Troubleshooting

System Suitability

Sounds simple, but what is required?

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This month's "LC Troubleshooting" column is inspired by a reader's question I received recently. She asked me what system-suitability tests were required for a liquid chromatography (LC) method. Unfortunately, this is like the situation in one of my math classes where the professor would make a big jump in logic and with a smirk, write "Q.E.D." on the blackboard — the proof is left to the student. We are given some guidelines in the various regulations, but establishment of system-suitability criteria is left up to the chromatographer. I would like to take a look at some of the guidelines and then give my opinion about what these mean to those of us who make our living doing chromatography.

USP

The *United States Pharmacopeia* (*USP*) is a well-referenced source of authoritative guidelines for chromatography of drug substance and drug product samples. The *USP* states (1):

> System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based upon the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such.

It goes on to mention resolution, column efficiency, and peak symmetry as measurements that can be made, but it makes no recommendation as to requirements for these parameters. For precision, the *USP* states (1):

> Unless otherwise specified in the individual monograph, data from five replicate injections of the analyte are used to calculate relative standard deviation (S_R) if the requirement is 2.0% or less; data from six replicate injections are used if the relative standard deviation requirement is more than 2.0%.

The *USP* tells us that the parameters in the monograph (method) do not need to be followed if other suitable operating conditions are chosen. And the final requirement, "No sample analysis is acceptable unless the requirements of system suitability have been met," tells us that we had better have some system-suitability test or we could be subject to regulatory action.

Helpful or not? Yes, the *USP* tells us that a system-suitability test must be run, that it should have some defined parameters, and that it should test the entire system with a real or surrogate sample. No, except for the guideline on precision, we are left on our own to define the system-suitability tests.

ICH

The *USP* is not the only source of information. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was formed to provide a uniform set of guidelines for international use by the pharmaceutical industry. The ICH has released a number of Guidance for Industry documents to summarize the current thinking of the organization. One of these guidances, "Q2B: Validation of Analytical Procedures: Methodology" (2), has a section devoted to system-suitability testing. Lest you get your hopes up on a definitive set of rules, here is the section quoted in its entirety:

> System suitability testing is an integral part of many analytical procedures. The tests are based upon the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suit-

ability test parameters to be established for a particular procedure depend upon the type of procedure being validated. See pharmacopeias for additional information.

The second sentence is identical to the one in the *USP*'s description. We do not gain much from the ICH except that the ICH and *USP* agree that the system should be tested as a whole.

FDA

The United States Food and Drug Administration (FDA) also issues guidances summarizing current thinking about various subjects under its jurisdiction. For workers who must measure drug concentrations in biological materials, a primary document is the "Guidance for Industry: Bioanalytical Method Validation" (3). This document includes just one sentence under the section "Application of Validated Method to Routine Drug Analysis":

> System suitability: Based upon the analyte and technique, a specific SOP [standard operating procedure] (or sample) should be identified to ensure optimum operation of the system used.

Not much help here, either. I did not check the United States Environmental Protection Agency (EPA) or other regulatory agency documents, but I suspect that I would find statements similar to this one.

Compendial Methods

The *USP* is the document that comes the closest to giving us some specific guidelines. As a last effort to get an idea of a specific example of system suitability, I decided to see what one of the *USP* methods had to say. Using the random-access selection criteria, I flipped open the *USP* and read the method that looked back at me, analysis of nitrofurantoin (4). The portion relating to system suitability says:

> Chromatograph the standard preparation, adjusting the operating parameters so that the retention time of the nitrofurantoin peak is about 8 minutes and the peak heights are about half full-scale. The resolution, R, between acetanilide and nitrofurantoin is not less than 3.0, and the relative standard deviation determined from the ratio of the peak responses in replicate injections is not more than 2.0%.

In this example, there are requirements for retention, resolution, response, and precision. These are several of the parameters suggested in the generic descriptions of system suitability considered earlier in this column.

Let us put this information from various sources plus practical laboratory experience together and see if we can come up with some more concrete ideas about how to design a system-suitability test. Consider several of the possible parameters first.

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Retention

Retention time is one of the easiest measurements to make and track in an LC run. It also is important that retention time be fairly constant, because the data system uses retention time to identify peaks; peaks that drift outside a certain retention time window might not be reported by the data system. However, retention time *per se* is not important and might vary from one nominally identical instrument setup to another. For system suitability, it is good to set a retention-time window or approximate value, as was specified in the nitrofurantoin method. This will allow for some variation in mobile-phase composition from batch to batch or a gradual change in column characteristics.

Resolution

Resolution, the separation between two peaks, is one of the most critical system-suitability parameters. System suitability, in one way of thinking, is a minivalidation that shows that the method still is valid for use. Usually, separation of one or more key peaks from other peaks is the objective of an LC method. Selecting resolution as a system-suitability parameter is one way to ensure that the critical separation is possible under the current conditions. Setting an easily attainable "resolution greater than" specification allows more flexibility than stating a specific value of resolution that might be difficult to obtain when conditions change slightly.

Response

Response, in terms of peak height or area, might or might not be an important system-suitability parameter to include in your tests. If your method is a stability-indicating assay, bioanalytical method, or other method that must detect trace concentrations of analyte, you need assurance that you can reach the detection limits necessary. In this case, a system-suitability test should include one or more injections at the lower method limit. On the other hand, if the method is used to determine only high concentrations, such as confirming content uniformity, response might not be important. As long as there is a reasonably sized peak, the method should work well. This looks to be the case for the nitrofurantoin example cited earlier — half-scale peaks are not very restrictive, but they ensure that a decent-sized peak is present.

LOD, LLOQ, and S/N

The limit of detection (LOD), lower limit of quantification (LLOQ), and signal-to-noise ratio (S/N) all relate to the quality of the detector signal at low concentrations of sample. This is another way to look at detector response. If the method is used for trace analysis, such as a stability-indicating assay, an impurities assay, or a bioanalytical assay, it is important to ensure that the method performs at the lower end. Methods that fall in this category often include one or more samples at the lowest concentration of interest to verify the LOD, LLOQ, and S/N.

Plate Number

Many workers include a column plate number as part of a system-suitability requirement. Personally, I do not put much stock in the plate number as a diagnostic. Peak response and resolution are critical parameters that depend indirectly upon plate number, so if you have these parameters as part of your test, there is not much point in measuring the plate number. For gradient methods, plate numbers are difficult to determine, and a peak width at half-height might be a more appropriate parameter. If you choose to include the plate number as a suitability parameter, be liberal with the requirement so that normal column deterioration can occur without failing suitability, or you will end up replacing columns with plenty of useful life left.

Peak Tailing

Tailing peaks can destroy a separation and reduce sensitivity below required levels.

They also can be good indicators of column deterioration or errors in mobile-phase preparation. In other cases, peak tailing might not be very important, such as when only one or two peaks are present and excess resolution exists. If the peaks in your separation tend to tail and this will have a negative impact on the method performance, include a tailing factor requirement in the system-suitability test.

Precision

Precision measurements define how reproducible the results are and give you confidence in the data you will gather. If the method uses external standardization, precision measurements assure that the autosampler is delivering the same volume each time and that sample preparation provides a consistent yield. When internal standardization is used, the internal standard will compensate for some instrument imprecision, and a precision measurement might not be necessary. Generally, six replicate injections will give you a very good idea of the precision of the method.

Accuracy

Accuracy is the measurement of how close an experimental value is to the true value. LC and LC–mass spectrometry (LC–MS) methods almost always use a standard of known concentration for comparison to unknown samples using either external or internal standardization techniques. Running a standard curve at the beginning of a run sequence or injecting replicate standards for a single-point calibration will establish the accuracy of the method. Because this normally is part of the method itself, accuracy often is not included in system suitability.

Pressure

Many laboratories set pressure limits, above which it is not recommended to run a method. For example, in my laboratory, we like to keep the pressure less than approximately 3000 psi. This helps reduce wear of system components, which increases as the pressure goes up. Also, the first sign of column failure often is an increase in pressure. For this reason, we include a pressure check as part of the system suitability in most methods to help reduce the chance of column failure or system over-pressure during a run sequence.

Blanks

Samples that do not contain any analyte can be used to determine carryover and confirm reagent purity. Such samples often are injected immediately following a high concentration standard to measure carryover. Depending upon their purpose, blank samples can comprise a blank extracted matrix, selected reagents, or just the injection solvent.

Priming Injections

Some methods require one or more priming injections before the retention, response, or tailing settles down to a constant value. This might be the case when some of the sample components are retained strongly on the column and act to deactivate unwanted interaction sites. If priming injections are required for your method, these generally should be run before the system-suitability test.

Use of Quality Control Samples

Quality control samples are spiked samples of known concentration that are interspersed with study samples during a run sequence. By back-calculating the assay value of quality control samples against a standard curve, you can show that the method is performing as desired. Some regulatory guidelines (for example, refer-

ence 3) specify performance of quality control samples, such as all quality controls above the LLOQ must be within ±15% of the standard curve response. Generally, quality control samples are not considered part of the system-suitability tests.

Summary

The preceding list of possible system-suitability tests is by no means exhaustive. If all of these tests were run for every method, there would be no time to run actual samples. It is up to the method developer or analyst to determine which set of tests will provide the most assurance that the method is running as expected. The number of tests and specific results will depend upon the application. The previous nitrofurantoin example listed a typical set of requirements: resolution, retention, precision, and response. A cleverly designed system-suitability test should get the most information out of a minimum number of injections. For example, if you do not need precision data, one injection at the upper limit of the method followed by an extracted blank and an LLOQ sample might be sufficient to generate retention, response, carryover, reagent purity, peak tailing, and pressure measurements.

You should set the system-suitability requirements so that they can be met easily if the method is working right but will fail if there is a method problem. Test requirements that are too stringent might not make the method any more reliable, and might only serve to delay the analysis of important samples. The regulatory agencies make one thing clear: system suitability should test the entire chromatographic system, not individual modules. One way of thinking about the system-suitability test is to consider it a minivalidation run just before each set of samples is run. When designed and used properly, system suitability should save you time and money — you will not waste time trying to analyze samples with a method that is not working correctly.

References

(1) *USP 27/NF 22*, United States Pharmacopoeial Convention, Rockville, Maryland, p. 2281 (2003).

(2) International Conference on Harmonization, "Guidance for Industry: Q2B Validation of Analytical Procedures: Methodology," (www.fda.gov/cedr/guidance/index.htm), p. 10 (1996).

(3) U.S. FDA, "Guidance for Industry: Bioanalytical Method Validation," (www.fda.gov/cedr/guidance/index.htm), p. 14 (2001).

(4) *USP 27*, p. 1324 (2003).

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