

Test Descriptions and Specifications of SystemsQT for e2695 eAlliance Systems with a 2489/2998 Detector

This document describes qualification tests and lists specifications for the Empower® Systems Qualification Tool (SystemsQT™). Use this SystemsQT with e2695 eAlliance® systems with firmware 3.03 (minimum) installed with a 100 µL or 250 µL syringe that include a 2489 or 2998 detector controlled by Empower 2 with Feature Release 5 (minimum) or Empower 3 base software (minimum).

SystemsQT tests

The following manually performed and automated qualification tests are run on this system dependent on its configuration:

1. Manually performed OQ tests run before the automated tests:
 - a. Sample heater/cooler temperature accuracy
 - b. Column heater or column heater/cooler temperature accuracy
 - c. Flow rate accuracy
2. Detector response check (this is not a test and there is no pass/fail acceptance criterion)
3. Automated tests:
 - a. System readiness check
 - b. System precision 100 µL (only for e2695 eAlliance systems equipped with a 100 µL syringe)
 - c. System precision 250 µL (only for e2695 eAlliance systems equipped with a 250 µL syringe)
 - d. Wavelength accuracy
 - e. Detector linearity and sensitivity
 - f. Injector linearity and accuracy
 - g. Injector carryover
 - h. Flow rate linearity and accuracy
 - i. Compositional precision
 - j. Detector noise and drift
 - k. Signal-to-noise

Manually performed OQ tests

Sample heater/cooler temperature accuracy

Sample heater/cooler temperature accuracy is determined by measuring the temperature within the sample heater/cooler at a specific location (the sensor) and comparing it against the set-point programmed on the instrument. The test requires a calibrated temperature measuring device that is accurate to $\pm 1.0^{\circ}\text{C}$. Two temperature set-points are tested.

Failing results

If the sample heater/cooler temperature accuracy test results fail to fall within specifications, take these actions:

- Ensure the probe of the temperature measurement device is in the proper location.
- Ensure the set temperature is at least 5.0°C above or below the ambient room temperature.

Upon solving the problem, identify the cause and resolution in the system log, and then rerun the Sample heater/cooler temperature accuracy test.

Column heater or column heater/cooler temperature accuracy

If the eAlliance is equipped with a column heater or column heater/cooler, the column heater or column heater/cooler temperature accuracy is determined by measuring the temperature within the instrument at a specific location (the sensor) and comparing it against the set-point programmed on the instrument. The test requires a calibrated temperature measuring device that is accurate to $\pm 1.0^{\circ}\text{C}$. Two temperature set-points are tested.

Failing results

If the column heater or column heater/cooler temperature accuracy test results fail to fall within specifications, take these actions:

- Ensure the probe of the temperature measurement device is in the proper location.
- Ensure the set temperature is at least 5.0°C above or below the ambient room temperature.

Upon solving the problem, identify the cause and resolution in the system log, and then rerun the column heater or column heater/cooler temperature accuracy test.

Flow rate accuracy

Flow rate accuracy is determined by timing the delivery of a set volume of mobile phase at a specified flow rate and system pressure into a certified volumetric flask. A calibrated stop watch and Class A volumetric flask are used for the measurements. The specification for flow rate accuracy includes the flask accuracy and operator error allowance.

Failing results

If the flow rate accuracy test results fail to fall within specifications, take these actions:

- Look for leaks in the fluid lines and inspect all tubing connections.

- Prime the solvent lines.

Ensure the mobile phase is sufficiently degassed.

Ensure the check valves are working properly.

Upon solving the problem, identify the cause and resolution in the system log, and then rerun the flow rate accuracy test.

Detector response check

This is not a manual or automated test. There is no pass/fail acceptance criterion. To ensure that the caffeine peak height reflects the UV detector's full linear range, this check calculates the injection volume that will produce a caffeine peak between 1.80 to 2.20 absorbance units (AU) for 2998 or 1.80 to 2.40 absorbance units (AU) for 2489 for the system readiness check and the UV detector linearity and sensitivity test.

The detector response check sample set includes an equilibration step followed by two blank injections of mobile phase to equilibrate the system. This is followed by one injection of a caffeine solution. The result is processed and a report is produced that shows the injection volume that reflects the UV detector's full linear range which will be used for the system readiness check and the UV detector linearity and sensitivity test.

Automated tests

Requirement: Perform the automated tests after the manually performed OQ tests have successfully completed.

Note: If a required peak is not found, the results table will show the retention time from the processing method. The related custom field is then calculated based on the processing method's retention time.

System readiness check

Notes:

- The system readiness check is not part of the SystemsQT qualification. It merely ensures that the system is equilibrated and prepared for qualification.
- Since the system readiness check is not a qualification test, there is no RM RS report. However, the RM report will be available for printing but does not include the signature template.

Requirement: Ensure the system is equilibrated before running the system readiness check.

Run a system readiness check sample set method before you run any qualification sample sets. You must obtain results specifying that the system is ready before continuing with any qualification sample sets.

The system readiness check sample set includes an equilibration step followed by three injections of a caffeine and uracil solution, all at the same concentration and injection volume. Deviation in the retention time, area, and height of the peaks is expressed by the %RSD (relative standard deviation) and should be minimal in a well-functioning system. The %RSD of the area and height gives the injection precision, and the %RSD of the retention time is the measure of system precision. Additionally, the retention time of uracil is controlled by a time specification. The uracil peak should fall within this specification in a well-functioning system.

Determining the system ready/system not ready criteria

For the system readiness check, the caffeine results tables include a custom field that checks whether the system readiness check falls within the specifications for retention time, area, height reproducibility, and minimum height for the caffeine peak and the average retention time for the uracil peak before any

qualification test can run. When it does, the words "System Ready" appear. When it does not, the words "System Not Ready" appear. If any required peak is missing, the phrase "Not All Peaks Found" appears.

Examine the system readiness check reports to ensure these conditions are met:

- ♦ All three uracil and caffeine peaks are identified in the chromatogram. When they do not appear, refer to Table 1 for the processing method for the system readiness check that needs to be updated with the updated retention times. When you save the revised methods, include a comment that retention times were updated based on the system readiness check.
- ♦ The chromatographic peak results table names caffeine and uracil as peaks and the peak appears in the table three times.

System not ready results

When the system not ready results fail to fall within specifications, take these actions:

- ♦ Ensure the system is equilibrated.
- ♦ Check for leaks or bubbles in the syringe.
- ♦ Check that the solvent reservoirs are not empty.
- ♦ Check that the sample vials contain sufficient sample.
- ♦ Prime the solvent lines and the needle wash, and purge the injector.
- ♦ Perform a static leak test of the e2695 separations module.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the system readiness check using the wizard.

Allowable processing method changes

Table 1 shows the allowable processing method changes when "Not all peaks found" is reported. Retention time, minimum height, minimum area, and peak width are allowed to change in the processing method to detect the peak.

Table 1. Qualification tests and associated processing methods

Test Name	Processing Method
System Readiness Check	01a System Readiness Check PM
System Precision (for both 100 µL and 250 µL syringe)	020 System Precision PM
Wavelength Accuracy	030 Wavelength Accuracy PM
Detector Linearity and Sensitivity	04b Detector Lin Sens Area PM 04b Detector Lin Sens Ht PM
Injector Linearity and Accuracy	050 Injector Lin Acc PM
Injector Carryover	060 Injector Carryover PM
Flow Rate Linearity	070 Flow Rate Lin Acc PM
Compositional Precision	080 Compositional Prec PM
Signal-to-Noise	100 Signal to Noise PM

System precision test

System precision is determined by making six injections of a caffeine solution, all at the same concentration and injection volume. The chromatograms should show the caffeine peaks and the retention time, area, and height. The deviation of these six chromatograms is expressed by the %RSD, which should be minimal in a well-functioning system. The %RSD is used to determine the precision of the injector and system; the %RSD of the area and height gives the injector precision, and the %RSD of the retention time is a measure of the system precision.

Determining pass/fail criteria

For the system precision test, the corresponding custom fields check whether the test falls within specifications. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

If any required peak is missing, the phrase "Not all Peaks Found" appears. Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on System Precision test results.

Failing results

When the system precision test results fail to fall within specifications, take these actions:

- ♦ Ensure the system is equilibrated.
- ♦ Check for leaks or bubbles in the syringe.
- ♦ Check that the solvent reservoirs are not empty.
- ♦ Check that the sample vials contain sufficient sample.
- ♦ Prime the solvent lines and the needle wash, and purge the injector.
- ♦ Perform a static leak test of the e2695 separations module.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the system precision test using the wizard.

Wavelength accuracy test

The absorbance detector wavelength accuracy verification test consists of multiple parts. The first part is built into the detector and performed automatically. It verifies wavelength accuracy during each detector power-on sequence. This test utilizes a fixed wavelength Balmer emission energy line found in the deuterium lamp spectrum at 656 nm. If this emission line is not located at its expected location, an error message is displayed and normal operation cannot proceed. Waters absorbance detectors provide additional wavelength verification as part of their power-on diagnostic with an erbium nitrate filter that is placed into the light path to verify additional wavelengths at approximately 257 nm, 379 nm and 521 nm. All diagnostics mentioned above are confirmed to be within ± 1 nm of established values. The wavelength accuracy diagnostic can be performed from the instrument's front panel or from the instrument console (where available) at any time when the instrument is not running a chromatographic method.

In combination with the power-on diagnostic, the SystemsQT provides additional accuracy verification across a range of 205 nm to 656 nm by determining the λ_{max} values for caffeine at 205 and 273 nm (NIST traceable secondary standard). The ± 2 nm pass/fail criterion for this part of the test is determined by the sum of the ± 1 nm instrument accuracy specification plus the ± 0.5 nm wavelength accuracy tolerance for the test solution used, rounded up to the next whole number of ± 2 nm.

The SystemsQT part of the wavelength accuracy test for the absorbance detector is demonstrated by measuring the UV spectrum of caffeine. Caffeine is a well-characterized compound with two UV absorption maxima, one at 205 nm and one at 273 nm. Wavelength accuracy of the 2998 detector is determined by

performing two injections of caffeine standard and extracting seven 2-D channels ranging from 202 nm to 208 nm in 1nm increments from the first injection, and seven 2-D channels ranging from 270 nm to 276 nm in 1 nm increments from the second injection. Wavelength accuracy of the 2489 detector is determined by performing 14 injections of a caffeine standard ranging from 202 nm to 208 nm in 1 nm increments for the first seven injections, and from 270 nm to 276 nm in 1 nm increments for the second seven injections. Wavelength accuracy must fall within ± 2 nm of specified wavelengths.

Determining pass/fail criteria

For wavelength accuracy, the corresponding custom fields check whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

Ensure that the results table contains seven rows showing caffeine with areas, heights, and wavelengths reported. If any required peak is missing, the phrase "Not All Peaks Found" appears. Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on the wavelength accuracy test results.

Failing results

When the wavelength accuracy test results fail to fall within specifications, take these actions:

- ♦ Check that the correct sample concentrations were in the correct sample tray position in the sample manager.
- ♦ Clean the UV detector's flow cell. Refer to the Operator's Guide for the detector.

If the failure persists, the detector's optics bench requires servicing by a Waters® service representative, or you must return it to Waters for repair.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the wavelength accuracy test using the wizard.

Detector linearity and sensitivity test

The detector linearity test determines whether a detector's absorbance response is linear within its specified range. The sample set injects a series of caffeine standards at different concentrations using a constant injection volume. Empower plots area and height against the sample amount and calculates the coefficient of determination (r^2) to measure the linearity of the detector's response.

The detector's area and height sensitivity is determined by dividing the area and height by the amount, applying the caffeine standards used to determine detector linearity. The apparent sensitivity, S , is not constant over the dynamic range of the instrument, but the magnitude of the %RSD of the sensitivity is a good measure of the degree of variation. When sensitivities are measured at values of absorbance that fall on both the linear and curved portions of the calibration curve, the %RSD of the sensitivity provides a good measure of the linear dynamic range.

There is a built-in carousel test for the e2695 when you perform the detector linearity and sensitivity test. This is because that test uses five different vial locations.

Determining pass/fail criteria

For detector linearity and sensitivity, the custom field checks whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

Ensure that the calibration plots for area and height each contain five points. Ensure that the results table contains five rows showing caffeine with areas and heights are reported. If any required peak is missing, the phrase "Not All Peaks Found" appears. Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on the detector linearity and sensitivity test results.

Failing results

When the detector linearity and sensitivity test results fail to fall within specifications, take these actions:

- ♦ Check that the correct sample concentrations were in the correct sample tray position in the autosampler chamber.
- ♦ Check that solvent lines are at the bottom of the reservoirs.
- ♦ Confirm that no air bubbles are present in the flow cell.
- ♦ Confirm that the system precision test results fall within the specification.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the detector linearity and sensitivity test using the wizard.

Injector linearity and accuracy test

The injector linearity and accuracy test verifies whether delivered injection volumes are linear and accurate within specified criteria. The test injects different injection volumes of one concentration from the same vial. Empower plots the area versus the volume injected to determine injector linearity. The accuracy is determined by the y-intercept of the linearity plot.

Determining pass/fail criteria

For injector linearity and accuracy, the custom field checks whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

Ensure that the results table contains six rows showing caffeine with areas reported. If any required peak is missing, the phrase "Not All Peaks Found" appears. Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on the injector linearity and accuracy test results.

Failing results

When the injector linearity and accuracy test results fail to fall within specifications, take these actions:

- ♦ Check for leaks or bubbles in the injector syringe or seals.
- ♦ Check that the solvent reservoirs are not empty.
- ♦ Check that the sample vials contain sufficient sample.
- ♦ Prime the solvent lines and purge the injector.
- ♦ Perform a static leak test of the e2695 separations module.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the injector linearity and accuracy test using the wizard.

Injector carryover test

The injector carryover test determines the presence of sample carryover from one injection to the next. The injector carryover test starts with two pre-blank (mobile phase) injections to demonstrate that the system is free of unexplained or "ghost" peaks. This is followed by three injections of a 0.01% carryover caffeine standard (0.4 µg/mL caffeine). A challenge solution containing 4.0 mg/mL of caffeine is injected, followed by three post-blank injections. The caffeine peak in the three post-blank injections is quantitated (if detected) against the 0.01% carryover standard.

Determining pass/fail criteria

For the injector carryover test, the custom field checks whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears. If the peak is not found for post blank 1, the peak type will be displayed as "Missing Peak" and the percent carryover will have the phrase as "Not Detected" and the injector carryover test is considered to "Pass".

Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on the injector carryover test results.

Failing results

When the injector carryover test results fail to fall within specifications, take these actions:

- ♦ Ensure that you followed the steps in the SystemsQT Instrument and System Qualification Setup PDF procedure for the e2695 eAlliance system that is available on the SystemsQT Projects/Documentation DVD.
- ♦ Check that the needle wash line is in the 100% methanol solvent reservoir and the line is at the bottom of the reservoir.
- ♦ Check that the solvent reservoirs are not empty.
- ♦ Check that the solvent lines are not kinked or blocked.
- ♦ Prime the needle wash.
- ♦ Check that the blank vials are in the correct positions in the sample tray.

Requirement: If the carryover test is repeated, use new blank vials containing fresh blank solution and place them in the corresponding vial positions.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the injector carryover test using the wizard.

Flow rate linearity and accuracy test

The flow rate linearity test determines whether the solvent manager's flow rate is linear. Flow rate linearity is determined by injecting a constant volume of uracil solution (which elutes in the void volume) and varying the flow rate. The plot generated is the plot of 1/void time versus flow rate (where void time = uracil retention time), which measures the flow rate linearity. Flow rate accuracy is calculated by dividing the absolute value of the y-intercept by the slope and multiplying by 1000 (to convert from mL to μ L).

Determining pass/fail criteria

For flow rate linearity and accuracy, the custom field checks whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

Ensure that the results table contains four rows showing caffeine with areas reported. If any required peak is missing, the phrase "Not All Peaks Found" appears. Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on the flow rate linearity and accuracy test results.

Failing results

When the flow rate linearity test results fail to fall within specifications, take these actions:

- Look for a leak in the fluid lines and inspect all tubing connections.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the flow rate linearity and accuracy test using the wizard.

Compositional precision test

The compositional precision test determines whether a solvent management system can deliver a solvent mixture within acceptable criteria. The sample is injected three times using four different combinations of solvent reservoirs. If the proportioning valves are operating properly, there should be minimal variation in the retention times of caffeine. The SystemsQT project determines this by measuring the %RSD (relative standard deviation) of the retention time.

Determining pass/fail criteria

For compositional precision, the custom field checks whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

Ensure that the results table contains three rows showing caffeine with areas reported for each solvent combination. If any required peak is missing, the phrase "Not All Peaks Found" appears. Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on the compositional precision test results.

Failing results

Note: This test is extremely sensitive to ambient conditions if there is no column heater. Ensure that the chromatographic system is not under a heating or cooling vent. The results of this test can be greatly affected if the chromatographic system is located near a door that is opened and closed often.

When the compositional precision test results fail to fall within specifications, take these actions:

- Look for leaking seals or failing check valves. Perform a diagnostic test on the solvent manager.
- Ensure that all solvent lines are primed.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the compositional precision test using the wizard.

Detector noise and drift test

Data for noise and drift is collected for 15 minutes. The average peak-to-peak noise (equivalent to ASTM 685-79) is calculated for 30-second intervals from 3 to 15 minutes. The detector average noise, in μAU , is reported. Detector drift is calculated for the 3 to 15-minute interval. The drift, in mAU/Hr , is reported.

Determining pass/fail criteria

For detector noise and drift, the custom field checks whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

Failing results

When the detector noise and drift test results fail to fall within specifications, take these actions:

- ♦ Ensure that you followed the SystemsQT Instrument and System Qualification Setup PDF procedure, which appears on the SystemsQT Projects/Documentation DVD.
- ♦ Look for baseline noise cycling (short term, 30 to 60 seconds).
- ♦ Look for flow fluctuations. Stabilize fluctuations by inspecting for leaks and repairing them.

- ♦ Look for radio frequency noise (short- or long-term cycling) and eliminate the interference.
- ♦ Look for ambient temperature fluctuations, and if present, stabilize the ambient temperature.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the detector noise and drift test using the wizard.

Signal-to-noise

For the signal-to-noise test, the sample set injects a caffeine standard and signal-to-noise ratio is determined from a 1 minute range of 30 second segments. The value for signal-to-noise is derived from peak height divided by peak-to-peak noise on the same chromatogram.

Determining pass/fail criteria

For signal-to-noise, the custom field checks whether the test falls within the specification. When it does, the word "Pass" appears. When it does not, the word "Fail" appears.

Ensure that the results table contains one row showing caffeine with height and peak-to-peak noise reported. If any required peak is missing, the phrase "Not All Peaks Found" appears. Refer to Table 1 on page 4 for allowed processing method changes and possible resolution. When saving the revised methods, include a comment that these values were updated based on the signal-to-noise test results.

Failing results

When the signal-to-noise test results fail to fall within specifications, take these actions:

- Ensure that you followed the SystemsQT Instrument and System Qualification Setup PDF procedure, which appears on the SystemsQT Projects/Documentation DVD.
- Look for baseline noise cycling (short term, 30 to 60 seconds).
- Look for flow/pressure fluctuations. Stabilize fluctuations by inspecting for leaks and repairing them.
- Look for radio frequency noise (short- or long-term cycling) and eliminate the interference.
- Look for ambient temperature fluctuations, and if present, stabilize the ambient temperature.

Upon solving the problem, identify the cause and resolution in the system log. Then rerun the signal-to-noise test using the wizard.

Manual OQ test specifications

The following table lists the specifications for manual OQ tests.

Manual OQ test specifications:

Test Parameter	Specification	Unit of Measure
Sample heater/cooler temperature accuracy	±4.0 °C of set-point temperature	°C
Column heater or column heater/cooler temperature accuracy	±2.0 °C of set-point temperature	°C
Flow rate accuracy at 5 mL/min	4.94 to 5.06 mL/min	mL/min

Automated OQ test specifications

The following table lists the specifications for automated e2695 Alliance OQ tests.

Automated e2695 Alliance OQ test specifications:

Test Parameter	Specification	Unit of Measure
System readiness check	RT %RSD ≤ 1.0 Area %RSD ≤ 1.0 Height %RSD ≤ 1.5	percent
System readiness check	Uracil RT range 0.47 – 0.95	minutes
System precision (with 100 μL syringe)	RT %RSD ≤ 1.0 Area %RSD ≤ 0.50 Height %RSD ≤ 1.5	percent
System precision (with 250 μL syringe)	RT %RSD ≤ 1.0 Area %RSD ≤ 1.0 Height %RSD ≤ 1.5	percent
Wavelength accuracy	205 ± 2 , 273 ± 2	nm
Detector linearity (area and height)	$R^2 \geq 0.990$	N/A
Detector sensitivity (area and height)	Sensitivity %RSD ≤ 4.50	percent
Injector linearity	$R^2 \geq 0.9990$	N/A
Injector accuracy	Accuracy $\leq 1.0 \mu\text{L}$	μL
Injector carryover	≤ 0.01	percent
Flow rate linearity	$R^2 \geq 0.90$	N/A
Flow rate accuracy	Accuracy ≤ 100	$\mu\text{L}/\text{min}$
Compositional precision	RT %RSD ≤ 3.0	percent
Detector noise (2998)	≤ 80	μAU
Detector noise (2489)	≤ 60	μAU
Detector drift	≤ 10	mAU/Hr
Signal-to-noise	> 6000	N/A