

System Suitability Tests For Quantitative Chromatographic Methods

1. Introduction

Despite the importance of the system suitability test to ensure the performance of the analytical system, in many cases this is overlooked by scientists when they develop analytical methods. This article will summarize the strategy used to develop a sound system suitability test. In addition, different approaches to set suitable limits will be discussed.

2. Definition

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application. The USP Chromatography General Chapter states:

"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 on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such."

3. System Suitability, Operational Qualification And Data Acceptance Criteria

3.1 Operational Qualification (OQ)

Within the analytical community, system suitability is sometimes confused with the operational qualification performed during instrument IQ/OQ/PQ. Operational qualification is used to demonstrate that all components of the instrument, and/or the complete instrument system, are meeting performance standards (i.e., specifications). The testing methodology for operational qualification is specific to instrumental performance. In order to ensure that the chromatographic system is tested in a manner not affected by the analytical method, the system is usually qualified in a well-controlled environment. Therefore, the analytical HPLC column is removed in order to remove its contribution to the variability of the system, and a simple mobile phase should be used in the OQ of HPLC systems.

However, unlike operational qualification, the system suitability test is method specific. The system, which should already be qualified during IQ/OQ/PQ, is tested using the test conditions described in the method. Operational qualification focuses on the analytical instrument performance. System suitability, however, encompasses the complete testing system including instrument, reagents, columns and analysts.

3.2 Data Acceptance Criteria

In some analytical methods, there are data acceptance criteria that describe the criteria used to accept or reject analytical data. For example, if multiple sample preparations are prepared from a homogeneous sample composite, the precision among the replicate results (e.g. %RSD) may be used to determine the acceptability of the results. Higher than expected variability among sample replicates may indicate possible preparation error or instrumental error, therefore the results would not be acceptable.

Even though part of the system suitability tests may be performed during the analysis (see more discussion later), system suitability is generally tested before proceeding with the actual sample analysis. On the other hand, data acceptability criteria are limits used to evaluate the sample results after the sample analysis.

4. Evolution Of System Suitability

Similar to the analytical method development, the system suitability test strategy should be revised as the analysts develop more experience with the assay. In general, consistency of system performance (e.g., replicate injections of the standard) and chromatographic suitability (e.g. tailing factor, column efficiency and resolution of the critical pair) are the main components of system suitability.

4.1 Early Stage Of Method Development

During the early stage of the method development process some of the more sophisticated system suitability tests may not be practical due to the lack of experience with the method. In this stage, usually a more "generic" approach is used. For example, evaluation of the tailing factor to check chromatographic suitability, and replicate injections of the system suitability solution to check injection precision may be sufficient for an HPLC impurities assay.

In the early method development, it may be useful to perform some additional system suitability tests to evaluate the system performances under different method conditions. This information will help to develop an appropriate system suitability test strategy in the future.

4.2 As The Method Matures

As more experience is acquired for this method, a more sophisticated system suitability test may be necessary. For an HPLC impurities method intended to be stability indicating, a critical pair for resolution determination should be identified. The critical pair is defined as the two peaks with the least resolution in the chromatographic separation. Generally, a minimum resolution limit is defined for the critical pair to ensure that the separations of all other impurities are acceptable. All critical factors that will significantly impact the method performance will need to be identified. Therefore, if the resolution test results exceed the acceptance limit, the critical factors can be adjusted to optimize the system performance.

If % organic in the mobile phase has a significant impact on the resolution of the critical pair, organic composition in the mobile phase can be adjusted within a predetermined range to achieve the acceptable resolution. Therefore, system suitability strategy not only consists of the tests and limits, but also the approach used to optimize system performance when the original test result exceeds the limit.

In addition, if the method demands high method sensitivity (e.g. to analyze very low impurity levels), a detector sensitivity solution may be required to demonstrate suitable signal-to-noise from the HPLC system. These system suitability tests, combined with the typical replicate injections of the standard solution, may be used to demonstrate the system suitability for this method.

4.3 Long Term System Suitability Strategy

During the final stage of method development, there is a need to define the long-term strategy for system suitability requirements, and the practicalities for all laboratories using this method. If the system suitability test involves the use of any reference sample (i.e. isolated and characterized impurity), the laboratory needs to have enough supply of this reference sample to complete the system suitability test. However, maintaining the supply of this reference sample in the long term is usually not an easy task. If the reference sample is a degradation product of the drug substance, it is desirable to generate the reference sample *in-situ* by artificially degrading the drug substance in order to streamline the method. Therefore, extensive investigations must be done to evaluate the best approach to generate the reference sample, and to identify the critical factors needed to ensure that the degradation process is reproducible.

5. How To Set Limits

Numerous approaches can be used to set limits for system suitability tests. This depends on the experience with the method, material available and personal preference. During method development, it may be useful to perform some system suitability tests with no acceptance limit. Firstly, it is premature to set any limit during the very early stage of method development. Secondly, since experimental conditions will be varied intentionally during method development, collecting system suitability data in these experiments will help the analyst to evaluate the impact of results generated under different method conditions. This information will be used to set appropriate system suitability limits in the future.

5.1 Default Values from Regulatory Guidelines

There are numerous guidelines which detail the expected limits for typical chromatographic methods. In the current FDA guidelines on "Validation of Chromatographic Methods" , the following acceptance limits are proposed as initial criteria:

Parameter	Limit
Capacity factor	$k' > 2$
Injection precision	RSD < 1% for $n \geq 5$
Resolution	$R_s > 2$
Tailing factor	$T \leq 2$
Theoretical plate	$N > 2000$

These suggested limits may be used as a reference to set up the initial system suitability criteria in the early method development process.

5.2 Method Validation Results

Making use of the method validation results is yet another approach. During the robustness testing of method validation, critical method parameters such as mobile phase composition, column temperature are varied to mimic the day-to-day variability. Therefore, the system suitability results from these robustness experiments should reflect the expected range for the system suitability results. As a result, the limits for system suitability tests can be determined from these experiments.

This is a very effective approach since the required system suitability results can be generated during method validation, and no special study is required. However, these results only reflect the expected performance of the system, but not necessarily the minimum "performance standard" for acceptable results. For example, the minimum resolution of the critical pair from method validation may be 3.5; however, a resolution of 2.0 may still be acceptable as long as they are baseline resolved, and all other chromatographic parameters remain acceptable.

5.3 Simulated Conditions

Ideally the analyst should observe the results from a "deteriorating" system and determine the situations under which the results are no longer acceptable. One way to simulate the deterioration of the system is to use an old or artificially degraded column in the analysis. Typically, a column can be degraded artificially by numerous injections or heating at extreme pH conditions. These old columns will provide the information about the changes

in separation, peak shape and the impact of results from the less-than-ideal chromatography. If the results are adversely affected by the changes in column performance (e.g. unacceptable precision of results due to overlapping peaks), the system suitability results from these experiments will help to determine the limits for system suitability criteria.

This approach facilitates the investigation of the worst case scenario, which reflects minimum performance standard used to ensure that the chromatography is not adversely affected.

6. Points Of Consideration

6.1 Retention Time

Instrumental factors affecting retention time include dwell volume, flow rate and column temperature. The analyst should therefore expect retention time to vary in different instruments. However, in most cases, change in retention time has no direct impact on the results. As a result, it is not advisable to use retention time (or retention time window) as part of the system suitability criteria. If the analyst would like to demonstrate suitable retention characteristics in the analysis, relative retention or capacity factor may be acceptable.

6.2 Equivalent Column

Most of the analytical methods allow equivalent columns to be used to ensure that the analyst is not restricted to a particular column brand or supplier. One good way to determine the equivalence of columns from different suppliers is to allow alternate columns to be used as long as they pass system suitability criteria. Before this strategy can be used, the system suitability tests should be developed to ensure that different aspects of the column performance are covered. For example, in addition to the resolution and column efficiency tests, a qualitative comparison of the impurity profile and retention order with the results from the reference column may be required.

6.3 Quality Control Sample

The use of a quality control sample is strongly recommended in the system suitability strategy. Unlike most of the other system suitability tests that focus on sample analysis, results generated for the quality control sample can be used to demonstrate the suitability of the complete analytical system including sample preparation and sample analysis.

6.4 System Suitability during the Analysis

Historically, system suitability tests were completed before proceeding with sample analysis. However, in order to ensure that the system performance is consistent throughout, additional system suitability tests may be performed during the analysis. For example, if the method has very demanding detector sensitivity requirement, the detector sensitivity solution may need to be analyzed before and after the run to ensure satisfactory detector performance throughout the analysis.

7. Conclusion

Determination of system suitability parameters and limits will depend upon where the method is in the method development process. The criteria to be used for system suitability tests at each stage of method development will vary with the requirements of the method and its intended application.