PAL SYSTEM
Ingenious sample handling

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

Imprint

Legal Statements

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1 Syringes, Gravimetric Specifications

1.1 Repeatability

Specificatons:

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 [µL]	Injected Volume [µL]	Specification
100 µL, G: 22	20	80	Full Loop Injection: RSD ≤ 0.1 %
100 µL, G: 22	20	10	Partial Loop Injection: RSD ≤ 0.15 %

Conditions:

Full Loop Injection: n=10, 4 x overfill
Sample: Benzophenone, 2 mg/L in MeOH : H_2O = 80 : 20 v/v
Partial Loop Injection: n=10,
Sample: Benzophenone, 4 mg/L in MeOH : H_2O = 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 [µL]	80	10
Fill Speed [µL/s]	10	10
Inject Speed [µL/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 [µL]	50	50
Air Volume [µL]	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 [µL]	50	50
Valve Clean with Solvent 2 [µL]	50	50

LC Method:

Gradient	Isocratic: 20 % A : 80 % B v/v A = (H_2O + 0.1 % TFA), B = (CH_3CN + 10 % H_2O + 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	$R^2 \geq 0.999$

Conditions:

Sample: Benzophenone, 2 mg / L in MeOH : H_2O = 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 repeatabilty.

2.3　HPLC Injection Repeatability for LCMS-P Tool

Specifications for LCMS-P Tool:

Syringe	Loop size [µL]	Injected Volume [µL]	Specification
100 µL	20	80	Full Loop Injection: RSD ≤ 0.1 %
100 µL	20	10	Partial Loop Injection: RSD ≤ 0.5 %
100 µL	2	8	Full Loop Injection: RSD ≤ 0.1 %
100 µL	2	1	Partial Loop Injection: RSD ≤ 0.5 %

Conditions:

Full Loop Injection: n=10, 4 x overfill
Sample for 2 µL Loopsize : Caffein, 80 µg/mL in H_2O : ACN = 90 : 10 v/v + 0.1 % TFA
Sample for 20 µL Loopsize : Caffein, 8 µg/mL in H_2O : ACN = 90 : 10 v/v + 0.1 % TFA

Partial Loop Injection: n=10,
Sample for 2 µL Loopsize : Caffein, 80 µg/mL in H_2O, 2µL Loop : ACN = 90 : 10 v/v + 0.1 % TFA
Sample for 20 µL Loopsize : Caffein, 8 µg/mL in H_2O, 20 µL 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 [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.

LC Method:

Gradient	Isocratic: 100 % A A = H_2O : 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_2O + 10% CH_3CN + 0.1% Trifluoroacetic acid
Wash Solvent 2: CH_3CN + 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	Despense 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_2O + 0.1 % TFA), B = (CH_3CN + 10 % H_2O + 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 seperate injector valve.
Wash Solvent 1: H_2O + 10% CH_3CN + 1.0% Formic acid
Wash Solvent 2: CH_3CN + 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 PAL3NdlSeal)
	A = (H_2O + 0.1 % TFA), B = (CH_3CN + 10 % H_2O + 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_{14}	≤ 0.6 %
C_{15}	≤ 0.6 %
C_{16}	≤ 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 [µL]	1
Air Volume [µL]	1
Vial Penetration Depth [mm]	30
Pre Clean with Solvent 1	3
Pre Clean with Sample	1
Fill Volume [µL]	3
Fill Speed [µL/s]	1
Fill Strokes	5
Pullup Delay [ms]	2000
Injector Penetration Depth [mm]	40
Inject Speed [µL/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 [°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 [°C]	300
Temperature Programme	80 °C for 1 min, then 25 °C/min to 200 °C for 1 min

Column

Constant Flow

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

FID

Heater [°C]	300
H_2 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 µm, 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	≤ 40	not detectable
C15	≤ 40	not detectable
C16	≤ 40	not detectable

Conditions:

Alkane Mix, 6.0 g/L in cyclohexane, 1 µL injected
2 mL vials filled to 50 %
Wash Solvent: Cyclohexane

PAL Sample Control Method: GC Liquid Injection General

Sample Volume [µL]	1
Air Volume [µL]	1
Vial Penetration Depth [mm]	30
Pre Clean with Solvent 1	3
Pre Clean with Sample	1
Fill Volume [µL]	3
Fill Speed [µL/s]	1
Fill Strokes	5
Pullup Delay [ms]	2000
Injector Penetration Depth [mm]	40
Inject Speed [µL/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

6 Analytical Data GC Headspace

6.1 Repeatability

Specifications:

Syringe	% RSD
Headspace 2500 µL	≤ 1

Conditions:

n=7
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:

Oven

Equilibration Time [min]	0.5
Max Temperature [°C]	300

200 °C for 2.5 min, isotherm

SSL (Split / Splitless Injector Model)

Mode	Split
Heater [°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_2 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

6.3 GC Needle Discrimination

Specifications	Conditions
Peak area C40/C20 ≥ 0.98	Restek Florida Mix, 1 μL injected, 100 ms fast split/spliless injection

Conditions:

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

PAL Sample Control Method: GC Liquid Injection General

Sample Volume [µL]	1
Air Volume [µL]	1
Vial Penetration Depth [mm]	30
Pre Clean with Solvent 1	1
Pre Clean with Sample	1
Fill Volume [µL]	3
Fill Speed [µL/s]	1
Fill Strokes	10
Pullup Delay [ms]	2000
Injector Penetration Depth [mm]	35
Inject Speed [µL/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_2 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)

7 Dilutor Tool, Gravimetric Specifications

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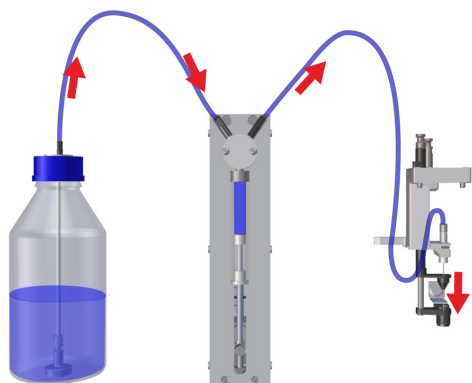
Delivery mode

Specifications:

Volume	Specification
50 µL	RSD ≤ 0.5 %
100 µL	RSD ≤ 0.15%
1000 µL	RSD ≤ 0.1 %
10'000 µL	RSD ≤ 0.1 %

Conditions:

n=9, Standard Dilutor Module (dilutor syringe: 1 mL, gauge 22 needle, 750 µL 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 [µL/s]	300
Dispense Flow Rate [µL/s]	110
Number of Samples	9
Pullup Delay [s]	1
Dispense Delay [s]	1
Solvent Volume [µL]	50, 100, 1000, 10'000

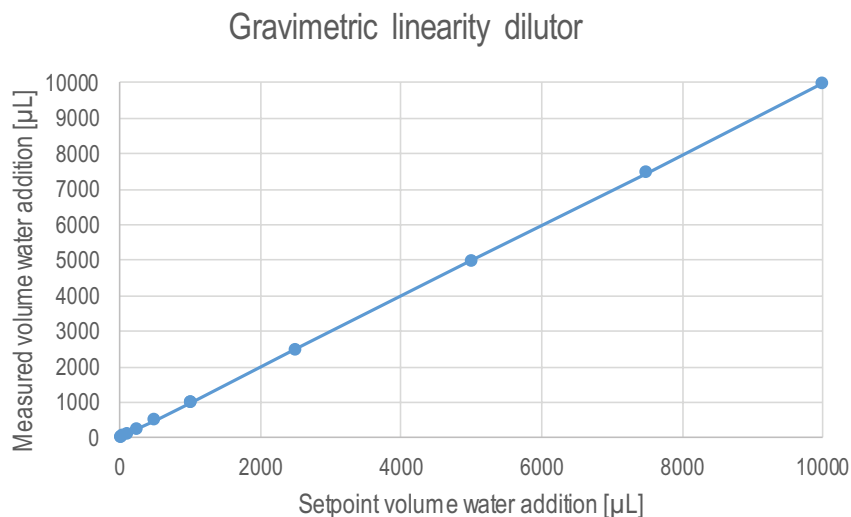**PAL Sample Control Method: Gravimetric Precision Dilutor Deliver Liquid**

Free dispense

7.2   Linearity deliver liquid mode

Specifications:

Volume Range	Specification
10 - 10'000 µL	$R^2 \geq 0.999$

Gravimetric linearity dilutor

Conditions:

Standard Dilutor Syringe (1 mL), measured with 3 modules
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 [µL/s]	300
Dispense Flow Rate [µL/s]	110
Pullup Delay [s]	1
Dispense Delay [s]	1
Solvent Volume $[1,...,10]$ [µL]	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)
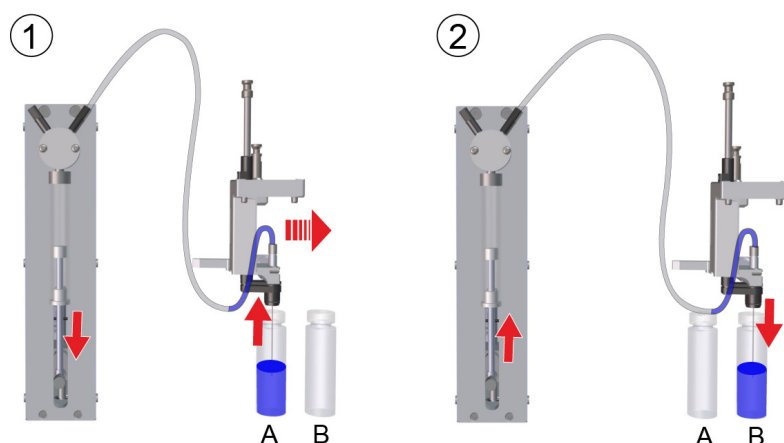
Transfer mode

Specifications:

Volume	Specification
10 µL	RSD ≤ 1.5%
50 µL	RSD ≤ 0.5%
100 µL	RSD ≤ 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 [µL]	3
Solvent Volume [µL]	10, 50, 100
Rear Air Gap [µL]	10
Vial Penetration Depth [mm]	10 (free dispense)
Aspirate Flow Rate [µL/s]	5
Dispense Flow Rate [µL/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 Loopsize: Caffein, 80 µg/ mL in H_2O : ACN = 90 : 10 v/v + 0.1 % Trifluoroacetic acid
Injection Valve: Vici Valco Cheminert C2V-2006D-CTC (0.4 mm 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
Wash Solvent 1: H_2O + 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_2O : ACN = 90 : 10 v/v+ 0.1 % TFA B= H_2O : 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 Acetonaphtone (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_2O + 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_2O : ACN = 90 : 10 v/v + 0.1 % TFA B= H_2O : 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

For more information on the PAL System visit:

www.palsystem.com