

## Specifications

### PAL RTC / RSI including Series II (LC & GC)



## Imprint

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# 1 Syringes, Gravimetric Specifications

## 1.1 Repeatability

### Specifications:

Syringe Type	Specification
10 µL, Gauge: 22s	RSD ≤ 1 %
10 µL, Gauge: 26s	RSD ≤ 1 %
100 µL, Gauge: 22	RSD ≤ 0.1 %
100 µL, Gauge: 26s	RSD ≤ 0.1 %

### Conditions:

n=7 x 8 µL transferred with 10 µL syringe

n=7 x 100 µL transferred with 100 µL syringe

Balance used: Mettler Toledo, New Classic MF, MS105DU (d = 0.01 mg)

### PAL Sample Control Method: Gravimetric Precision

Parameter	10 µL Syringe	100 µL Syringe
Number of Vials	7	7
Number of Transfers	1	1
Transfer Volume [µL]	8	100
Fill Speed [µL/s]	1	10
Eject Speed [µL/s]	50	200
Fill Volume [µL]	5	50
Fill Strokes	3	3
Air Volume [µL]	0.5	0
Pullup Delay [ms]	3000	3000
Dispense Delay	NA	NA

## 1.2 Linearity

### Specifications:

Syringe Type	Specification
10 µL, Gauge: 22s	$R^2 \geq 0.999$
10 µL, Gauge: 26s	$R^2 \geq 0.999$
100 µL, Gauge: 22	$R^2 \geq 0.999$
100 µL, Gauge: 26s	$R^2 \geq 0.999$
100 µL, LCMS-P Tool	$R^2 \geq 0.999$

### Conditions:

2, 4, 6, 8, 10 µL transferred with 10 µL syringe, 3 replicates

20, 40, 60, 80, 100 µL transferred with 100 µL syringe, 3 replicates

Balance used: Mettler Toledo, New Classic MF, MS105DU (d=0.01 mg)

### PAL Sample Control Method: Gravimetric Linearity

Parameter	10 µL Syringe	100 µL Syringe
Number of Vials	5	5
Number of Transfers	1	1
Volume Increment [µL]	2	20
Fill Speed [µL/s]	1	10 (5)*
Eject Speed [µL/s]	50	200 (20)*
Fill Volume [µL]	5	50
Fill Strokes	3	3 (0)*
Air Volume [µL]	0	0
Pullup Delay [ms]	3000	3000

\* Numbers in brackets are valid for the LCMS-P Tool only, all other numbers are valid for standard syringes and LCMS-P Tool.

## 2 Analytical Specifications HPLC

### 2.1 HPLC Injection Repeatability for standard syringes

#### Specifications for standard syringes:

Syringe	Loop size [ $\mu\text{L}$ ]	Injected Volume [ $\mu\text{L}$ ]	Specification
100 $\mu\text{L}$ , G: 22	20	80	Full Loop Injection: RSD $\leq$ 0.1 %
100 $\mu\text{L}$ , G: 22	20	10	Partial Loop Injection: RSD $\leq$ 0.15 %

#### Conditions:

Full Loop Injection: n=10, 4 x overfill

Sample: Benzophenone, 2 mg/L in MeOH : H<sub>2</sub>O = 80 : 20 v/v

Partial Loop Injection: n=10,

Sample: Benzophenone, 4 mg/L in MeOH : H<sub>2</sub>O = 80 : 20 v/v

Injection Valve: Vici Valco Cheminert C72VX-1676D-CTC

#### PAL Sample Control Method: LC Injection General

Parameter	Full Loop	Partial Loop
Sample Volume [ $\mu\text{L}$ ]	80	10
Fill Speed [ $\mu\text{L/s}$ ]	10	10
Inject Speed [ $\mu\text{L/s}$ ]	10	1
Pre Inject Delay [ms]	500	500
Post Inject Delay [ms]	500	2000
Injector Penetration Speed [mm/s]	40	40
Fill Strokes	3	3
Fill Volume [ $\mu\text{L}$ ]	50	50
Air Volume [ $\mu\text{L}$ ]	0	0
Pullup Delay [ms]	2000	2000
Dispense Delay	1000	1000
Pre Clean with Solvent 1	1	1
Pre Clean with Solvent 2	1	1
Post Clean with Solvent 1	1	1
Post Clean with Solvent 2	1	1
Valve Clean with Solvent 1 [ $\mu\text{L}$ ]	50	50
Valve Clean with Solvent 2 [ $\mu\text{L}$ ]	50	50

## LC Method:

Gradient	Isocratic: 20 % A : 80 % B v/v A = (H <sub>2</sub> O + 0.1 % TFA), B = (CH <sub>3</sub> CN + 10 % H <sub>2</sub> O + 0.1 % TFA)
Runtime [min]	3.0
Flow [mL/min]	1.00
Detection [nm]	UV 258
Flow cell	L: 60 mm; Volume: 4 µL
Column	Phenomenex Kinetex 2.6 µm C8 100A, 100 x 4.6 mm
Precolumn	Phenomenex SecurityGuard ULTRA Cartridges, C8 for 4.6 mm Columns
Column temperature [°C]	40

## 2.2 HPLC Injection Linearity for standard syringes

### Specifications for standard syringes:

Syringe Type	Loop size [µL]	Specification
100 µL, Gauge: 22	100	R <sup>2</sup> ≥ 0.999

### Conditions:

Sample: Benzophenone, 2 mg / L in MeOH : H<sub>2</sub>O = 80 : 20 v/v, n=3.

### PAL Sample Control Method: LC Injection General

Parameter	100 µL Syringe
Sample Volume [µL]	20, 30, 40, 50, 60
Fill Speed [µL/s]	10
Inject Speed [µL/s]	10
Pre Inject Delay [ms]	500
Post Inject Delay [ms]	2000
Injector Penetration Speed [mm/s]	40
Fill Strokes	3
Fill Volume [µL]	50
Air Volume [µL]	0
Pullup Delay [ms]	2000
Dispense Delay	1000
Pre Clean with Solvent 1	1
Pre Clean with Solvent 2	1
Post Clean with Solvent 1	1
Post Clean with Solvent 2	1
Valve Clean with Solvent 1 [µL]	50
Valve Clean with Solvent 2 [µL]	50

### LC Method:

As for repeatability.



## 2.3 HPLC Injection Repeatability for LCMS-P Tool

### Specifications for LCMS-P Tool:

Syringe	Loop size [ $\mu\text{L}$ ]	Injected Volume [ $\mu\text{L}$ ]	Specification
100 $\mu\text{L}$	20	80	Full Loop Injection: RSD $\leq$ 0.1 %
100 $\mu\text{L}$	20	10	Partial Loop Injection: RSD $\leq$ 0.5 %
100 $\mu\text{L}$	2	8	Full Loop Injection: RSD $\leq$ 0.1 %
100 $\mu\text{L}$	2	1	Partial Loop Injection: RSD $\leq$ 0.5 %

### Conditions:

Full Loop Injection: n=10, 4 x overfill

Sample for 2  $\mu\text{L}$  Loopsizes : Caffein, 80  $\mu\text{g}/\text{mL}$  in  $\text{H}_2\text{O}$  : ACN = 90 : 10 v/v + 0.1 % TFA

Sample for 20  $\mu\text{L}$  Loopsizes : Caffein, 8  $\mu\text{g}/\text{mL}$  in  $\text{H}_2\text{O}$  : ACN = 90 : 10 v/v + 0.1 % TFA

Partial Loop Injection: n=10,

Sample for 2  $\mu\text{L}$  Loopsizes : Caffein, 80  $\mu\text{g}/\text{mL}$  in  $\text{H}_2\text{O}$ , 2 $\mu\text{L}$  Loop : ACN = 90 : 10 v/v + 0.1 % TFA

Sample for 20  $\mu\text{L}$  Loopsizes : Caffein, 8  $\mu\text{g}/\text{mL}$  in  $\text{H}_2\text{O}$ , 20  $\mu\text{L}$  Loop : ACN = 90 : 10 v/v + 0.1 % TFA

Injection Valve: Vici Valco Cheminert C72VX-1676D-CTC

### PAL Sample Control Method: LC Injection LCMS Standard General

Parameter	Full Loop	Partial Loop
Sample Volume [ $\mu\text{L}$ ]	80 (8)*	10 (1)*
Rear Air Gap [ $\mu\text{L}$ ]	3	3 (1)*
Front Air Gap [ $\mu\text{L}$ ]	3	3 (1)*
Rear Volume [ $\mu\text{L}$ ]	5	5
Front Volume [ $\mu\text{L}$ ]	5	5
Fill Speed [ $\mu\text{L}/\text{s}$ ]	5	5
Inject Speed [ $\mu\text{L}/\text{s}$ ]	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 [ $\mu\text{L}$ ]	80	80
Valve Clean with Solvent 2 [ $\mu\text{L}$ ]	80	80
Stator Wash	Off	Off
Clean Valve Flow Rate [ $\mu\text{L}/\text{s}$ ]	20	20

\* Numbers in brackets are valid for 2  $\mu\text{L}$  loop size only, all other numbers are valid for both loop sizes.

## LC Method:

Gradient	Isocratic: 100 % A A = H <sub>2</sub> O : ACN = 90 : 10 v/v + 0.1 % TFA
Runtime [min]	2.0
Flow [mL/min]	0.5
Detection [nm]	UV 273
Flow cell	L: 60 mm; Volume: 4 µL
Column	HALO C18; 2.1 x 50 mm; 2.7 µm
Column temperature [°C]	40
HPLC System	Agilent 1290 Infinity

## 3 HPLC Injections out of a Small Sample Volume

### Specifications:

3 injections of 1.5 µL can be performed from a sample of 5 µL  
(with suitable sample containers, e.g. 96 well MTP with conical 200 µL insert)

## 4 HPLC Carry-over Data

### 4.1 HPLC Carry-over Data for standard syringes

#### Specifications for standard syringes:

Chlorhexidine / Fast Wash Station

Syringe	Carry-over in blank
100 µL X-Type, G: 22	≤ 0.02 %

#### Conditions:

The highly concentrated solution of Chlorhexidine (600 µg/mL) is injected in a separate injector valve.  
Wash Solvent 1: H<sub>2</sub>O + 10% CH<sub>3</sub>CN + 0.1% Trifluoroacetic acid  
Wash Solvent 2: CH<sub>3</sub>CN + 0.1% Trifluoroacetic acid

#### PAL Sample Control Method: LC Injection Standard General

Parameter			
Pre Clean with Solvent 2	1	Source Penetration [mm]	-1
Pre Clean with Solvent 1	1	Post Clean with Solvent 2	3
Pre Clean with Sample	2	Post Clean with Solvent 1	3
Filling Speed [µL/s]	5	Fill Speed during Post Clean [µL/s]	*30/15
Sample Volume [µL]	8	Valve Clean with Solvent 2 [µL]	160
Injection Volume [µL]	8	Valve Clean with Solvent 1 [µL]	160
Front Volume [µL]	0	Fill Speed during Valve Clean [µL/s]	*30/15
Injection Speed [µL/s]	5	Injection Speed during Valve Clean [µL/s]	20
Pre Inject Delay [ms]	500	Pullup Delay [ms]	2000
Post Inject Delay [ms]	2000	Dispense Delay [ms]	1000

\*30/15: FillSpeed SYHX-208311 (Gauge 22) = 30 µL/s; SYH-208312 (Gauge 22S) = 15 µL/s  
(Cycle Time ≈ 4 1/2 min)

## LC Method:

Gradient	Isocratic: 77 % A : 23 % B v/v A = (H <sub>2</sub> O + 0.1 % TFA), B = (CH <sub>3</sub> CN + 10 % H <sub>2</sub> O + 0.1 % TFA)
Runtime [min]	3.0
Flow [mL/min]	0.50
Detection [nm]	UV 254
Flow cell	L: 60 mm; Volume: 4 µL
Column	HALO C18; 2.1 X 50 mm; 2.1 µm
Column temperature [°C]	30

## 4.2 HPLC Carry-over Data for LCMS-P Tool

### Specifications for LCMS-P Tool / Chlorhexidine:

	Carry-over in blank 1	Comment
LCMS-P Tool; 100 µL; G: 22	≤ 0.004 %	
Cycle Time [min]	≤ 1	without valve toggle

### Conditions:

The highly concentrated solution of Chlorhexidine (600 µg/mL) is injected in a separate injector valve.

Wash Solvent 1: H<sub>2</sub>O + 10% CH<sub>3</sub>CN + 1.0% Formic acid

Wash Solvent 2: CH<sub>3</sub>CN + 1.0% Formic acid

### PAL Sample Control Method: LC Injection LCMS General

Parameter			
Sample Volume [µL]	7	Pullup Delay [ms]	2000
Front Volume [µL]	5	Source Penetration [mm]	-1
Rear Volume [µL]	5	Post Clean with Solvent 2	2
Front Air Gap [µL]	3	Post Clean with Solvent 1	2
Rear Air Gap [µL]	3	Stator Wash	On
Fill Speed [µL/s]	5	Stator Wash Delay [s]	120
Injection Speed [µL/s]	5	Valve Clean with Solvent 2 [µL]	90
Pre Inject Delay [ms]	500	Valve Clean with Solvent 1 [µL]	90
Post Inject Delay [ms]	2000		

## LC Method:

Gradient	t=0 min	77% A / 23% B
	t=2.0 min	77% A / 23% B
	t=3.0 min	100% B
	t=4.0 min	100% B
	t=4.5 min	77% A / 23% B
	t=6.0 min	77% A / 23% B

With Pal Needle Seal (Part number PAL3NdISeal)

A = (H<sub>2</sub>O + 0.1 % TFA), B = (CH<sub>3</sub>CN + 10 % H<sub>2</sub>O + 0.1 % TFA)

Runtime [min]	6.0
Flow [mL/min]	0.50
Detection [nm]	UV 254
Flow cell	L: 60 mm; Volume: 4 µL
Column	HALO C18; 2.1 x 50 mm; 2.1 µm
Column temperature [°C]	40

## 5 Analytical Data GC Liquid Injection

### 5.1 Repeatability

#### Specifications:

Alkane	RSD [Area]
C <sub>14</sub>	≤ 0.6 %
C <sub>15</sub>	≤ 0.6 %
C <sub>16</sub>	≤ 0.6 %

#### Conditions:

Agilent FID Performance Test Solution, PN: 18710-60170  
Evaluation Sample Kit, 0.218 g/L C14 / C15 / C16 in hexane  
2 mL vials filled with 0.5 mL sample

## PAL Sample Control Method: GC Liquid Injection General

Sample Volume [ $\mu\text{L}$ ]	1
Air Volume [ $\mu\text{L}$ ]	1
Vial Penetration Depth [mm]	30
Pre Clean with Solvent 1	3
Pre Clean with Sample	1
Fill Volume [ $\mu\text{L}$ ]	3
Fill Speed [ $\mu\text{L/s}$ ]	1
Fill Strokes	5
Pullup Delay [ms]	2000
Injector Penetration Depth [mm]	40
Inject Speed [ $\mu\text{L/s}$ ]	50
Pre Inject Delay [ms]	4000
Post Inject Delay [ms]	2000
Post Clean with Solvent 1	3

### GC Method:

#### SSL (Split / Splitless Injector Model)

Mode	Split
Heater [ $^{\circ}\text{C}$ ]	280
Pressure [kPa]	52.268
Total Flow [mL/min]	137.6
Septum Purge Flow [mL/min]	5
Gas Saver [mL/min]	20 after 2 min
Split Ratio	50:01:00
Split Flow [mL/min]	130

#### Oven

Equilibration Time [min]	0.5
Max Temperature [ $^{\circ}\text{C}$ ]	300
Temperature Programme	80 $^{\circ}\text{C}$ for 1 min, then 25 $^{\circ}\text{C}/\text{min}$ to 200 $^{\circ}\text{C}$ for 1 min

#### Column

Constant Flow	
Pressure [kPa]	52.268
Flow [mL/min]	2.6
Average Velocity [cm/s]	50.19

#### FID

Heater [ $^{\circ}\text{C}$ ]	300
H <sub>2</sub> Flow [mL/min]	30
Air Flow [mL/min]	400
Make-up Flow [mL/min]	25

## GC Column / GC Injector Liner

Column Phenomenex ZB5 L:15 m, ID: 0.32 mm, df:0.25  $\mu$ m, 5 % diphenyl, 95 % dimethyl polysiloxane

Liner Agilent: Split Liner 4 mm x 6.3 x 78.5, glass wool non deactivated

## 5.2 Carry-over

### Specifications:

Alkane	Carry-over in blank 1 [ppm]	Carry-over in blank 2 [ppm]
C14	$\leq 40$	not detectable
C15	$\leq 40$	not detectable
C16	$\leq 40$	not detectable

### Conditions:

Alkane Mix, 6.0 g/L in cyclohexane, 1  $\mu$ L injected

2 mL vials filled to 50 %

Wash Solvent: Cyclohexane

### PAL Sample Control Method: GC Liquid Injection General

Sample Volume [ $\mu$ L]	1
Air Volume [ $\mu$ L]	1
Vial Penetration Depth [mm]	30
Pre Clean with Solvent 1	3
Pre Clean with Sample	1
Fill Volume [ $\mu$ L]	3
Fill Speed [ $\mu$ L/s]	1
Fill Strokes	5
Pullup Delay [ms]	2000
Injector Penetration Depth [mm]	40
Inject Speed [ $\mu$ L/s]	50
Pre Inject Delay [ms]	4000
Post Inject Delay [ms]	2000
Post Clean with Solvent 1	3

### GC Method:

Identical Method as for Repeatability

## 5.3 GC Needle Discrimination

Specifications	Conditions
Peak area C40/C20 $\geq$ 0.98	Restek Florida Mix, 1 $\mu$ L injected, 100 ms fast split/spliless injection

### Conditions:

Restek Florida mix PN 31266, C8, C10, C12, C14, ...C40, 500  $\mu$ g/mL in hexane, diluted to 10 ppm with n-hexane  
 Syringe: PN: 207837, Hamilton 10  $\mu$ L, Gauge 26s, 57 mm needle length, Point Style AS

### PAL Sample Control Method: GC Liquid Injection General

Sample Volume [ $\mu$ L]	1
Air Volume [ $\mu$ L]	1
Vial Penetration Depth [mm]	30
Pre Clean with Solvent 1	1
Pre Clean with Sample	1
Fill Volume [ $\mu$ L]	3
Fill Speed [ $\mu$ L/s]	1
Fill Strokes	10
Pullup Delay [ms]	2000
Injector Penetration Depth [mm]	35
Inject Speed [ $\mu$ L/s]	fast injection mode
Pre Inject Delay [ms]	fast injection mode
Post Inject Delay [ms]	fast injection mode
Post Clean with Solvent 1	1

### GC Method:

#### Oven

Equilibration Time [min]	0.5
70 °C for 1 min, then 25 °C/min to 340 °C for 1 min	

#### SSL (Split / Splitless Injector Model)

Mode	Splitless
Heater [°C]	280
Mode	Constant Flow, 2.5 mL/min (He)
Septum Purge Flow [mL/min]	5
Gas Saver [mL/min]	20 after 2 min
Split Flow [mL/min]	80
Splitless Time [min]	0.8

#### GC Column

Constant Flow	
Flow [mL/min]	2.5



## FID

Heater [°C]	330
H <sub>2</sub> Flow [mL/min]	35
Air Flow [mL/min]	350
Make-up Flow [mL/min]	30

## GC Column / GC Injector Liner

Column	Thermo PN 26080001, 5 % diphenyl, 95 % dimethyl polysiloxane L: 7 m, ID: 0.32 mm, df: 0.25 µm
Liner	Thermo PN 453A1925 (ID 4 mm, with glass wool, 4.0 x 6.3 x 78.5)

# 6 Analytical Data GC Headspace

## 6.1 Repeatability

### Specifications:

Syringe	% RSD
Headspace 2500 $\mu\text{L}$	$\leq 1$

### Conditions:

n=7

iso-Octane, 5  $\mu\text{L}$  out of micro capillary in a 20 mL headspace vial, sealed immediately  
500  $\mu\text{L}$  injected

### PAL Sample Control Method: Headspace Injection General

Sample Volume [ $\mu\text{L}$ ]	500
Incubation Temperature [ $^{\circ}\text{C}$ ]	90
Incubation Time [s]	600
Syringe Temperature [ $^{\circ}\text{C}$ ]	100
Fill Speed [ $\mu\text{L}/\text{s}$ ]	100
Pullup Delay [ms]	10000
Pre Inject Delay [ms]	2000
Inject Speed [ $\mu\text{L}/\text{s}$ ]	100
Injector Penetration Depth [mm]	40
Post Inject Delay [ms]	5000
Purge Time [s]	90

### GC Method:

Oven

Equilibration Time [min]	0.5
Max Temperature [ $^{\circ}\text{C}$ ]	300

200  $^{\circ}\text{C}$  for 2.5 min, isotherm

SSL (Split / Splitless Injector Model)

Mode	Split
Heater [ $^{\circ}\text{C}$ ]	250
Pressure [kPa]	56.204
Total Flow [mL/min]	17.55
Septum Purge Flow [mL/min]	5
Gas Saver [mL/min]	20 after 2 min
Split Ratio	1:250
Split Flow [mL/min]	12.5

GC Column

Constant Flow	
Pressure [kPa]	56.204
Flow [mL/min]	0.05
Average Velocity [cm/s]	12.978

#### FID

Heater [°C]	300
H <sub>2</sub> Flow [mL/min]	30
Air Flow [mL/min]	400
Make-up Flow [mL/min]	25

#### GC Column / GC Injector Liner

Column	Agilent: Retention Gap, FS deactivated, 0.100 mm 5 m
Liner	Thermo: Straight Split Liner, 990 µL volum, 4 mm ID

## 6.2 Carry-over

### Specifications:

Syringe	Carry-over in blank 1 [ppm]	Carry-over in blank 2 [ppm]
Headspace 2500 µL	≤ 1000	≤ 500

### Conditions:

iso-Octane, 5 µL out of micro capillary in a 20 mL headspace vial, sealed immediately  
500 µL injected

### PAL Sample Control Method: Headspace Injection General

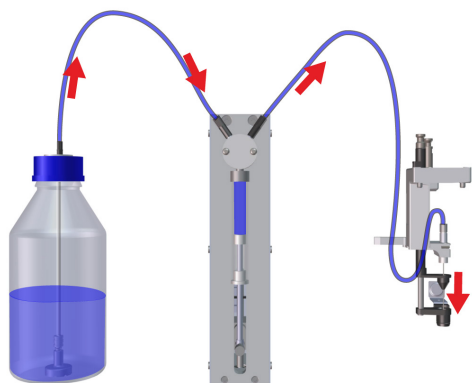
Sample Volume [µL]	500
Incubation Temperature [°C]	90
Incubation Time [s]	600
Syringe Temperature [°C]	100
Fill Speed [µL/s]	100
Pullup Delay [ms]	10000
Pre Inject Delay [ms]	2000
Inject Speed [µL/s]	100
Injector Penetration Depth [mm]	40
Post Inject Delay [ms]	5000
Purge Time [s]	90

### GC Method:

Identical to Method Repeatability

# 7 Dilutor Tool, Gravimetric Specifications

## 7.1 Repeatability deliver liquid mode (liquid is dispensed from a container connected to the dilutor valve)



Delivery mode

### Specifications:

Volume	Specification
50 $\mu\text{L}$	RSD $\leq$ 0.5 %
100 $\mu\text{L}$	RSD $\leq$ 0.15%
1000 $\mu\text{L}$	RSD $\leq$ 0.1 %
10'000 $\mu\text{L}$	RSD $\leq$ 0.1 %

### Conditions:

n=9, Standard Dilutor Module (dilutor syringe: 1 mL, gauge 22 needle, 750  $\mu\text{L}$  transfer tubing)

Liquid: Water

Balance used: Mettler Toledo, New Classic MF, MS105DU (d = 0.01 mg)

Dilutor Priming Volume [mL]	1.5
Vial Penetration Depth [mm]	10 (free dispense)
Aspirate Flow Rate [ $\mu\text{L/s}$ ]	300
Dispense Flow Rate [ $\mu\text{L/s}$ ]	110
Number of Samples	9
Pullup Delay [s]	1
Dispense Delay [s]	1
Solvent Volume [ $\mu\text{L}$ ]	50, 100, 1000, 10'000

### PAL Sample Control Method: Gravimetric Precision Dilutor Deliver Liquid

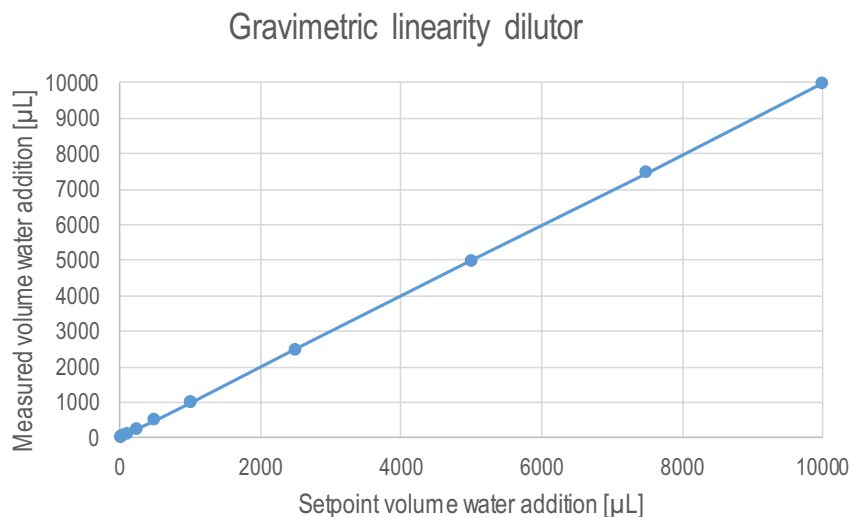


Free dispense

## 7.2 Linearity deliver liquid mode

### Specifications:

Volume Range	Specification
10 - 10'000 $\mu\text{L}$	$R^2 \geq 0.999$



### Conditions:

Standard Dilutor Syringe (1 mL), measured with 3 modules

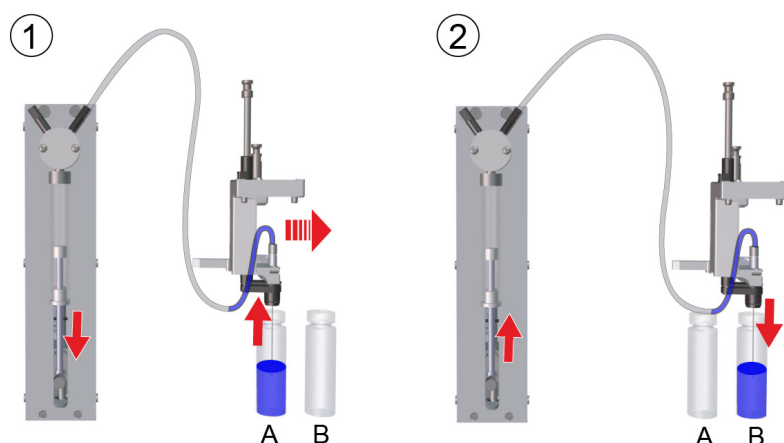
Liquid: water

Balance used: Mettler Toledo, New Classic MF, MS105DU ( $d = 0.01 \text{ mg}$ )

Dilutor Priming Volume [mL]	1.5
Vial Penetration Depth [mm]	10 (free dispense)
Aspirate Flow Rate [ $\mu\text{L}/\text{s}$ ]	300
Dispense Flow Rate [ $\mu\text{L}/\text{s}$ ]	110
Pullup Delay [s]	1
Dispense Delay [s]	1
Solvent Volume [1,...,10] [ $\mu\text{L}$ ]	10, 25, 50, 100, 250, 500, 1000, 2500, 5000, 7500, 10'000

### PAL Sample Control Method: Gravimetric Linearity Dilutor Deliver Liquid

## 7.3 Repeatability transfer mode (liquid is aspirated from a container on the deck and transferred to a container on the deck)



Transfer mode

### Specifications:

Volume	Specification
10 $\mu\text{L}$	RSD $\leq$ 1.5%
50 $\mu\text{L}$	RSD $\leq$ 0.5%
100 $\mu\text{L}$	RSD $\leq$ 0.5%

### Conditions:

n=9, Standard Dilutor Syringe (1 mL)

Liquid: Water

Balance used: Mettler Toledo, New Classic MF, MS105DU (d = 0.01 mg)

Dilutor Priming Volume [mL]	2.2
Front Air Gap [ $\mu\text{L}$ ]	3
Solvent Volume [ $\mu\text{L}$ ]	10, 50, 100
Rear Air Gap [ $\mu\text{L}$ ]	10
Vial Penetration Depth [mm]	10 (free dispense)
Aspirate Flow Rate [ $\mu\text{L/s}$ ]	5
Dispense Flow Rate [ $\mu\text{L/s}$ ]	20
Pullup Delay [s]	3
Dispense Delay [s]	3

### PAL Sample Control Method: Gravimetric Precision Dilutor Transfer Liquid

# 8 Pipette Tool, Gravimetric Specifications

## 8.1 Repeatability, accuracy and linearity, single dispense, 200 µL Pipette Tool

Dispense Volume	Repeatability	Accuracy
20 µL	RSD ≤ 1.5 %	≤ 2 %
100 µL	RSD ≤ 0.5 %	≤ 1 %
200 µL	RSD ≤ 0.5 %	≤ 1 %

n=7

Linearity:

20, 40, 80, 120, 160, 200 µL:  $R^2 \geq 0.999$

### Conditions:

Pipette Tool 200 µL, measured with two different tools

Liquid: water, 23 °C, density 0.997538 g/mL

Balance used: Precisa, XB 120A (d=0.1 mg)

PAL System 200 µL tips



Liquid Class Parameter	Liquid Class: Water (Single Dispense)
Airgap, front [µL]	0
Airgap, rear [µL]	100.0
Aspirate delay [ms]	300
Aspirate flow rate [µL/s]	100.0
Correction factor	1.04
Dispense delay [ms]	100
Dispense flow rate [µL/s]	100.0
Volume offset [µL]	1.6
Volume, front [µL]	0
Volume, rear [µL]	0

### PAL Sample Control Method: Aspirate Dispense with Liquid Class\_Rev1

## 8.2 Repeatability, multi dispense, 200 µL Pipette Tool

200 µL have been aspirated.

Dispense Volume	Repeatability
4 times 40 µL	RSD ≤ 2 %

5 times n=4

### Conditions:

Pipette Tool 200 µL

Liquid: water, 23 °C, density 0.997538 g/mL

Balance used: Precisa, XB 120A (d=0.1 mg)

PAL System 200 µL tips



**Free Dispense**

Liquid Class Parameter	Liquid Class: Water (Multi Dispense)
Airgap, front [µL]	5
Airgap, rear [µL]	0
Aspirate delay [ms]	300
Aspirate flow rate [µL/s]	100.0
Correction factor	1.04
Dispense delay [ms]	100
Dispense flow rate [µL/s]	500.0
Volume offset [µL]	1.6
Volume, front [µL]	2.0
Volume, rear [µL]	20.0

### **PAL Sample Control Method: Aspirate and Multi-Dispense with Liquid Class\_Rev1**



## 8.3 Repeatability, accuracy and linearity, single dispense, 1000 µL Pipette Tool

Dispense Volume	Repeatability	Accuracy
100 µL	RSD ≤ 0.5 %	≤ 0.5 %
500 µL	RSD ≤ 0.3 %	≤ 0.5 %
1000 µL	RSD ≤ 0.3 %	≤ 0.5 %

n=7

Linearity:

50, 100, 300, 500, 700, 1000 µL:  $R^2 \geq 0.999$

### Conditions:

Pipette Tool 1000 µL, measured with two different tools

Liquid: water, 23 °C, density 0.997538 g/mL

Balance used: Precisa, XB 120A (d=0.1 mg)

PAL System 1000 µL tips



Liquid Class Parameter	Liquid Class: Water (Single Dispense)
Airgap, front [µL]	0
Airgap, rear [µL]	20.0
Aspirate delay [ms]	300
Aspirate flow rate [µL/s]	100.0
Correction factor	1.026
Dispense delay [ms]	100
Dispense flow rate [µL/s]	500.0
Volume offset [µL]	5.9
Volume, front [µL]	0
Volume, rear [µL]	0

### PAL Sample Control Method: Aspirate Dispense with Liquid Class\_Rev1

## 8.4 Repeatability, multi dispense, 1000 µL Pipette Tool

1000 µL have been aspirated.

Dispense Volume	Repeatability
4 times 200 µL	RSD ≤ 1 %

5 times n=4

### Conditions:

Pipette Tool 1000 µL

Liquid: water, 23 °C, density 0.997538 g/mL

Balance used: Precisa, XB 120A (d=0.1 mg)

PAL System 1000 µL tips



**Free Dispense**

Liquid Class Parameter	Liquid Class: Water (Single Dispense)
Airgap, front [µL]	5
Airgap, rear [µL]	0
Aspirate delay [ms]	300
Aspirate flow rate [µL/s]	100.0
Correction factor	1.026
Dispense delay [ms]	100
Dispense flow rate [µL/s]	750
Volume offset [µL]	5.9
Volume, front [µL]	2
Volume, rear [µL]	50

### **PAL Sample Control Method: Aspirate and Multi-Dispense with Liquid Class\_Rev1**

# 9 Pipette Tool, HPLC Specifications

## 9.1 HPLC Injection Repeatability, 200 µL Pipette Tool

Specifications for 200 µL pipette tool

Pipette Tool	Loop size [µL]	Injected Volume [µL]	Specification
200 µL	5	25	RSD ≤ 0.3 %

### Conditions:

Full Loop Injection: n= 10, 5x overfill

Sample for 5 µL Loops: Caffeine, 80 µg/ mL in H<sub>2</sub>O : ACN = 90 : 10 v/v + 0.1 % Trifluoroacetic acid

Injection Valve: Vici Valco Cheminert C2V-2006D-CTC (0.4 mm bore size)

### PAL Sample Control Method: Aspirate with Liquid Class and Inject

#### Parameter:

FrontVolume [µL]	5
InjectionVolume [µL]	25
CleanInjectorTime [s]	1
CleanStatorTime [s]	1

#### Fixed Method Parameters:

InjectionSpeed [µL/s]	10
PreInjectDelay [s]	2
PostInjDelay [s]	3
FlowRate (wash pumps) [mL/s]	0.55

Wash Solvents used for Pipette Wash Module

Wash Solvent 1: H<sub>2</sub>O + 10% Acetonitrile + 0.1% Trifluoroacetic acid

Wash Solvent 2: Acetonitrile + 0.1% Trifluoroacetic acid

#### LC Method:

Gradient	Isocratic 100 % A : 0 % B v/v A= H <sub>2</sub> O : ACN = 90 : 10 v/v+ 0.1 % TFA B= H <sub>2</sub> O : ACN = 0 : 100 v/v+ 0.1 % TFA
Runtime [min]	3
Flow [mL/min]	0.5
Detection [nm]	UV 273
Flow cell	L: 60 mm, Volume: 4 µL
Column	HALO C18; 2.1 x 50 mm; 2.7 µm
ColumnTemperature [°C]	40
HPLC System	Agilent 1290 Infinity

## 9.2 HPLC Carry Over Data, 200 µL Pipette Tool

Specifications for 200 µL pipette tool Acetonaphthone

Pipette Tool	Carry Over in blank 1
200 µL	≤ 0.004 %

### Conditions:

The highly concentrated solution of Acetonaphthone (600 µg/mL) is injected in a separate injector valve

Injection Valve: Vici Valco Cheminert C2V-2006D-CTC (0.4mm bore size)

### PAL Sampe Control Method: Aspirate with Liquid Class and Inject

#### Parameter:

FrontVolume [µL]	5
InjectionVolume [µL]	25
CleanInjectorTime [s]	1
CleanStatorTime [s]	1

#### Fixed Method Parameters:

InjectionSpeed [µL/s]	10
PreInjectDelay [s]	2
PostInjDelay [s]	3
FlowRate (wash pumps) [mL/s]	0.55

Wash Solvents used for Pipette Wash Module

**Solvent 1:** H<sub>2</sub>O + 10 % Acetonitrile + 0.1 % Trifluoroacetic acid

**Solvent 2:** Acetonitrile + 0.1 % Trifluoroacetic acid

#### LC Parameters:

Gradient	Isocratic 60 % A : 40 % B v/v A= H <sub>2</sub> O : ACN = 90 : 10 v/v + 0.1 % TFA B= H <sub>2</sub> O : ACN = 0 : 100 v/v+ 0.1 % TFA
Runtime [min]	3
Flow [mL/min]	0.5
Detection [nm]	UV 247
Flow cell	L: 60 mm, Volume: 4 µL
Column	HALO C18; 2.1 x 50 mm; 2.7 µm
ColumnTemperature [°C]	30
HPLC System	Agilent 1290 Infinity



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For more information on the PAL System visit:

[www.palsystem.com](http://www.palsystem.com)



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