This month’s “LC Troubleshooting” column is inspired by a reader’s question that 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 maths classes where the professor would make a big jump in logic and with a smirk, write “QED” 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 analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend upon the type of procedure being validated. See pharmacopeias for additional information.

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 Harmonization 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 several Guidance for Industry documents to summarize the current thinking of the organization. One of these guidances, “Q2B: Validation of Analytical Procedures: Methodology”, 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 analysed constitute an integral system that can be evaluated as such. System suitability 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”. 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 System Suitability.

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Sounds simple but what is required?
For system suitability, it is good to set a retention-time window or approximate value...
No sample analysis is acceptable unless the requirements of system suitability have been met.

establish the accuracy of the method. Because this is normally part of the method itself, accuracy is often 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 is often 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 are often 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 situation 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 should generally 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 (e.g. reference 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 analyse samples with a method that is not working correctly.

**References**

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

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