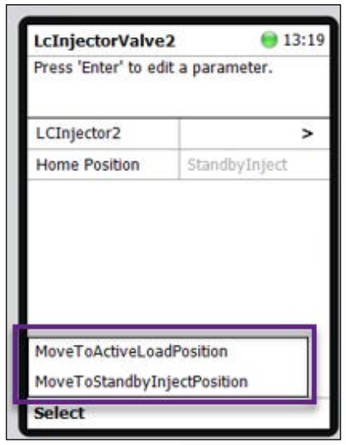
12.2 Test LC/MS-P Pump Module through Tool

Teach the waste position above a graduated cylinder
Options/Setup/Pump Modules/Pump n (Pump Module) ➔ Check Pump
Choose a default flow rate of 600 µl/s and 7 ml
Measure if the volume in the cylinder is +/- 20 %





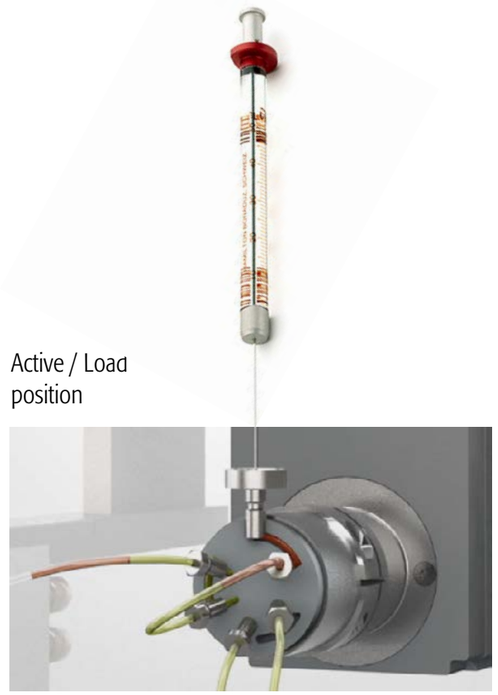
13. Injection Valve Check



It is recommended to remove the waste line and the loop (port 2 and 6) for this test.

- Fill the syringe with water or appropriate wash solvent and insert it into the injector.
- Manually dispense liquid into the valve.
- Observe whether the liquid flows to the correct port.

Stand-by / Inject position



Active / Load position

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