

**HPLC SYSTEMS****OO OPTIONAL TESTS AND VARIANCES****Extra Tests for OO**

The following tests are NOT INCLUDED in the standard OO for HPLC but can be ordered as EXTRA COST TESTS. Select the check boxes on the right and attach this document to your OO EQP documentation for a record of qualification conditions.

Key: Fixed setpoints/limits Variances allowed

Test	Setpoints and Parameters	Limits	Include
Injection Linearity (UV/UV-Vis)	Select five injection volumes; constant concentration of caffeine standard	Coefficient of determination (r^2) ≥ 0.99900 R/F precision RSD $\leq 5.00\%$	
Injection Linearity (CD)	Select five injection volumes; constant concentration of sodium chloride standard	Coefficient of determination (r^2) ≥ 0.99500 R/F precision RSD $\leq 10.00\%$	
Injection Response (UV/UV-Vis)	Same as Injection Precision	Average area $\geq 1,200,000$ and $\leq 1,800,000$ counts (For standard cell with 20 ul injection; result is corrected for path length and attenuation.)	
Extended Wavelength Accuracy (UV/UV-Vis, analytical and capillary scale systems)	Up to 4 wavelengths (only 361 is selected by default; others are optional) 361 nm (max) 416 nm (max) 451 nm (max) 537 nm (max)	Accuracy ≤ 2 nm	
Solvent Selection Valve (UV/UV-Vis; only if installed; for multiple valves)	Select port positions 1, 2, and 3	Pos. 1 PH $<$ Pos. 2 PH $<$ Pos. 3 PH Pos. 2 PH $\geq 1.2 \times$ Pos. 1 PH Pos. 3 PH $\geq 1.2 \times$ Pos. 2 PH (PH is peak height)	
Column Selection Valve (only if installed; for multiple valves)	Select column numbers 1, 2, and 3 (3 is N/A for 2-column position valves)	Pos. 1 Pr $<$ Pos. 2 Pr $<$ Pos. 3 Pr Pos. 2 Pr $\geq 1.2 \times$ Pos. 1 Pr Pos. 3 Pr $\geq 1.2 \times$ Pos. 2 Pr (Pr is pressure)	

Test Design and Rationale**Injection Linearity**

Description: Injection linearity of variable volume HPLC injector systems is normally not critical to quantitative or qualitative analysis. Most HPLC analytical methods use fixed and only nominal injection volumes and do not use variable volume injections within a single analysis. However, some users may wish to use variable volume injection if the linearity is demonstrated.

Procedure: Five injections of increasing volumes of the same traceable caffeine standard are made. Injection linearity is calculated from the coefficient of determination (r^2) of the peak areas versus injection volume. Also, % RSD of the response factor for all five peaks is calculated. The choice of volumes is user selectable. It should be compatible with the system configuration and representative of the usage conditions. See the table on next page for the default values used if no others are provided.

Injection Response

Description: The accuracy of the injected volume is normally not critical to quantitative or qualitative analysis. Most HPLC analytical methods use fixed and only nominal injection volumes and results are not affected by even moderate inaccuracy in actual injected volume. However, it may be important for comparability between systems and transferring methods, and it is useful as a diagnostic for establishing that the correct injection syringe/loop/device is installed.

Procedure: A known traceable caffeine standard is injected six times (in the precision tests) and the average response is calculated. The injection response is the mean of the average areas corrected for sample concentration, cell path length, and attenuation, and the response within an acceptance window indicates correct volume injected.

Extended Wavelength Accuracy

Description: Wavelength accuracy is critical for accuracy of quantitative and qualitative analysis. Wavelength accuracy is also important for comparability between systems and transferring methods.

Procedure for UV absorbance detector (UV/UV-Vis, PDA, etc.): A traceable holmium oxide standard is used to determine the wavelength accuracy. In one procedure, for certain models, the holmium oxide is trapped in the flow cell and a programmable timetable is used to determine the wavelength maxima for selected wavelengths (from 361, 416, 451, and 537 nm). For other models (for example, DAD and PDA), a holmium oxide injection is made and a spectrum is acquired. The spectral maxima are determined directly from the scan or the table of scan results. The wavelength accuracy is determined as the absolute difference between the measured and certified wavelength values.

Agilent-recommended Volumes for Injection Linearity

Detector Types: UV/UV-VIS, CD (Units: µl)					
Max Volume	Volume for Injection				
	# 1	# 2	# 3	# 4	# 5
5	1	2	3	4	5
10	2	4	6	8	10
20	1	5	10	15	20
25	1	5	10	15	25
40	5	10	20	30	40
50	5	10	25	40	50
100	5	10	20	50	100
250	10	50	150	200	250
500	50	100	250	400	500
900	50	100	200	500	900
1000	50	100	200	500	1000
2000	100	500	1000	1500	2000
Detector Type: UV/UV-VIS Surveyor (Units: µl)					
100	20	40	50	60	70

Allowed Variance Ranges

The simplest and most common occurrence is the Agilent Recommended test program - whereby the acceptance and approval refers to the fixed standards qualification tests and setpoints as recorded in the Agilent Recommended EQP. In this case, verbal confirmation of approval after customer review is sufficient for Agilent service to proceed with scheduling and delivery.

Agilent defines variances as changes to the default recommended values (as stated in the Agilent Recommended EQP) that fall within a range of well-defined allowable changes. These changes are considered to be within the intended use range of the system under test. The tables below show the allowed variance ranges for the test setpoints that can be configured. Agilent reserves the right to warrant conformance only when the tests definition lay within the maximum and minimum values shown below.

Note: **Red** tests are additional (not part of standard test program); the same minimum and maximum values apply to Agilent and non-Agilent systems.

Analytical Scale

CSV: Column Selection Valve	IL: Injection Linearity	PFA&P: Pump Flow Accuracy and Precision
CTA: Column Temperature Accuracy	IPCO: Injection Precision, Carry Over	SSV: Solvent Selection Valve
CTS: Column Temperature Stability	IR: Injection Response	STA: Sample Temperature Accuracy
EWLA: Extended Wavelength Accuracy	ND: Noise and Drift	WLA: Wavelength Accuracy

Test	Configuration	Setpoint				Units
		Min	Agilent Default	Non-Agilent Default	Max	
PFA&P	HPLC flow 1	0.100	0.500	0.500	5.000	ml/min
	HPLC flow 2	0.100	5.000	3.000	5.000	ml/min
	Agilent G4220B, Waters Acquity BSM, Waters Acquity UHPLC, or Thermo Surveyor MS pumps flow 1	0.100	0.500	0.500	2.000	ml/min
	Agilent G4220B, Waters Acquity BSM, Waters Acquity UHPLC, or Thermo Surveyor MS pumps flow 2	0.100	2.000	2.000	2.000	ml/min
	UHPLC Agilent flow 1	0.100	0.500	N/A	5.000	ml/min
	UHPLC Agilent flow 2	0.100	5.000	N/A	5.000	ml/min
	UHPLC non-Agilent flow 1	0.100	N/A	0.500	2.000	ml/min
	UHPLC non-Agilent flow 2	0.100	N/A	2.000	2.000	ml/min
	GE HPLC pump flow 1	0.100	N/A	1.000	5.000	ml/min
	GE HPLC pump flow 2	0.100	N/A	5.000	5.000	ml/min
	UHPLC Agilent with ULD kit flow 1	0.100	0.500	N/A	5.000	ml/min
	UHPLC Agilent with ULD kit flow 2	0.100	1.500	N/A	5.000	ml/min
CTA	G7116A #1	50.0	60.0	N/A	70.0	°C
	G7116A #2	10.0*	40.0	N/A	49.99	°C
	1120/1220 system, G7130A #1	50.0	60.0	N/A	80.0	°C
	1120/1220 system, G7130A #2	30.0	40.0	N/A	49.99	°C
	1220 system with G4294B detector #1	50.0	60.0	N/A	80.0	°C
	1220 system with G4294B detector #2	35.0	40.0	N/A	49.99	°C
	All others #1	50.0	80.0	60.0	80.0	°C
	All others #2	10.0*	40.0	40.0	49.99	°C
CTS	G7116A	10.0*	40.0	N/A	70.0	°C
	1120/1220 system, G7130A	30.0	40.0	N/A	80.0	°C
	1220 system with G4294B detector	35.0	40.0	N/A	80.0	°C
	All others	10.0*	40.0	40.0	80.0	°C
IPCO	UV/UV-Vis, ≤ 400 bar	1	20	20	2,000	ul
	UV/UV-Vis, > 400 bar	1	10	10	2,000	ul
	UV/UV-Vis, Agilent G4277A/G4278A	1	2	N/A	2,000	ul
	UV/UV-Vis, Dionex AS-AP/AS-DV	10	N/A	25	500	ul
	RID	1	20	20	2,000	ul
	RID, Waters UHPLC inj. With SO	1	N/A	10	2,000	ul
	RID, Agilent G4277A/G4278A	1	2	N/A	2,000	ul
	CD, with Dionex AS-AP/AS-DV injector	10	N/A	25	500	ul
	CD, with other injectors	1	25	25	2,000	ul
	FLD	1	5	5	5	ul
	CAD	1	N/A	10	20	ul
ELSD	1	20	20	2,000	ul	
ND	UV/UV-Vis	N/A	254	254	N/A	nm
	RID, CD, ELSD, CAD	N/A	N/A	N/A	N/A	nm
STA	All	4.0	4.0	5.0	40.0	°C
WLA	UV/UV-Vis	N/A	205, 245, 273	205, 273	N/A	nm
WLA	FLD	N/A	350, 397	350, 397	N/A	nm
IL	UV/UV-Vis	1	Inj. vol**	Inj. vol**	2,000	ul
IR	UV/UV-Vis, ≤ 400 bar	1	20	20	2,000	ul
	UV/UV-Vis, > 400 bar	1	10	10	2,000	ul
	UV-UV-Vis, Agilent G4277A/G4278A	1	2	N/A	2,000	ul
	UV/UV-Vis, Dionex AS-AP/AS-DV	10	N/A	25	500	ul

Test	Configuration	Setpoint				Units
		Min	Agilent Default	Non-Agilent Default	Max	
EWLA	UV/UV-Vis	N/A	361, 416, 451, 537***	361, 416, 451, 537***	N/A	nm
CSV	All	1	Port #**	Port #**	8	N/A
SSV	UV/UV-Vis	1	1, 7, 12	1, 7, 12	12	N/A

* Typically 10°C below ambient

** Varies

*** Only 361 is selected by default; others are optional

Capillary Scale

#	Capillary Scale Test	Agilent Setpoint			Units
		Min	Default	Max	
1a	Pump Flow Accuracy and Precision #1 - 20 ul flow sensor	0.001	0.010	0.010	ml/minute
2a	Pump Flow Accuracy and Precision #2 - 20 ul flow	0.011	0.020	0.020	ml/minute
1b	Pump Flow Accuracy and Precision #1 - 100 ul flow	0.001	0.010	0.010	ml/minute
2b	Pump Flow Accuracy and Precision #2 - 100 ul flow	0.011	0.100	0.100	ml/minute
1c	Pump Flow Accuracy and Precision #1 - no flow sensor	0.001	0.500	2.500	ml/minute
2c	Pump Flow Accuracy and Precision #2 - no flow sensor	0.001	2.000	2.500	ml/minute
7	Column Temperature Accuracy #1	50.0	80.0	80.0	°C
8	Column Temperature Accuracy #2	10.0*	40.0	49.99	°C
9	Column Temperature Stability	10.0*	40.0	80.0	°C
10	Injection Precision, Carry Over	1	5	2,000	ul
11	Noise and Drift	N/A	254	N/A	nm
12	Sample Temperature Accuracy	4.0	4.0	40.0	°C
13	Wavelength Accuracy	N/A	205, 245, 273	N/A	nm
A1	Extended Wavelength Accuracy	N/A	361, 416, 451, 537***	N/A	nm

* Typically 10°C below ambient

** Only 361 is selected by default; others are optional

Preparative Scale

#	Preparative Scale Test	Setpoint				Units
		Min	Agilent Default	Non-Agilent Default	Max	
1	Pump Flow Accuracy and Precision #1	2.0	10.0	10.0	500.0	ml/minute
2	Pump Flow Accuracy and Precision #2	2.0	20.0	20.0	500.0	ml/minute
3	Injection Carry Over	1	300	300	5,000	ul
4	Sample Temperature Accuracy	4.0	4.0	5.0	40.0	°C
5	Wavelength Accuracy	N/A	205, 245, 273	205, 273	N/A	nm
A1	Solvent Selection Valve UV/UV-Vis	1	1, 7, 12	1, 7, 12	12	N/A
A2	Column Selection Valve	1	Port #*	Port #*	8	N/A

* Varies

For a fully tailored operational qualification program using all the flexibility of Agilent CrossLab, contact your local Agilent representative with your OQ test specification requirements. Fees may apply.

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www.agilent.com/crosslab/compliance-steps

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