LC Troubleshooting

Performance Qualification of LC Systems

Gerald Hall and John W. Dolan

Chromatographers can use a simple set of tests to check system performance.

Users of liquid chromatography (LC) systems have two compelling reasons to ensure that their systems are working properly. First, good science — why would anyone want to perform LC experiments on a system that isn't working at its best? Second, the government regulations for the laboratory environments in which many analysts work require testing to confirm that the instruments are doing their jobs. One of the most common series of tests is the installation, operational, and performance qualification tests familiar to most laboratory workers. Installation and operational qualification generally are performed by the vendor when a new system is installed. Performance qualification — a periodic test that ensures that an instrument operates as specified — often is the responsibility of users.

This month's "LC Troubleshooting" discusses a performance qualification test designed by PerkinElmer Analytical Instruments (Shelton, Connecticut). For the purposes of this column, we've modified the test somewhat so that it can apply to most, if not all, LC systems. The philosophy of this process is to make the test as simple and noninvasive as possible and to provide a realistic measurement of the quality of an LC system's performance. The test limits suggested in this column are realistic for an all-up test, and the test can be conducted in less than 4 h. Tests of individual components under ideal conditions could yield tighter specifications, but they generally cannot be achieved under normal operating conditions. Users, of course, are free to set other acceptance criteria.

Setup 1: Pump and Detector Tests

The first set of tests measures the ability of the pumping system to deliver and proportion the solvent accurately. For these tests, the column is removed and replaced with a piece of 0.005- or 0.007-in. i.d. tubing long enough to generate approximately 1000 psi (70 bar) back pressure when pumping water at 1 mL/min. This flow rate ensures enough pressure so that the check valves and pump seals are working in a realistic pressure environment. Reservoir A is filled with high performance liquid chromatography–grade water, and reservoir B is filled with 0.5% acetone in water (some users prefer 0.1% acetone). If the system supports four solvents, place the inlet line for C in the A reservoir and D in the B reservoir. Set the detector at 240 nm and the flow rate at 1 mL/min. Purge each reservoir line with solvent, then set the pump for 100% A (water). The detector should warm up for at least 30 min before collecting data.

Determine detector noise and drift: Record the baseline signal for at least 15 min. It might be necessary to make an injection (for example, 1 µL of water) to start the data-collection process. While the baseline is being run, collect the detector effluent in a 10-mL volumetric flask and carefully time how long it takes to collect 10.0 mL. These data are used to calculate the true flow rate. A flow rate of 1.0 mL/min ±1% (0.01 mL) is acceptable. Also observe the pump pressure during this period (some data systems will record pump pressure). A pressure variation of as much as ±30 psi (2 bar) is acceptable.

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Measure the detector noise by drawing lines to capture most of the peak-to-peak baseline noise, as Figure 1 illustrates. Measure the size of the noise envelope and compare it with the detector specification.

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(Some data systems will make a noise calculation automatically.) If the detector noise is less than or equal to fivefold the manufacturer's specification, the detector can be considered to be operating acceptably.

Draw a line through the center of the baseline signal to establish an average signal over time (see Figure 1). Determine the drift over a 15-min period and compare this number with the detector specification. If the drift is within fivefold of the specification, the detector can be considered suitable for use. The accompanying sidebar "Example Calculations" shows how to determine noise and drift.

Determine pump performance: Program the system controller to deliver the solvent mixtures in the step test, as specified in Tables I and II; if C and D reservoirs are unavailable, skip these steps. Run the program while collecting data. Some systems might require a dummy injection of mobile phase to start the data-collection process.

When the step test is complete, calculate the height of each step by comparing the (100–0%) steps with the intermediate steps. Each step should be within ±2% of the set point. Figure 2 shows the results from a pump step test.

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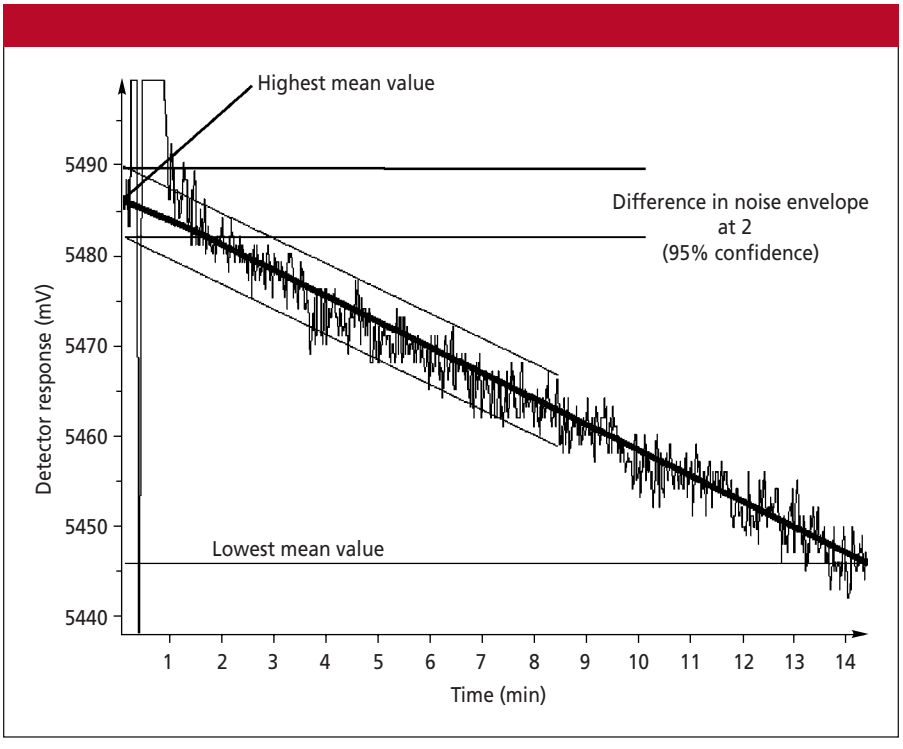

Figure 1: Detector noise and drift.

Example Calculations

$$\text{Noise} = (5.490\ \text{mV} - 5.482\ \text{mV})\left(\frac{1\ \text{V}}{1000\ \text{mV}}\right)\left(\frac{1\ \text{AU}}{1\ \text{V}}\right) = 8.0 \times 10^{-6}\ \text{AU} \quad [1]$$

$$\text{Drift} = \left(\frac{5.486\ \text{mV} - 5.446\ \text{mV}}{15\ \text{min}}\right)\left(\frac{60\ \text{min}}{1\ \text{h}}\right)\left(\frac{1\ \text{V}}{1000\ \text{mV}}\right)\left(\frac{1\ \text{AU}}{1\ \text{V}}\right) = 1.6 \times 10^{-4}\ \text{AU/h} \quad [2]$$

Although the next evaluation is not part of the PerkinElmer test, if an LC system is to be used for gradient operation, we recommend determining the dwell volume (gradient delay) at this point. Run a linear gradient; for example, 0–100% B in 20 min. Determine the time for the point on the curve at which the baseline is halfway between the initial and final plateaus (the 50% point) and subtract one-half the gradient time (10 min, in this case). The remaining time is the dwell time, which, when multiplied by the flow rate, will give the dwell volume for the system.

Setup 2: Autosampler and Detector Performance

Stop the pump and replace the contents of the A reservoir with 75:25 (v/v) methanol–water, and purge at least 30 mL through the system. Replace the restrictor tubing with a C18 column. The PerkinElmer test uses a 30 mm × 4.6 mm, 3-µm d_p column; other column configurations can be used, but the run times and retention times will need to be adjusted accordingly. Set the flow for 1.5 mL/min. The tests described here use a commercial universal test mixture (PerkinElmer), but users can make a similar mixture. The important component is anthracene, which is used to determine wavelength accuracy (see Figure 3 for an example chromatogram).

Table I: Step test program

Step	Time (min)	Flow Rate (mL/min)	A (%)	B (%)
0	5.0	5.0	100	0
1	1.0	5.0	100	0
2	3.0	5.0	0	100
3	3.0	5.0	10	90
4	3.0	5.0	48	52
5	3.0	5.0	52	48
6	3.0	5.0	90	10
7	3.0	5.0	100	0

Table II: Optional step test program

Step	Time (min)	Flow Rate (mL/min)	C (%)	D (%)
0	5.0	5.0	100	0
1	1.0	5.0	100	0
2	3.0	5.0	0	100
3	3.0	5.0	10	90
4	3.0	5.0	90	10
5	3.0	5.0	100	0

Wavelength accuracy: If a variable-wavelength UV detector is to be used, program five separate 3-min runs with wavelengths of 249, 250, 251, 252, and 253 nm. If a secondary wavelength is required, program three additional injections of the test mixture at 348, 350, and 352 nm. If a diode-array detector is to be used, analysts can perform a single run that covers the same (or a greater) wavelength range. Run the program and make 10-µL injections of the test mixture. Measure the area of anthracene for each run (the retention time should be approximately 2.3 min with the

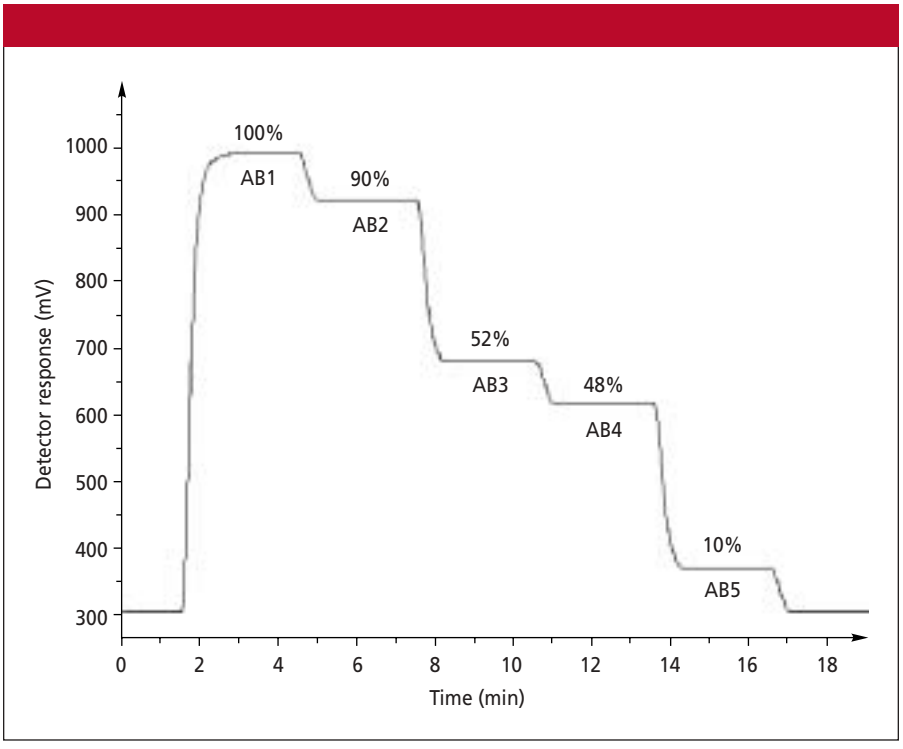

Figure 2: Pump step test.

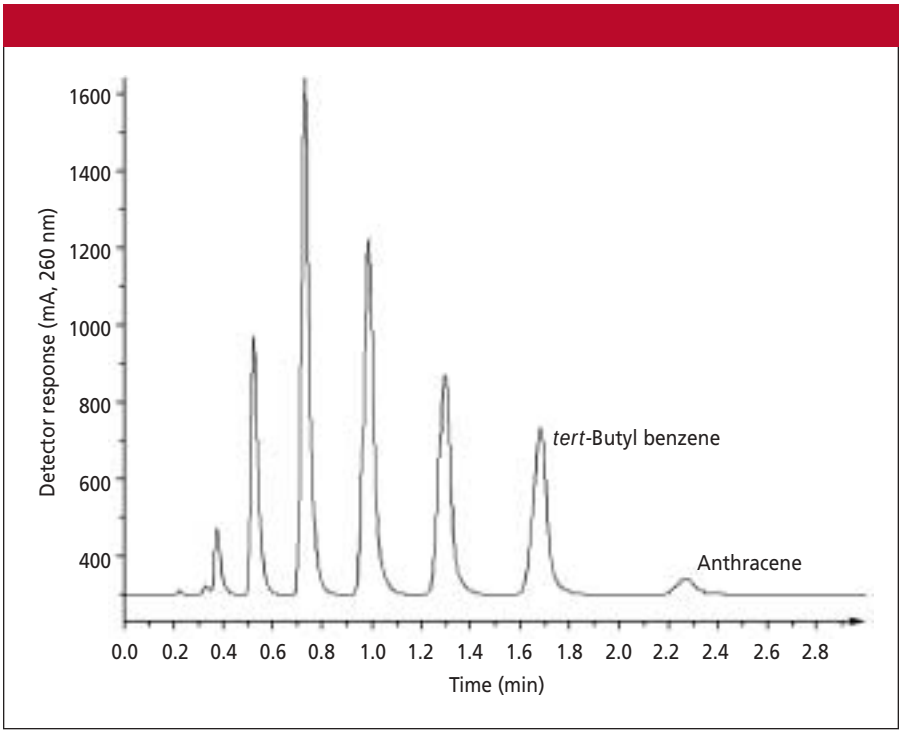

Figure 3: Example chromatogram.

30-mm column). The wavelength for maximum response should be 251 nm ±2 nm. Anthracene has a secondary maximum at 350 nm.

Autosampler performance: Set the detector wavelength to 260 nm, but keep the balance of the settings as above. Program the system to make six injections of 10 µL of the test mix, one injection of 10 µL of water, and one injection each of 5, 10, 20, 30, and 50 µL of the test mix. (We will assume that the system has a 100-µL or larger injector loop; the volumes can be adjusted so that the largest injection is 50% or less of the loop volume for this test.) Perform the runs and measure the peak area of anthracene for each run.

The autosampler precision is determined by calculating the relative standard deviation (RSD) for the area of the six 10-µL replicates. An RSD of 1% or less is acceptable. Retention reproducibility should be 0.5% RSD or less for the same injections. Carryover can be determined from the ratio of the anthracene peak in the water injection to the preceding anthracene peak area. A carryover of 0.1% or less is acceptable. The linearity of the injector for partial injections is determined by calculating the linear regression of the anthracene peak area for the 5-µL through the 50-µL injections. A linearity (r^2) of 0.998 or more is acceptable.

Documentation

The confirmation of proper system performance is incomplete until it has been fully documented. Prepare a set of tables similar to Tables III–V. Each table should include fields to record instrument identification information, test dates, and signatures of the analysts performing the tests. If any of the tests fail, users should make appropriate repairs and rerun the tests.

Regular testing of LC system performance using a test such as the one described in this column should be performed often enough that the risk of data loss from nonconformance is minimized. The frequency of testing is the choice of an individual laboratory's staff. In LC Resources' laboratory (McMinnville, Oregon), workers perform a similar test on every LC system once every three months; other laboratories might choose to undertake the tests yearly.

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If you would like more information about this holistic testing procedure, contact Gerald Hall at PerkinElmer.

Acknowledgments

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Table III: Pump performance

Test	Specification	Result	Pass–Fail
Power up and initialization diagnostics	Pass		
Flow-rate accuracy	1.0 mL/min ± 1%		
Pressure pulsation	≤30 psi		
Composition accuracy	≤2% absolute		
90% A–B	88–92%		
52% A–B	50–54%		
48% A–B	46–50%		
10% A–B	8–12%		
90% C–D	88–92%		
10% C–D	8–12%		
Dwell volume	As measured		
Retention-time reproducibility	≤0.5% RSD		

Table IV: Detector performance

Test	Specification	Result	Pass–Fail
Power up and initialization diagnostics	Pass		
Noise	5× Factory specifications		
Drift	5× Factory specifications		
Wavelength accuracy for anthracene	251 nm ± 2 nm		

Table V: Autosampler performance

Test	Specification	Result	Pass–Fail
Power up and initialization diagnostics	Pass		
Precision (n = 6)	≤1.0% RSD		
Carryover	≤0.1%		
Linearity in partial injections (r^2)	≥0.998		

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