ANALYTICAL SCALE HPLC AGILENT SYSTEMS OPERATIONAL QUALIFICATION

The Measure of Confidence

Agilent Enterprise Edition Compliance Services

Standard OQ Test Suite

This document describes the test program for qualifying LC analytical-scale Agilent systems; the following table lists all OQ tests.

Note: Some test conditions differ depending on the intended operating pressure range; UHPLC test names are orange. And for multiple-detector systems, the Injection Precision and Carry Over tests are performed for one detector in the standard test program. They can be run for additional detectors as optional tests for a nominal fee.

Key:	Fixed HPLC setpoints/limits	UHPLC setpoints	Variance allowed

Test	Setpoints and Parameters	Limits
Pump Flow Accuracy and Precision (≤ 400 bar)	Flow Rate 1: 0.500 ml/minute Flow Rate 2: 5.000 ml/minute Flow Rate 2: 2.00 ml/minute (G4220B)	Accuracy ≤ 5.00% Precision ≤ 0.50%
Pump Flow Accuracy and Precision (> 400 bar)	Flow Rate 1: 0.500 ml/minute Flow Rate 2: 5.000 ml/minute Flow Rate 2: 2.00 ml/minute (G4220B)	Accuracy ≤ 3.00% Precision ≤ 0.50%
Column Temperature Accuracy and Stability	Temperature 1: 80.0°C Temperature 2: 40.0°C Temperature 2: 60.0°C (1120, 1220 systems) Stability measured at temperature 2	Diff. from setpoint ≤ 3.0°C (≥ 60°C) Diff. from setpoint ≤ 2.0°C (< 60°C) Stability ≤ 1.0°C
Wavelength Accuracy (UV-Vis)	Wavelength 1: 205 nm (max) Wavelength 2 : 245 nm (min) Wavelength 3: 273 nm (max)	Accuracy ≤ 2 nm
Wavelength Accuracy (FLD)	Wavelength 1: 350 nm (max) Wavelength 2: 397 nm (max)	Accuracy ≤ 3 nm
Noise and Drift (VWD)	ASTM baseline noise Slope of regression fit for drift	Noise: ≤ 0.040 mAU Drift ≤ 0.500 mAU/hr
Noise and Drift (DAD, MWD) (≤ 400 bar)	ASTM baseline noise Slope of regression fit for drift	Noise: ≤ 0.050 mAU Drift ≤ 5.000 mAU/hr
Noise and Drift (DAD, > 400 bar)	ASTM baseline noise Slope of regression fit for drift	Noise: ≤ 0.050 mAU Drift ≤ 5.000 mAU/hr Noise: ≤ 0.030 mAU (G4212A/B) Drift: ≤ 3.000 mAU/hr (G4212A/B)
Noise and Drift (MWD, > 400 bar)	ASTM baseline noise Slope of regression fit for drift	Noise: ≤ 0.050 mAU Drift: ≤ 5.000 mAU/hr
Noise and Drift (RID)	ASTM baseline noise Slope of regression fit for drift	Noise: ≤ 10.000 nRIU Drift ≤ 400.000 nRIU/hr
Noise and Drift (ELSD)	ASTM baseline noise Slope of regression fit for drift	Noise: ≤ 2.000 mV Drift ≤ 5.000 mV/hr

Test	Setpoints and Parameters	Limits
Noise and Drift (CD)	ASTM baseline noise Slope of regression fit for drift	Noise: ≤ 0.100 uS Drift ≤ 10.000 uS/hour
Signal to Noise (UV-Vis ≤ 400 bar)	Signal height is divided by ASTM baseline noise for known concentration and known conditions.	Signal to noise ≥ 3,000
Signal to Noise (UV-Vis > 400 bar)	Signal height is divided by ASTM baseline noise for known concentration and known conditions.	Signal to noise ≥ 3,000 Signal to noise ≥ 10,000 (G4212A/B)
Signal to Noise (RID)	Signal height is divided by ASTM baseline noise for known concentration and known conditions.	Signal to noise ≥ 2,000
Signal to Noise (FLD)	Signal height of Raman peak is divided by noise at different wavelength in flat region of emission spectrum.	Signal to noise ≥ 400
Injection Precision (UV-Vis, RID; ≤ 400 bar)	Injection volume on column: 20 ul	Height RSD ≤ 2.00% Area RSD ≤ 1.00%
Injection Precision (UV-Vis over 400 bar)	Injection volume on column: 10 ul	Height RSD ≤ 2.00% Area RSD ≤ 1.00%
Injection Precision (ELSD)	Injection volume on column: 20 ul	Height RSD ≤ 3.00% Area RSD ≤ 3.00%
Injection Precision (FLD)	Injection volume on column: 5 ul Injection volume on column: 2 ul (1290 w/ CTC samplers)	Height RSD ≤ 2.00% Area RSD ≤ 2.00% Area RSD ≤ 1.00% (CTC samplers)
Injection Precision (CD)	Injection volume on column: 25 ul	Height RSD ≤ 2.00% Area RSD ≤ 1.00%
Injection Carry Over (UV-Vis, RID)	Injection volume on column: 20 ul Injection volume on column: 2 ul (1290 w/ CTC samplers)	Height carry over ≤ 0.40% Area carry over ≤ 0.20%
Injection Carry Over (UV-Vis > 400 bar)	Injection volume on column: 10 ul Injection volume on column: 2 ul (1290 w/ CTC samplers)	Height carry over ≤ 0.20% Area carry over ≤ 0.10%
Injection Carry Over (FLD)	Injection volume on column: 5 ul Injection volume on column: 2 ul (1290 w/ CTC samplers)	Height carry over ≤ 0.40% Area carry over ≤ 0.20%
Injection Carry Over (CD ≤ 400 bar)	Injection volume on column: 25 ul	Height carry over ≤ 1.00% Area carry over ≤ 1.00%
Response Linearity (UV-Vis)	Five concentrations of certified reference standard	Coefficient of determination (r2) ≥ 0.99900 R/F precision ≤ 5.00% RSD
Response Linearity (RID, CD)	Five concentrations of certified reference standard	Coefficient of determination (r2) ≥ 0.99500 R/F precision ≤ 10.00% RSD
Gradient Composition Accuracy (UV-Vis, CD)	20, 40, 60, and 80% steps	Accuracy ≤ 2.00%
Gradient Composition Noise and Drift (UV-Vis)	20, 40, 60, and 80 % steps	Composition noise ≤ 2.00% Composition drift ≤ 2.00%
Gradient Composition Noise and Drift (CD)	20.00, 40.00, 60.00, and 80.00% steps	Composition noise ≤ 3.50% Composition drift ≤ 3.50%
Gradient Composition Linearity (UV-Vis, CD)	Linear gradient from 100% to 0%; at start, 50:50 zone, end	Coeff. of det. (r2) ≥ 0.99900 Coeff. of det. (r2) ≥ 0.99000 (CD) Coeff. of det. (r2) ≥ 0.997 (1290 or 1260 600 bar pump with AIV)

Test	Setpoints and Parameters	Limits
Sample Temperature Accuracy	Temperature: 4.0°C Samples four vials of water in different tray positions	Diff. from setpoint ≥ -2.0°C and ≤ 5.0°C
Fraction Collection (only if installed)	Select fraction collector 1, 2, or 3; select peak- or time-based collection mode	Peak presence (qualitative)
Valve Solvent Selection (UV-Vis; only if installed; first valve included, subsequent valves for extra fee)	Select port positions 1, 2, and 3	Pos. 1 PH < Pos. 2 PH < Pos. 3 PH Pos. 2 PH ≥ 1.2 x Pos. 1 PH Pos. 3 PH ≥ 1.2 x Pos. 2 PH (PH is peak height)
Valve Column Switching (only if installed; first valve is included, subsequent valves for extra fee)	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 ≥ 1.2 x Pos. 1 Pr Pos. 3 Pr ≥ 1.2 x Pos. 2 Pr (Pr is pressure)

See corresponding attachments for recommended tests definitions for non-Agilent, capillary scale, and preparative scale systems,

Test Design and Rationale

Note: This test design covers UV absorbance, fluorescence, evaporative light scattering, refractive index, and conductivity detectors; isocratic, binary, tertiary, and quaternary pumps; most autosampler models; and fraction collectors. MS tests are covered in a separate document.

Many GMP/GLP enforcement agency inspectors now ask firms to provide a risk assessment of their equipment and computer systems plus a science-based rationale for subsequent validation and qualification testing.

GENERAL RISK STATEMENT: Any HPLC, LCMS, UHPLC, UHPLC_MS, GC, or GCMS system used for raw material testing or final drug product / medical device testing in GMP or used in formal GLP studies will likely fall into a HIGH RISK category. This risk assessment will imply the need for IQ & OQ & on-going qualification. ANY USER SPECIFIC RISK ANALYSIS SUPERCEDES THIS GENERAL RISK STATEMENT.

The rest of this section outlines the science-based rationale for each test in the Agilent hardware OQ plus a brief test design and procedure description.

The recommended set of hardware OQ tests described in this EQP derives from Agilent's interpretation of FDA, USP, and GAMP4 guidelines and other authoritative expert literature.

OQ test design incorporates both modular and holistic testing, which is a proven and regulatory acceptable approach. Direct metrology is used to test pump flow rates and thermal-controlled column compartment and autosampler modules. Holistic chemical testing is used for the evaluation of the following critical instrument characteristics: linearity, precision, signal-to-noise, and carry over.

Certified reference standards and calibrated traceable thermometers and digital flow meters are used.

Considering the number of setpoints, parameters, and conditions of each recommended OQ test, the proven concepts of worst case, range, and representative have been applied. If a property or characteristic is known to have its worst performance at one end of a range of use, this is the setpoint that should be tested and other setpoints are not required. If a property or characteristic has no known worst case, testing at the high and low points of the range of use is required. If there are too many possible use cases and conditions to realistically test (and none is a worst case), a representative sample for test is the best approach.

Pump Flow Accuracy and Precision

Description: Accuracy of flow is important for comparability between systems and transferring methods. Flow precision is critical for repeatability of peak height and area.

Procedure: A calibrated digital flow meter is attached to the waste line of the system flowing pure water at representative back pressure provided by a small guard column. Six readings are taken at each setpoint to determine the flow accuracy and precision. Flow accuracy is calculated as the absolute% difference of the mean of the six flow readings against the setpoint. The precision is calculated as the%RSD of the six flow readings. The two default setpoints (0.5ml/min and 5.0 ml/min) are evaluated in the core test. Extra setpoints and flexible test range are only available in customer-configured EQPs for flow, temperature, and some other tests. The repeat measurements of flow in the flow precision test eliminate the need for measurement of retention time precision (which is an indirect approach to determining flow precision).

Column Temperature Accuracy and Stability

Description: The thermostat accuracy is important for comparability between systems and transferring methods. Column temperature stability is critical for repeatability of peak height and area.

Procedure: A calibrated digital temperature meter and a proprietary probe are used to measure the temperature of the flowing eluent. With the use of a T-piece, the temperature probe is positioned to be in contact with the heated eluent. A typical column compartment temperature range of use is tested. At the high end of the range, after stabilization, the temperature accuracy is calculated as the absolute difference between what was measured and the setpoint. After completing this measurement at the low end of the range, six readings are taken every four minutes and temperature stability is calculated as the absolute difference between the highest and lowest measured temperatures. The temperature accuracy is calculated as the average of the six readings compared to the setpoint. All readings are reported in Celsius. Both sides of the Agilent column compartment are tested at the same time.

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, VWD, DAD, PDA, etc.): A traceable caffeine standard is used to determine the wavelength accuracy. In one procedure, for certain models, the caffeine is trapped in the flow cell and a programmable timetable is used to determine the wavelength maxima (205 and 273 nm) and minimum (245nm). For other models (for example, DAD and PDA), a caffeine injection is made and a spectrum is acquired. The spectral maxima and minimum 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.

Procedure for fluorescence detector: The detector cell is filled with pure water. Using a programmable timetable, the excitation (350 nm) and Raman band emission (397 nm) wavelengths are determined. The wavelength accuracy is determined as the absolute difference between the measured and theoretical peaks of Raman scattering (in nm).

Noise and Drift

Description: This test gives an indication of detector sensitivity and stability.

Procedure for UV absorbance detectors: Pumping water at 1 ml/min, the signal is monitored at a specified wavelength over a twenty minute period. The signal noise is calculated based on ASTM E685-93 as the average peak-to-peak noise in a number of signal segments. The drift is calculated as the slope of the linear regression for the signal.

Procedure for evaporative light scattering detectors: With no flow and the inlet to the detector capped, the signal is monitored over a twenty minute period. The signal noise is calculated based on ASTM E685-93 as the average peak-to-peak noise in a number of signal segments. The drift is calculated as the slope of the linear regression for the signal.

Procedure for refractive index detectors: Pumping water at 1 ml/min, the signal is monitored over a twenty minute period. The signal noise is calculated based on ASTM E685-93 as the average peak-to-peak noise in a number of signal segments. The drift is calculated as the slope of the linear regression for the signal.

Signal to Noise

Description: Sensitivity is a critical performance feature in quantitative and qualitative analysis. A signal-to-noise value of a representative compound at known concentration provides sensitivity statistics. This measurement is especially critical to establish level of detection.

Procedure for UV-Vis, RID: An evaluation standard is injected and the calculated height, divided by the ASTM noise monitored over a specified range, provides the signal-to- noise result.

Procedure for FLD: Using pure water in the flow cell, the signal is monitored at the emission maximum wavelength of the Raman band of water and then, using a timetable, switched to a no emission wavelength where the noise is monitored. Signal

to noise is calculated as the height of the Raman band peak divided by the monitored noise in a spectral region where no scattering is expected.

Injection Precision

Description: System precision is critical for accuracy of quantitation. Autosampler performance contributes to system precision.

Procedure: A short column is used to separate the evaluation standard from the void volume. Using a traceable standard, six injections from the same standard are made and the height, area, average height, average area,%RSD of height and%RSD of area are determined and calculated.

Injection Carry Over

Description: Low carry over from a previous injection is critical for accuracy of quantitative and reliability of qualitative analysis. This test challenges the injector system in the HPLC system.

Procedure: Following the six-injection precision test, a blank injection is made. The carry over result is calculated as a ratio of the area of any residual peak found in the blank injection to the area of the previous injection (expressed as a percentage).

Response Linearity

Description: The linearity of a detector is a critical parameter to establish for reliable and accurate quantitative results and is important for comparability between systems and transferring methods.

Procedure: A series of five traceable standards which represent typical concentrations range are injected and evaluated. The response linearity is calculated by determining the coefficient of determination (r2) of the peak areas versus concentration. It is now recognized that regression statistics alone are insufficient and non-sensitive indicators of linearity. Therefore, the% RSD of the response factors for all five peaks is also calculated. In addition, as an optional extra linearity statistic, ratios of peak areas in the set of five injections can be reported. For example, up to two ratios such as Peak 2 to Peak 1 and Peak 5 to Peak 2 can be selected in the EQP Record of Variances section.

Gradient Composition

Description: Accuracy and stability of solvent mixing online is critical for consistent and accurate quantitative analysis. Gradient composition is also important for comparability between systems and transferring methods.

Procedure: [Pre-requisite: UV-Vis or CD is installed.] An acetone tracer is used to determine the solvent gradient composition accuracy, stability, and linearity. The test challenges the system by making compositional changes from 0% to 100% in 20% increments. In addition, a linear ramp down from 100% to 0% is performed where the composition linearity is determined between ranges 95, 75, and 25%. All composition accuracies are calculated as the absolute difference between the mean composition at each setpoint and the theoretical composition. Stability is determined by the noise and drift at each composition step. Linearity is calculated from 95% to 5% in the linear portion of the gradient.

Sample Temperature Accuracy

Description: The thermostat accuracy is important for comparing systems and transfer methods.

Procedure: Four vials are filled with water and allowed to equilibrate to the temperature setpoint. Similar to the column compartment, the temperature of the water is measured using a traceable digital temperature meter and proprietary probe. Accuracy is determined as the difference between the measured temperature and the setpoint.

Fraction Collection

Only if installed

Description: It is important to demonstrate that a fraction collector can collect fractions based on peak detection or time.

Procedure: Two injections of a traceable standard are made and fractions are collected in peak-based or time-based mode. This is a qualitative test in which collected fractions are re-injected to prove that they are fractions of the traceable standard.

Valve Solvent Selection

Only if installed

Description: This test demonstrates that the valve is working correctly when a specific valve position is chosen.

Procedure (requires a UVD): Increasing acetone tracer solutions are introduced at three different valve positions. Their responses are monitored and their peak heights are recorded. There must be at least a 20% peak height differential between each peak for the test to pass.

Valve Column Switching

Only if installed

Description: This test demonstrates that the valve is not leaking when a specific valve position is chosen.

Procedure (does NOT require a UVD): Restriction capillaries are installed in the port and the system backpressure is monitored. There must be at least a 20% pressure differential between each position for the test to pass.

Updated: February 2015

www.agilent.com/chem/enterprise