

HPLC SYSTEMS

OO OPTIONAL TESTS AND VARIANCES

Agilent CrossLab Compliance Services



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From Insight to Outcome

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 (see the Injection Linearity section)	Coefficient of determination (r^2) ≥ 0.99900 R/F precision RSD $\leq 5.00\%$	<input type="checkbox"/>
	Caffeine standard concentrations (see the Injection Linearity section)	R/F precision RSD $\leq 7.50\%$ (Waters Acquity and Arc injectors)	
Injection Linearity (CD)	Select five injection volumes (see the Injection Linearity section)	Coefficient of determination (r^2) ≥ 0.99500 R/F precision RSD $\leq 10.00\%$	<input type="checkbox"/>
	Sodium chloride concentrations (see the Injection Linearity section)		
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 μL injection; result is corrected for path length and attenuation.)	<input type="checkbox"/>
Extended Wavelength Accuracy (UV/UV-Vis, analytical and capillary scale systems)	Some or all of the following wavelengths (no alternative wavelengths allowed): 361 nm (max) 416 nm (max) 451 nm (max) 537 nm (max) <i>N/A for Waters PDA</i>	Accuracy ≤ 2 nm	<input type="checkbox"/>
2D-LC Valve Precision (UV/UV-Vis)	Injection volume on column: 1.0 μL	$\leq 3.00\%$	<input type="checkbox"/>

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 (some or all of the following, no alternative wavelengths allowed: 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.

2D-LC Valve Precision

Only if 2D-LC system with multi-heart cutting (MHC) valves is installed.

Description: System precision is critical for accuracy of quantification. The performance of the 2D-LC and MHC valves contribute to the precision of the results in the second dimension.

Procedure: Ten injections of a traceable caffeine standard, collected in a single data file via an injection program, are transferred to ten different loops of the 2D-LC MHC valves. The transferred peaks are analyzed via the second dimension detector and the area plus its average and %RSD are determined and calculated.

Injection Linearity

Agilent-recommended Injection Volumes

For Response Linearity, the actual injected volumes can vary depending on the configured system's response as determined by the scouting run.

Detector Types: UV/UV-VIS, CD (µL)

Max Volume	# 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 (µL)

100	20	40	50	60	70
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Detector Type: UV/UV-VIS Vanquish (µL)

10	1	3	5	7	9
25	1	5	10	15	20
100	5	10	20	50	90

Standard Concentrations

For listed volumes, use the suggested concentrations. For other volumes, pick a standard that does not exceed the range of the detector or cause the peak to flat top when the largest injection volume is used.

Max Volume (µL)	Standard Concentrations (µg/mL)			
	UV/UV-Vis Detectors, Caffeine		CD Detectors, Sodium Chloride	
	10 mm flow cell	60 mm flow cell	Waters	All Others
2	Not recommended	Not recommended	Not recommended	Not recommended
5	200 µg/mL	50 µg/mL	500 µg/mL	800 µg/mL
10	200 µg /mL	50 µg/mL	100 µg/mL	800 µg/mL
20	100 µg /mL	25 µg/mL	100 µg/mL	500 µg/mL
40	50 µg/mL	5 µg/mL	50 µg/mL*	500 µg/mL
100	25 µg/mL	5 µg/mL	10 µg/mL	100 µg/mL
250	5 µg/mL	2 µg/mL	10 µg/mL	50 µg/mL*
900	2 µg/mL	0.5 µg/mL	1 µg/mL**	10 µg/mL
1000	2 µg/mL	0.5 µg/mL	1 µg/mL**	10 µg/mL
2000	1 µg/mL	Not recommended	1 µg/mL**	1 µg/mL**

* 10:1 dilution of 500 µg/mL std required.

** 10:1 dilution of 10 µg/mL std required.

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.

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 test definitions lay within the maximum and minimum values 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

2DVP: 2D-LC Valve Precision	IL: Injection Linearity	PFA&P: Pump Flow Accuracy and Precision
CSV: Column Selection Valve	IP* & CO*: Injection Precision, Carry Over	SSV: Solvent Selection Valve
CTA: Column Temperature Accuracy	IR: Injection Response	STA: Sample Temperature Accuracy
CTS: Column Temperature Stability	ND: Noise and Drift	WLA: Wavelength Accuracy
EWLA: Extended Wavelength Accuracy	* Includes online valve versions of tests	

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

Test	Configuration	Setpoint				Units
		Min	Agilent Default	Non-Agilent Default	Max	
	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
IP&CO	UV/UV-Vis, ≤ 400 bar	1	20	20	2,000	μL
	UV/UV-Vis, > 400 bar	1	10	10	2,000	μL
	UV/UV-Vis, Agilent G4277A/G4278A	1	2	N/A	2,000	μL
	UV/UV-Vis, Dionex AS-AP/AS-DV	10	N/A	25	500	μL
	RID	1	20	20	20	μL
	RID, Waters UHPLC inj. With SO	1	N/A	10	20	μL
	RID, Agilent G4277A/G4278A	1	2	N/A	20	μL
	CD, with Dionex AS-AP/AS-DV injector	10	N/A	25	500	μL
	CD, with other injectors	1	25	25	2,000	μL
	FLD	1	5	5	5	μL
	CAD	1	N/A	10	20	μL
	ELSD	1	20	20	2,000	μL
	ND	UV/UV-Vis	N/A	254	254	N/A
RID, CD, ELSD, CAD		N/A	N/A	N/A	N/A	nm
STA	Agilent (1220s only)	4.0	4.0 (5.0)	N/A	40.0	°C
	Waters ACQUITY	4.0	N/A	4.0	40.0	°C
	All others	4.0	N/A	5.0	40.0	°C
WLA	UV/UV-Vis	N/A	205, 245, 273	205, 273 / 241.1, 656.1**	N/A	nm
WLA	FLD	N/A	350, 397	350, 397	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
IL	UV/UV-Vis	1	Inj. vol***	Inj. vol***	2,000	μL
IR	UV/UV-Vis, ≤ 400 bar	1	20	20	2,000	μL
	UV/UV-Vis, > 400 bar	1	10	10	2000	μL
	UV-UV-Vis, Agilent G4277A/G4278A	1	2	N/A	2000	μL
	UV/UV-Vis, Dionex AS-AP/AS-DV	10	N/A	25	500	μL
EWLA	UV/UV-Vis	N/A	361, 416, 451, 537***	361, 416, 451, 537****	N/A	nm
2DVP	UV/UV-Vis	N/A	1.0	1.0	N/A	μL

* Typically 10°C below ambient

** Shimadzu LC-2030 VWDs

*** Varies

**** Select some or all of the listed wavelengths (no alternative wavelengths allowed)

Capillary Scale

#	Capillary Scale Test	Agilent Setpoint			Units
		Min	Default	Max	
1	Pump Flow Accuracy and Precision #1 - 20 µL flow sensor	0.005	0.010	0.010	mL/minute
	Pump Flow Accuracy and Precision #2 - 20 µL flow	0.011	0.020	0.020	mL/minute
2	Pump Flow Accuracy and Precision #1 - 100 µL flow	0.005	0.010	0.010	mL/minute
	Pump Flow Accuracy and Precision #2 - 100 µL flow	0.015	0.100	0.100	mL/minute
3	Pump Flow Accuracy and Precision #1 - no flow sensor	0.005	0.500	2.500	mL/minute
	Pump Flow Accuracy and Precision #2 - no flow sensor	0.005	2.000	2.500	mL/minute
4	Column Temperature Accuracy #1	50.0	80.0	80.0	°C
	Column Temperature Accuracy #2	10.0*	40.0	49.99	°C
5	Column Temperature Stability	10.0*	40.0	80.0	°C
6	Injection Precision, Carry Over	1	5	2,000	µL
7	Noise and Drift	N/A	254	N/A	nm
8	Sample Temperature Accuracy	4.0	4.0	40.0	°C
9	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

** Select some or all of the listed wavelengths (no alternative wavelengths allowed)

Preparative Scale

APH: Analytical pumphead	PPH: Preparative pumphead
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#	Preparative Scale Test	Setpoint				Units
		Min	Agilent Default	Non-Agilent Default	Max	
1	Pump Flow Acc. #1, Agilent G7161B, APH	1.0	10.0	N/A	50.0	mL/minute
	Pump Flow Acc. #2, Agilent G7161B, APH	1.0	20.0	N/A	50.0	mL/minute
	Pump Flow Prec. #1, Agilent G7161B, APH	1.0	10.0	N/A	20.0	mL/minute
	Pump Flow Prec. #2, Agilent G7161B, APH	1.0	20.0	N/A	20.0	mL/minute
2	Pump Flow Acc. #1, Agilent G7161B, PPH	4.0	10.0	N/A	200.0	mL/minute
	Pump Flow Acc. #2, Agilent G7161B, PPH	4.0	20.0	N/A	200.0	mL/minute
	Pump Flow Prec. #1, Agilent G7161B, PPH	4.0	10.0	N/A	20.0	mL/minute
	Pump Flow Prec. #2, Agilent G7161B, PPH	4.0	20.0	N/A	20.0	mL/minute
3	Pump Flow Acc. #1, all others	2.0	10.0	10.0	500.0	mL/minute
	Pump Flow Acc. #2, all others	2.0	20.0	20.0	500.0	mL/minute
	Pump Flow Prec. #1, all others	2.0	10.0	10.0	20.0	mL/minute
	Pump Flow Prec. #2, all others	2.0	20.0	20.0	20.0	mL/minute
4	Injection Carry Over	1	300	300	5,000	µL
5	Sample Temperature Accuracy	4.0	4.0	5.0*	40.0	°C
6	Wavelength Accuracy	N/A	205, 245, 273	205, 273 / 241.1, 656.1**	N/A	nm
7	Solvent Selection Valve, UV/UV-Vis	1	1, 7, 12	1, 7, 12	12	N/A
8	Column Selection Valve	1	Port #***	Port #***	8	N/A

* Agilent 1220 thermostats

** Shimadzu LC-2030 VWDs

*** 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.

www.agilent.com/chem/qualification

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