Tips and Hints

Tips and Hints for PAL Systems including general technical HPLC information

1. Valve Type and Characteristics

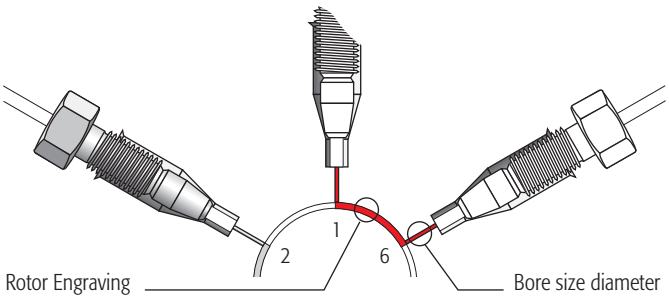
Valve P/N	Nr. of Ports Valve Type Vertical Port	Valve Bore Size in mm	Tube Connect. in inch	Stator P/N Material	Rotor P/N Material	Flow Rate Range	Remarks
C2V-3006-CTC-K	6 C Vertical Port	0.75	1/16	C2V-3C06 sst	C2-30R6 Valcon H	5 to 100 ml/min	Preparative application Bore size requires needle Gauge 19
DC6WK-CTC-K	6 W Vertical Port	0.40	1/16	na sst	SSAC6W Valcon H	0.5 to 5.0 ml/min	Valve for standard HPLC application. Column ID 4 mm, Flow 1ml/min 10 Port Valve available
C2V-2346D-CTC-K	6 C Vertical Port	0.40	1/16	C2V-2C46 PAEK	C2-23R6 Valcon E	0.5 to 5.0 ml/min	Biocompatible Valve for standard HPLC flow rates 10 Port Valve available
DCI4WK.5/1-K	4 W Vertical Port	0.40	1/16	na sst	SSACI4W.5/1 Valcon H	0.5 to 5.0 ml/min	4-Port Valve with Internal Loop Rotor interchangeable, Volumes 0.5 and 1.0 µl
PD7991	6 R Vertical Port	0.28	1/16	Ti-plated with SPC-1	PEEK blend RPC-10	10 to 500 µl/min	Valve for high pressure use 9000 psi / 600 bar
C2V-1006D-CTC-K	6 C Vertical Port	0.25	1/16	C2V-1C06 sst	C2-10R6 Valcon H	10 to 500 µl/min	Valve for Semi-Micro Columns ID 1 and 2 mm 10 Port Valve available
C2V-1346D-CTC-K	6 C Vertical Port	0.25	1/16	C2V-1C46 PAEK	C2-13R6 Valcon E	10 to 500 µl/min	Biocompatible Valve for Semi-Micro Columns ID 1 and 2 mm 10 Port Valve available
C2-0006D-CTC-K	6 C No Vertical Port	0.15	1/16	C-0C06 sst	C2-00R6 Valcon H	100 nl/min to 100 µl/min	Injection Valve for Micro Columns. Requires special bracket (P/N: PAL VHldr60)
C2-0346D-CTC-K	6 C No Vertical Port	0.15	1/16	C-0C46 PAEK	C2-03R6 Valcon E	100 nl/min to 100 µl/min	Biocompatible Injection Valve for Micro Columns. Requires special bracket (P/N: PAL VHldr60)
CN2-4346D-CTC	6 C No Vertical Port	0.10	1/32	CN2-4C46I PAEK	CN2-43R6 Valcon E	10 nl/min to 10 µl/min	Biocompatible Valve for column switching only. Stator sst. wetted parts PAEK

Key and Remarks to the Table "Valve Type and Characteristics":

P/N:
- "CTC": Specific modifications for CTC Analytics.
- "-K": indicates a kit: Containing Nuts, Ferrules, Needle Guide, Needle Seals and Teflon Waste Tubing

Valve Type:
- W-Type: Conical Rotary Valve, VICI / Valco
- C = Cheminert-Type : Flat Plate Rotary Valve, VICI / Valco
  Plumbing of the two different valve types are shown below.
- R = Rheodyne, Flat Plate Rotary Valve.

Bore Size: Diameter of inlet/outlet path of the valve connection port.
See graphic below.



Stator: sst = stainless steel, N60 (corresponds to sst 316)
PAEK Polymer related to well known PEEK material.

Rotor Material:
- Valcon H: Reinforced carbon fiber composite PTFE lubricated inert engineering polymer.
  Standard material from VICI/Valco.
- Valcon E: Polyaryletherketone/Teflon (PAEK/Teflon).
  Material if biocompatibility is required.
- Valcon T: Polyimide (Vespel)/PTFE/Carbon composite.
  A choice if high temperature is needed.

For material specific information and limitations consult VICI/Valco product information bulletin.

Pressure Rating:
- W-Type: 5000 psi / 340 bar.
- Cheminert Type: 5000 psi / 340 bar.
- Rheodyne PD 7991: 9000 psi / 600 bar

2. Valve Type, Bore Size versus Valve Volume

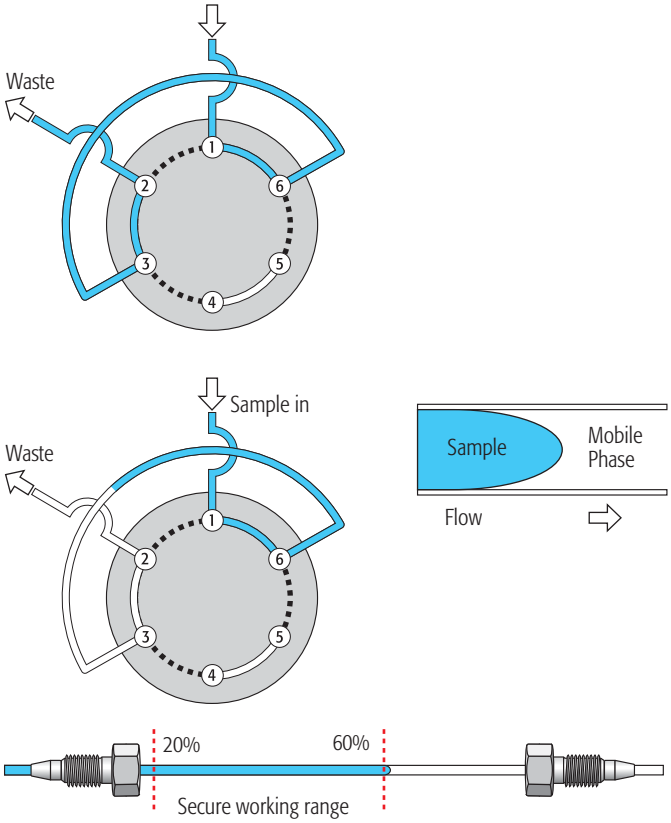
Valve Volume specification:
Loop Overfill (Full Loop): 2 Ports and 1 Engraving
Partial Loop Filling: 1 Port and 1 Engraving

Sample Loss at Injection, Valve Contribution (not overfill volume)
Loop Overfill (Full Loop): 2 Ports and 1 Engraving
Partial Loop Filling: 1 Port. Content of Engraving is injected.

Valve Bore Size and Valve Type	Volume for Port Gravure		Valve Volume for Loop Overfill	Valve Volume for Partial Loop
0.75 mm Cheminert, sst	Port: Gravure:	2725 nl 1173 nl	6623 nl	3898 nl
0.40 W-Type, sst	Port: Gravure:	155 nl 390 nl	700 nl	545 nl
0.40 mm Cheminert, sst	Port: Gravure:	220 nl 170 nl	610 nl	390 nl
0.40 mm Cheminert, PAEK	Port: Gravure:	345 nl 170 nl	860 nl	515 nl
0.25 mm Cheminert, sst	Port: Gravure:	75 nl 70 nl	220 nl	145 nl
0.25 mm Cheminert, PAEK	Port: Gravure:	130 nl 70 nl	330 nl	200 nl
0.15 mm Micro Cheminert, sst	Port: Gravure:	30 nl 40 nl	100 nl	70 nl
0.15 mm Micro Cheminert, PAEK	Port: Gravure:	40 nl 40 nl	120 nl	80 nl
0.10 mm Nano Cheminert, PAEK	Port: Gravure:	8 nl 13 nl	29 nl	21 nl

All values are theoretical calculated values. Values can be changed without notice. Values provided by VICI/Valco.
Rheodyne PD 7991: Valve Volume for Loop overfill 500 nl.

3. General Rules for Loop Filling



Full Loop injection:
Overfill Loop 3 to 5 times.

Example 1: Small Loop Size: Loop 20 µl. Volume needed to fill the 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:
Safe working Range: 20 to 60 % of Loop content.

Example: Small Loop Size: Loop 20 µl. 4 to 12 µl Sample Volume.

Loops with a larger volume can be filled in a range from 20 to maximum 80% of the loop content (200 µl or larger).

Disregarding the rules will result in bad repeatability.

The reason for these rules is the principle of the hydrodynamic flow pattern of the solvent front reaching the loop inlet and outlet.

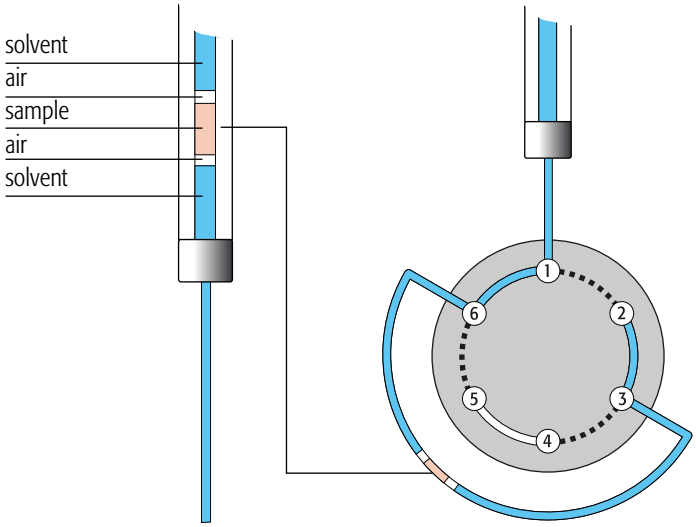
Injection Speed:
Example for a 20 µl Loop: Injection speed is 5 to 10 µl/sec.
A higher speed will cause turbulences in the loop which results in bad repeatability.
The Injection Speed is a PAL Method parameter and has to be adjusted for the type of solvent (viscosity and boiling point), loop size or rather loop internal diameter and the valve bore size.
See the recommended method parameters listed in the PAL Firmware Software Overview.

4. Low Volume Pick-up

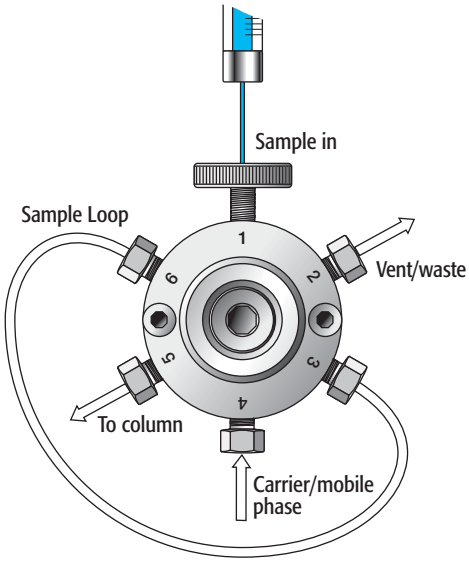
If a limited amount of sample solution is available, it is recommended to use a "low volume pick-up mode". The sample solution is embedded in a sandwich of solvent, separated by small air gaps. The sample plug is positioned in the middle of the loop.

Important:
Use a solvent with a lower elution power than the starting composition of the Mobile Phase gradient.

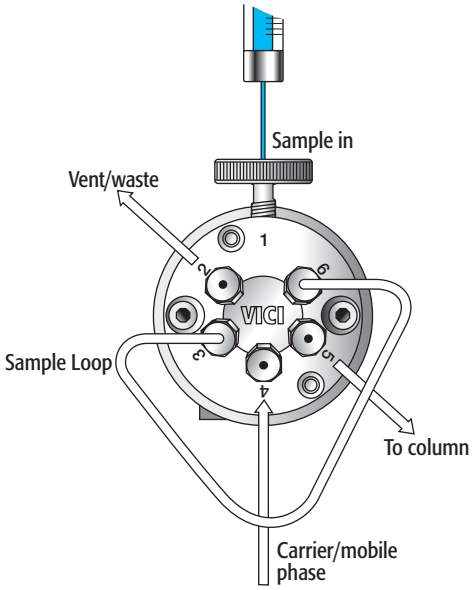
An example Macro for the Cycle Composer Software is available from any CTC Analytics Representative. The Macro is written with built-in flexibility to adapt for syringe, loop and sample volumes.



5. Plumbing Diagram for the W-and Cheminert- Valve Types



VICI/Valco W-Type Valve Conical Rotary Valves



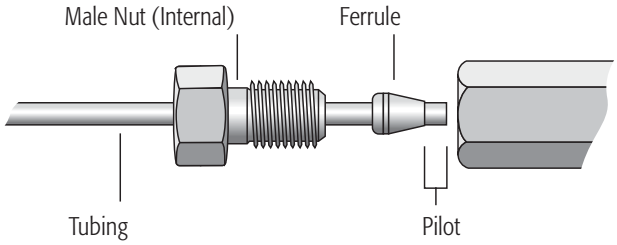
VICI/Valco Cheminert Type Valve Flat Plate Rotary Valve
(Biocompatible version available)
Rheodyne Valves have the same pattern as the Cheminert Type Valve.

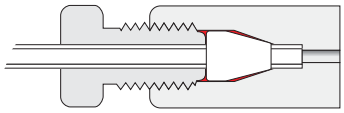
6. Nuts and Ferrules

- Do not use a Nut or a Ferrule from another vendor than specified with the product.
- Tightening the Nut, keep the tubing tight in position to ensure the correct Pilot distance.
- Do not over tighten the Nut/Ferrule.
- Do not reuse an installed Nut/Ferrule for any other connections.
- Eliminate trapped air by installing Nut/Ferrule in wetted Ports only.

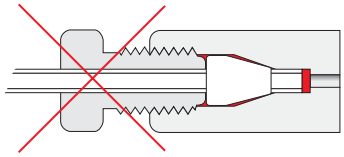
Disregarding these basic rules does lead to:

- Dead Volume.
- Peak deformation or Peak splitting.
- Carry over effect.

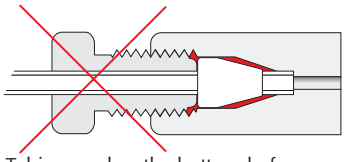




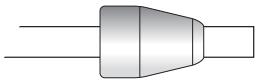
Tubing seats correctly at the bottom



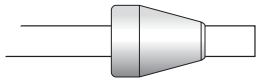
Tubing doesn't reach the bottom, introducing dead volume



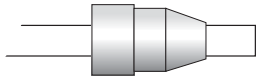
Tubing reaches the bottom before ferule seats



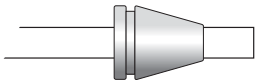
Valco



Parker



Rheodyne



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7. Tubing Internal Diameter versus Flow Rate

The tubing internal diameter has to be adjusted to the flow rate, the valve type and the application to avoid a high back pressure or chromatographic irregularities.

Tubing ID
Points to consider are:

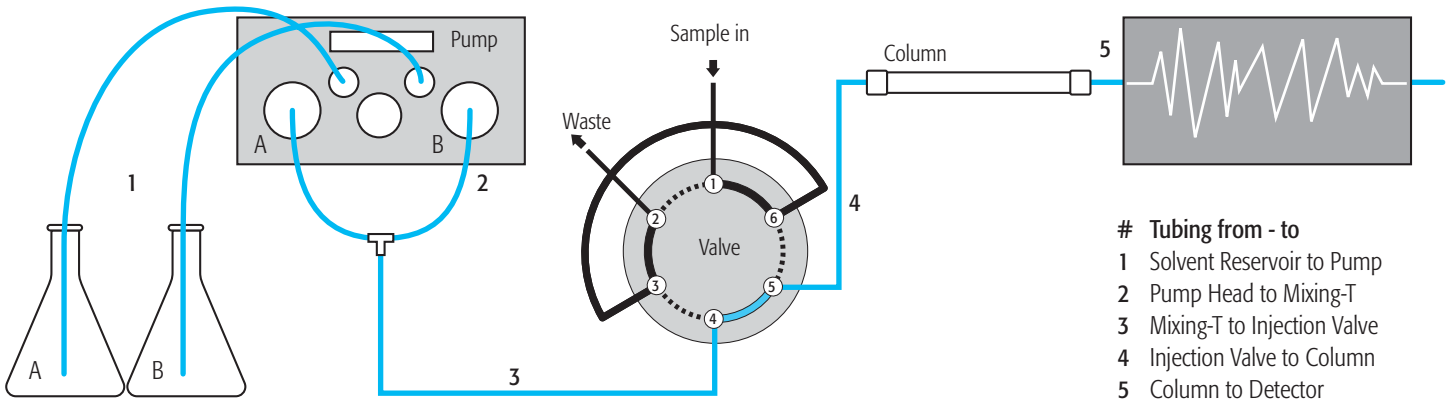
- Delay Volume of entire HPLC System
- Time needed for gradient to go active at Column inlet
- Adjust Tubing Diameters and length:
  - Solvent Reservoir to Pump: Cavitation?
  - Pump Head to Mixing-T: low backpressure
  - Mixing-T to Injection Valve: low backpressure; as short as possible
  - Valve to Column: ID as small as possible, considering the backpressure
  - Column to Detector: ID as small as possible (if possible smaller than # 4) considering the backpressure

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

Tube ID inch	Tube ID in mm	Tube Volume
0.040	1.00	78.55 µl
0.020	0.50	19.64 µl
0.010	0.25	4.91 µl
0.005	0.13	1.33 µl
0.0025	0.064	0.32 µl

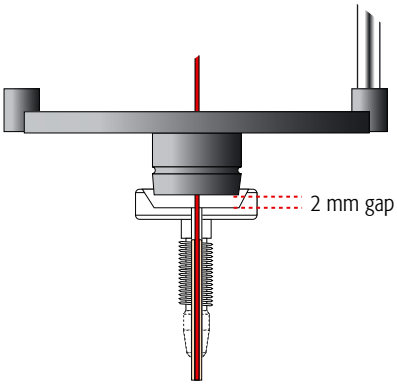
Valve Type Bore Size in mm	Flow Rate Range from - to	Tubing ID inch / mm	Tubing from - to # see diagram below
0.75 mm	5 to 100 ml/min preparative application	0.25" / 6.35 mm 0.040" / 1.0 mm	1: Reservoir 2-5: Same ID for entire HPLC sytem plumbing to avoid backpressure
0.40 mm	0.5 to 5 ml/min Standard HPLC Column ID 4 mm	0.125“ / 3.18 mm 0.020 “ / 0.50 mm 0.010" / 0.25 mm	1: Reservoir 2-3: Pump to Valve 4-5: Valve to Detector
0.25 mm	10 to 500 µl/min Standard HPLC Column ID 1 to 2 mm	0.040” / 1.0 mm 0.010” / 0.25 mm 0.005" / 0.13 mm	1: Reservoir 2-3: Pump to Valve 4-5: Valve to Detector
0.15 mm	100nl to 100 µl/min Micro Flow application	0.020” / 0.50 mm 0.020” / 0.50 mm 0.005” / 0.13 mm 0.005” / 0.13 mm	1: Reservoir 2: Pump Head to Mixing –T 3: Mixing-T to Valve 4-5: Valve to Detector
0.10 mm	10nl to 10 µl/min Nano Flow application	0.020” / 0.50 mm Fused Silica 50µm Fused Silica 50µm Fused Silica 25µm	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 ID's are recommendations only. Variations depend on the Application, Mobile Phase, Flow Rate, Column ID and Sample Load.



8. Needle Guide and Needle Seals

Positioning of Injection Unit on Valve Needle Guide
Position Injection Unit approx. 2 mm above the bottom of Valve Needle Guide.

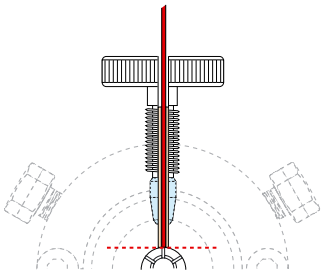


Effect if Injection Unit is not positioned correctly:

Loss of steps from the Z-stepper motor.
(PAL does recover steps in the moment of referencing the axis.)
Possibility that the next object in cycle is not detected (if the next step follows directly without referencing of axis. Example: Wash Station).

Needle Seal and Needle Guide

- Change the Needle Seal in regular intervals
- Do not use steel ferrules for a PAEK Valve Stator
- Select the corresponding Needle Seal/Guide as listed in the table below

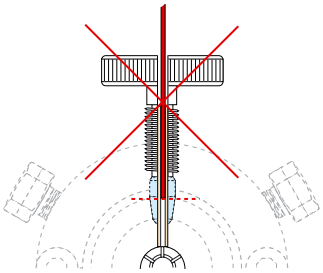


Effect if Needle Seal is not tight:

- Loss of sample or no sample transfer in valve
- Filling Injection Port (Port 1) with sample solution
- Carry Over

Needle Penetration in Valve Inlet Port

- Path: Menu/Utilities/Injectors/F3 – Move to Injector
- Activate "Needle Penetration"
- Turn the needle slowly down until a clear audible noise occurs
- Motor pressure release 2 steps up
- Check position again
  Correct position shown in graphic (Needle Seal and Needle Guide)



Needle Penetration in wrong position:

- If needle is too high: No seal possible. Carry over.
- If needle is too low: Needle tip hits top of valve body. Possible needle distortion.

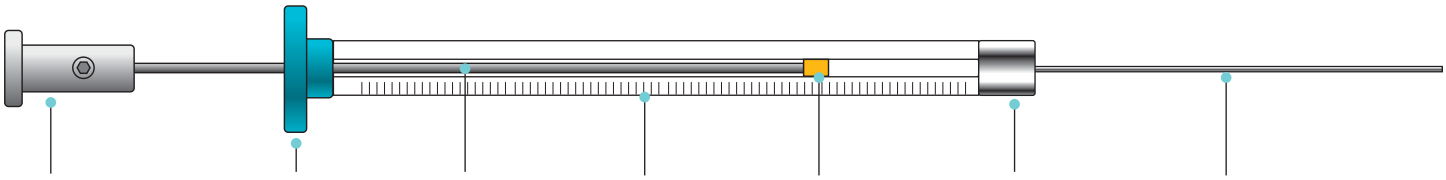
Needle Guide and Seals Replacement Information

P/N	Description	Remarks
PAL NdlSeal	Needle Seal Gauge 22	Transparent FEP tubing with sst Ferrule.
PAL NdlSeal-P	Needle Seal for PAEK Valve Gauge 22	PEEK Ferrule to protect PAEK valve stator. Transparent FEP tubing.
PAL NdlSeal-19	Needle Seal Gauge 19	Blue colored Teflon tubing to differentiate from Gauge 22, sst. Ferrule.
MV 30-12	Needle Guide for Gauge 22 Needles	sst Material.
MV 30-30	Needle Guide for Gauge 22 Needles	PEEK Material for PAEK Valve.
MV 30-52	Needle Guide for Gauge 19 Needles	Groove in the rim to differentiate from Gauge 22 type. sst. Material.

9. Syringes and Needles

CTC Analytics Syringe ***X***-Type developed for:

- High Throughput Applications — E***X***tra Long Life Time and E***X***tra Low Carry Over
- Biological Samples/ Drug Discovery/Proteomics — No Metal Contact
- Practically Zero Carry-Over — Inert Smooth Surface



Adjustable plunger, protects plunger tip from squeezing

Colour coded flange indicating needle internal diameter

Plunger stem sealed with temperature and solvent stable material

Glass barrel inner surface polished and sealed for inertness. Stable against organic solvents, acids and base (brine)

New inert plunger tip Polymer with significant longer lifetime

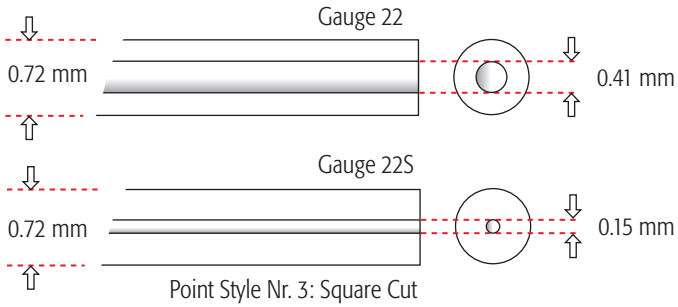
Needle fixation to barrel. No sample contact with glue or cement

Deactivated needle by extremely smooth inorganic glass layer, prevents metal contact. Stable against organic solvents, acids and base (brine)

Syringe max. Vol.	CTC	Hamilton			Syringe description			
µl	Article No.	P/N	Rev. No.	Description	Glass OD mm	Gauge	Point style Pst	Remarks
	X-Type Syringes, SyrX - for High Throughput and Biocompatible Applications							
25	SyrX G25-22S-3		01	1702 CTC (22S/3) inert	7.7	22S	3	Metal Flange: red color
100	SyrX G100-22S-3		01	1710 CTC (22S/3) inert	6.6	22S	3	Metal Flange: red color
100	SyrX G100-22-3		01	1710 CTC (22/3) inert	6.6	22	3	Metal Flange: blue color
	C-Type Syringes, SyrC, - Standard HPLC Syringes							
10	SyrC G10-22S-3	203194	02	1701 CTC (22S/3)	6.6	22S	3	Metal Flange
25	SyrC G25-22S-3	203075	03	1702 CTC (22S/3)	7.7	22S	3	Metal Flange
100	SyrC G100-22S-3	203077	03	1710 CTC (22S/3)	6.6	22S	3	Metal Flange
100	SyrC G100-22-3	203235	03	1710 CTC (22/3)	6.6	22	3	Metal Flange
250	SyrC G250-22-3	203079	03	1725 CTC (22/3)	7.7	22	3	Metal Flange
500	SyrC G500-22-3	203349	03	1750 CTC (22/3)	7.7	22	3	Metal Flange
1000	SyrC G1000-22-3	203081	02	1001 CTC (22/3)	7.7	22	3	Glass Flange
2500	SyrC G2500-22-3	203083	02	1002 CTC (22/3)	9.5	22	3	Glass Flange
5000	SyrC G5000-22-3	203085	02	1005 CTC (22/3)	13.5	22	3	Glass Flange

G = Gastight/ Plunger Teflon. | Plunger Tip for ***X***-Line: Teflon/Polymer Mix | Replacement Plungers are available at CTC.
X-Type Syringes are sold by CTC exclusively. Flange color indicates needle internal diameter. Example Gauge 22 or Gauge 22S.

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

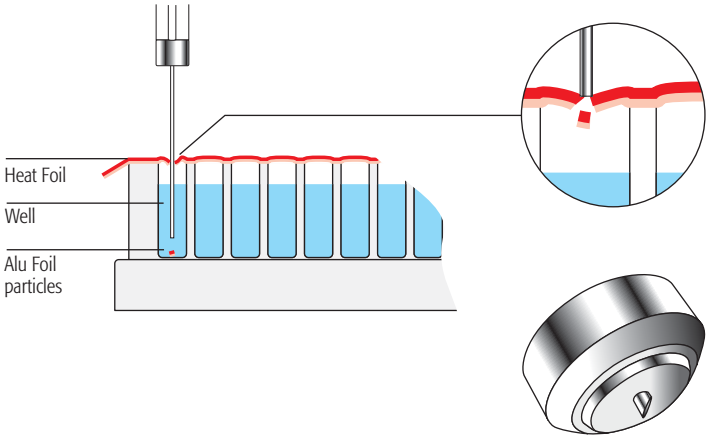
Needle Gauge	Fill Speed[1])	Time to Fill a Syringe	Needle Volume[2])
Gauge 22S	5 to 10 µl/sec	10 to 20 sec	0.90 µl
Gauge 22	200 µl/sec	0.5 sec	6.73 µl

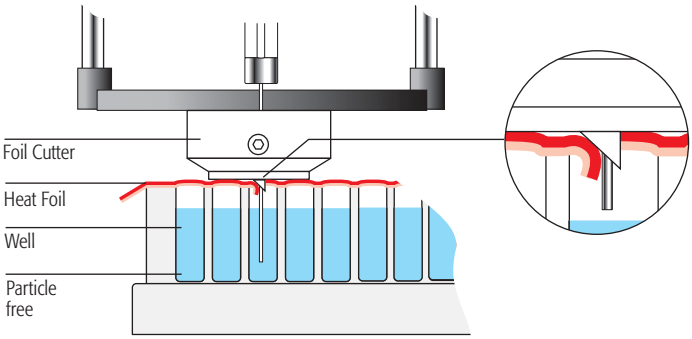
Example: 100 µl syringe

[1]) Maximum Fill Speed before cavitations is observed.
Fill Speed Example with Solvent Methanol / Water (1:1)

[2]) Needle Volume for 51 mm standard needle

10. Microtiter / Deepwell Plates and Piercing Foils





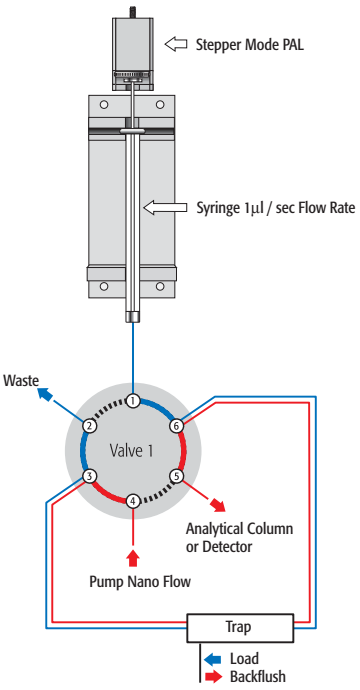
- Foil Cutter: Article Nr. MSZ 02-01
- Foil Cutter can be used for all type of heat foils
- Do not use the adhesive seal foils.
  Organic solvents, like DMSO, can dissolve glue.

11. Examples of Plumbing Diagrams for Micro/Nano Flow Applications

- Transferring sample in µl-range is easier to handle than in nl-range
- Transfer sample first on a trap to focus with a "high flow rate".
- Use partial loop filling to minimize loss of sample
- Adjust the sample and wash solvent to the chromatographic strength of Mobile Phase
- Use a back flash mode to optimize the transfer from the trap to the next device.
- First wash step for biological samples has to be with an aqueous media followed by an organic solvent.
  Last wash step has to be with an aqueous media. (Can be achieved with a "Pre-Wash" directly before the next injection.)
- Trap can be used for sample preparation.
  Examples:
  - Eliminate buffer/salt or high molecular compounds
  - Solid Phase Extraction (SPE)
  - Restricted Access Material (RAM)
  - Molecularly Imprint Polymer (MIP)
  - etc.

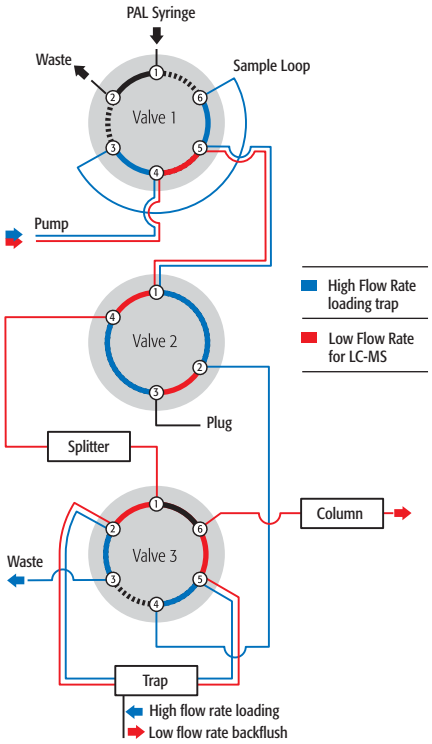
PAL System used as a Syringe Pump

- Loop is replaced with a cartridge or trap.
- PAL System used as a Syringe Pump to load the cartridge or trap.
- Slow deposition of the sample solution, 1 µl/sec.
- Advantage: Simple setup. Only one valve and one HPLC Pump required



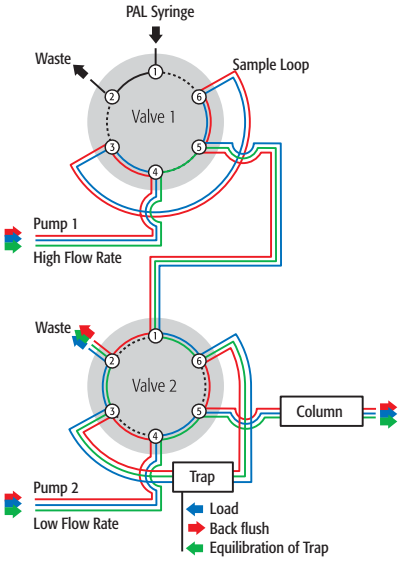
Valve System, Single Pump with Splitter

- Sample Transfer with high flow rate.
- Control of the analytical flow rate by the splitter device after valve switches.
- Place splitter as close as possible to the column.
- Advantage: One pump system required.
- Disadvantage: 3 valve setup. Splitter has to work reliable.



Valve System and 2 Pump Setup

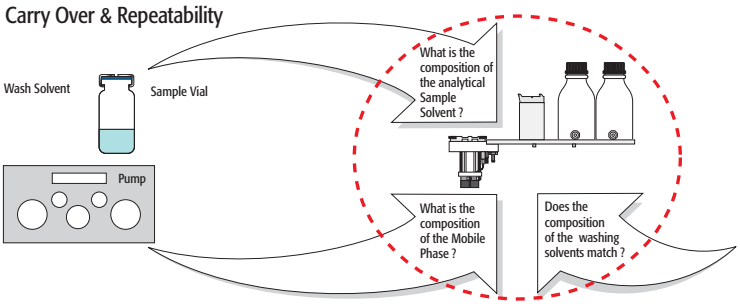
- Two pumps are operated independent from each other.
  High flow rate for fast sample transfer on trap
  Low flow rate optimized for LC-MS application.
- Mobile Phase from first pump does recondition the trap.
- Advantage: Conceptional clean setup.
  2 dedicated flow ranges (pumps).
- Disadvantage: 2 pumps are required.



Load Sample from Loop to Trap High Flow Rate	(Pump 1)
Backflush with Low Flow Rate on Column/Detector LC-MS	(Pump 2)
Equilibrate Trap with High Flow Rate.	(Pump 1)
Low Flow Rate supports analytical column and LC-MS	(Pump 2)

CARRY OVER | TROUBLE SHOOTING | CHECK LIST ...CTC ANALYTICS

Carry Over & Repeatability



Wah Steps for Biological Samples:

- 1st Wash Cycle: Aqueous Solvent
- 2nd Wash Cycle: Organic Solvent
- 1st Wash Cyle before next sample:
  Pre-Wash with Aqueous Solvent
  (eliminate Organic Solvents in Syringe and Valve)

#	Important points to control	Influence for Carry Over	Influence for Repeatability	Remarks
1	**Wash Solvents:** Does the washing solvent match?	!	!	*See graphic above*
	Washing solvent in Loop for next injection: Is the chromatographic strength adjusted to the Mobile Phase?		!	Avoid strong Organic Solvents remaining in Loop.
2	**Valve system?** Is the valve intact?	!	!	Are any leaks observed?
	Is the Waste line open?	!	!	At loop fill observe movement and speed of solvent front.
	Is the back pressure at normal level?	!	!	
	Can the valve be activated?	!	!	Menu/Utilities/Injectors/LC Vlv (F1)
	Is the Valve Type (dimensions) appropriate with the flowrate?	!	!	*See point 1*
3	**Needle Seal:** Is the seal tight?	!	!	Does the seal fit in size? Gauge 22/19? *See graphic point 8*
4	**Needle Penetration:** Is needle Penetration in valve inlet port adjusted?	!	!	Is the Injection Unit needle guide (Z-Axis) correct positioned? *See graphic point 8*
5	**Nuts and Ferrules:** Are only corresponding nuts and ferrules applied?	!	!	Are the tubing's cut square and are they open without a restriction?
	Are all connections renewed (Dead Volume)?	!	!	*See graphic point 6*
6	**Loop Injection:** Full Loop Injection: Is the Loop 3 to 5 times overfilled?		!	*See point 3*
	Partial Loop Filling: Is the rule of 20 to 60% of the loop content applied?		!	Consider also the Valve volume as indicated in *Point 2*
7	**Syringe:** Is the Plunger tight? Is the syringe intact?	!	!	
	Is a change to the ***X***-Type syringe indicated?	!	!	High throughput application? High affinity of a compound to metal?
8	**Material replacement:** Tubing: sst to PEEK or Fused Silica?	!	!	Relationship flow rate or compound affinity to metal. *See point 7*
	Valve Rotor: Is a change from Valcon H to Valcon E indicated?	!	!	*See point 1*
	Valve Body: Is a change from sst to PEEK indicated?	!	!	Observe material advantages and limitations. Product information is available from supplier.
9	**PAL Method Parameters:** Syringe Fill Speed: Cavitations?		!	Gauge 22S or 22? *See point 9*
	Pullup Delay: Is the time long enough?			For recommendations see the PAL Firmware Overview or the PAL User Manual.
	Injection Speed: is the loop fill speed too high?	!	!	
	Eject Speed: Is the syringe Eject Speed high enough ?		!	Eject speed is a syringe parameter. Air bubbles are ejected during Fill Strokes.
10	**HPLC Column:** Is the inlet frit clean/replaced?	!	!	HPLC System backpressure?
11	**HPLC System Parameters:** Column and Trap Is the selected equilibration time long enough? Is enough Tolerance timed?		!	General rule is to flush the column/trap 5 to 10 times with the starting condition of the gradient
12	**Detection and Integration:** Is the Peak Detection and Integration verified?		!	Peak tailing? Baseline assignment? S/N ratio? Area rejected? Peak Slope detection?
	Is the Signal within the dynamic linear Range of the Detector?		!	Linearity?

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