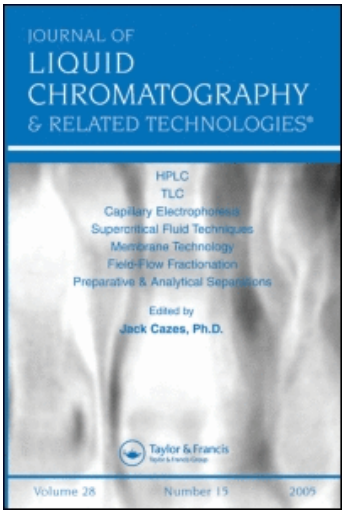

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SYSTEM SUITABILITY IN AN OPTIMIZED HPLC SYSTEM

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ABSTRACT

The relationship between calibration, validation and system suitability is demonstrated within the chemometric framework. The importance of system suitability is stressed and its interrelationship with mobile phase optimization is discussed. In addition, it is shown that sensitivity data generated in a mobile phase optimization study can be used to effectively set system suitability criteria and make improvements to an unacceptable chromatographic system.

INTRODUCTION

The term system suitability is often misused interchangeably with calibration and validation. However, the three terms are quite different and represent separate and distinct processes. While calibration (apparatus test) ensures the proper functioning of each individual component within the system, validation provides the proof that the performance characteristics of a

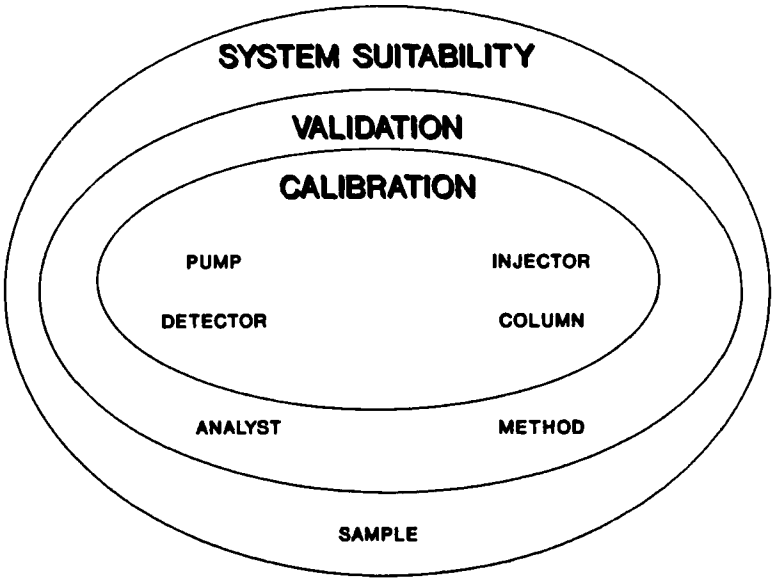

FIGURE 1 Domain for a HPLC Analytical System showing relationship between System Suitability, Validation and Calibration.

method (accuracy, precision, specificity, etc.) meet minimum requirements for its intended use (1). System suitability is the tool that ensures that the total analytical system is functioning properly at a given point in time. How these three processes interrelate has been reviewed recently (2) and can be observed in Figure 1.

As Figure 1 shows, each term encompasses progressively more domain of the analytical system. The starting point is the verification of component functionality. The validation, while it measures the performance of the method, can only be determined on

calibrated components. The system suitability test, if properly designed, then ensures the current system is comparable to the validated system and verifies the functionality of all system components. This reflects the two-fold purpose system suitability serves in a chromatographic system.

From a chemometric approach, these three processes (calibration, validation and system suitability) comprise the "quality evaluation loop" (3) and are critical to ensuring the integrity of the reported results. Methods for calibrating each system component have been reported in the literature (4-7). Likewise, protocols and data elements required for methods validation have also been reported (8-13). In contrast, system suitability has only received limited attention in the literature (2, 10-15). However, within the framework of the chemometric approach to the analytical process, system suitability is paramount to the long term quality of the results.

BACKGROUND OF SYSTEM SUITABILITY TEST

A system suitability test was first reported in the literature by King et. al. in 1974 (16). The test was originally envisioned to prevent the known variability of chromatographic components (pump, injector, column, detector) from adversely affecting official methods. In addition, by not designating specific component brands, an official endorsement of component superiority was not made. This allowed the analyst flexibility in choosing

appropriate chromatographic components that satisfied the system suitability test. However, this flexibility has been tempered by two realities.

First, in order to function adequately, many assays require a column with a certain selectivity (α) and efficiency (N). Since columns of the same type (i.e. C-18) vary from one manufacturer to another (17), such an assay may be column specific. Elucidation of the column brand through secondary sources (method originator, trial-and-error) is thus often required to successfully implement the official method.

Second, the system suitability test includes limiting parameters (precision, resolution, efficiency, capacity) that characterize the validated system. While compliance with these parameters will ensure comparability to the original method -- an important function of system suitability -- excessive chromatographic efficiency designed into the original method translates into minimum acceptable requirements for future users. To be useful to the analyst, system suitability should reflect minimum, not typical chromatographic requirements.

The importance of system suitability has been previously presented (10, 12) and has now become widely accepted. In the pharmaceutical industry, it has been adopted across the board (18) for determining the acceptability of a chromatographic system. However, while related to optimization criteria, its use in method development has been only after the selection of the final method.

A new perspective on system suitability (19) demonstrates the advantages of developing criteria earlier in the method development process. Since the information generated in a method optimization study is critical to the establishment of realistic system suitability criteria, the two operations can occur simultaneously to the advantage of both studies.

SYSTEM SUITABILITY: A NEW APPROACH

This new perspective is based on the premise that resolution needs to be only as high as will allow minimum baseline resolution. While this would seem to be intuitively obvious to any chromatographer, it has frequently not been followed in the past when system suitability criteria has been set. In many cases, a minimum resolution criteria has been established at a value that was typical for the assay in its final form. Setting suitability criteria in this manner will ensure comparability of a system to the originally validated method. However, this approach precludes the use of a system that provides baseline resolution but fails to meet an arbitrarily selected resolution minimum. For this reason, a new procedure has been established for developing system suitability criteria.

In order to develop meaningful system suitability criteria, the following three-step process should be used (19):

1) Determination of the sensitivity of the method to changes in chromatographic conditions.

2) Identification of suitability parameters that can monitor system functionality and determination of their minimum or maximum value.

3) Validation of the newly established suitability criteria for each formulation (dosage form) or each raw material (each synthetic pathway) that is assayed by the method.

Following this process, system suitability criteria can be established that will ensure comparability of the current system to the validated system, ensure proper functioning of the system, and prevent the requirement of excessive chromatographic efficiency. Data generated in a method optimization study can be used as input into steps #1 and #2 above.

SELECTION OF SYSTEM SUITABILITY CRITERIA

As has been previously studied (19), selection of appropriate system suitability criteria has not always been a well defined process. However, in the case of multiple peak chromatograms, resolution has almost universally been selected along with a precision criteria for establishing system suitability. Equation 1 below shows that resolution is the most powerful tool for testing chromatographic performance since it addresses efficiency (N), selectivity (α) and capacity (k'):

$$R_s = (1/4)(\alpha - 1)(N)^{0.5} [k'/(1 + k')] \quad (1)$$

Since optimization of the mobile phase is necessary only in the

instance were multiple peaks are present, then resolution and precision are appropriate criteria to select.

While the goal of chromatography is separation of bands, baseline resolution is the ideal for which all chromatographers strive. This is true for two reasons. First, as Figure 2a shows, baseline resolution avoids the inaccuracy involved in artificially drawing the baseline using perpendiculars and tangents. Second, Figure 2b shows that baseline resolution prevents the area from one peak being added to a second peak. The occurrence of both phenomena prevents the accurate quantitation of either peak.

Because of its simplicity, resolution is more often calculated by the formula given in Equation 2 (20):

$$R_s = \frac{2(t_2 - t_1)}{W_2 + W_1} \qquad (2)$$

where t and W represent retention times and peak widths in similar units. The disadvantage of using this equation is that it ignores peak tailing and assumes Gaussian peak shape (21). For Gaussian peaks, baseline resolution is normally achieved at a value of 1.5.

Despite this drawback, equation 2 is often used even when peaks are non-Gaussian and quite asymmetrical. Alternative approaches for determining system resolution have been published (21-22) but may be too complex for routine use. Thus, with non-Gaussian peaks, resolution factors in excess of this 1.5 value will be necessary to achieve baseline resolution.

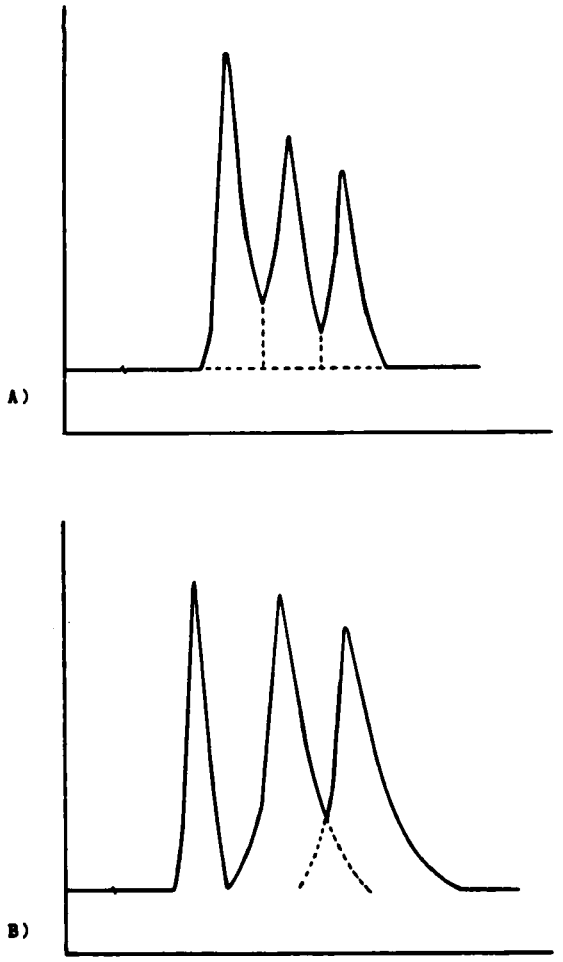

FIGURE 2 A) Chromatogram Showing Need for Tangents and Perpendiculars; B) Chromatogram Showing Peak Overlap.

With very complex mixtures, baseline resolution may not be obtainable. One of the methods for measuring percent of peak overlap (22-24) may be required to determine minimum criteria. However, if quantitation is not required, then baseline resolution will not be necessary.

METHOD OPTIMIZATION

HPLC method development has traditionally been a labor intensive, time consuming process. Characterized by repetitive manual manipulations of the mobile phase, methods were developed by a trial-and-error process requiring human decisions and interactions at every step. The introduction of instrument controllers has reduced the physical human involvement but the requirement for human cognition has remained. The advent of small, low cost computers has provided the electronic intelligence that can lead to totally automated search methods with little or no human interaction. However, the optimization criteria selected dramatically impacts the success of the optimization study (25-26).

Whether computer controlled or manually performed, mobile phase optimization involves the changing of mobile phase constituents and monitoring its effect on the chromatography. An important part of this optimization study is the elucidation of the chromatographic conditions to which a method is sensitive. These conditions are varied during the optimization study to

determine those set of chromatographic conditions that provide the optimal separation, i.e. maximum resolution in a minimum amount of time. However, the use of this sensitivity data in the development of system suitability criteria has previously gone unreported.

METHOD OPTIMIZATION AND SYSTEM SUITABILITY

While the goal of mobile phase optimization is retention, selectivity, and efficiency (27) the goal of system suitability is to ensure acceptable performance of the assay each time it is performed. Realizing that the "system" encompasses every component of a chromatograph, any of which may vary over time, the system suitability test certifies that the components as a whole are functioning properly. But as mentioned, unless system suitability criteria reflect system limitations, unnecessarily high requirements are placed on that system.

Figure 1 showed how calibration, validation and system suitability were related. Figure 3 shows how method optimization and system suitability are related. As Figure 3 shows, the sensitivity data generated in the optimization study is the same information needed in step #1 for establishing system suitability criteria. For determining the optimal system, this sensitivity data is used to maximize the resolution of the system. For setting system suitability criteria, this sensitivity data is used to minimize the system. With the optimized method set and system

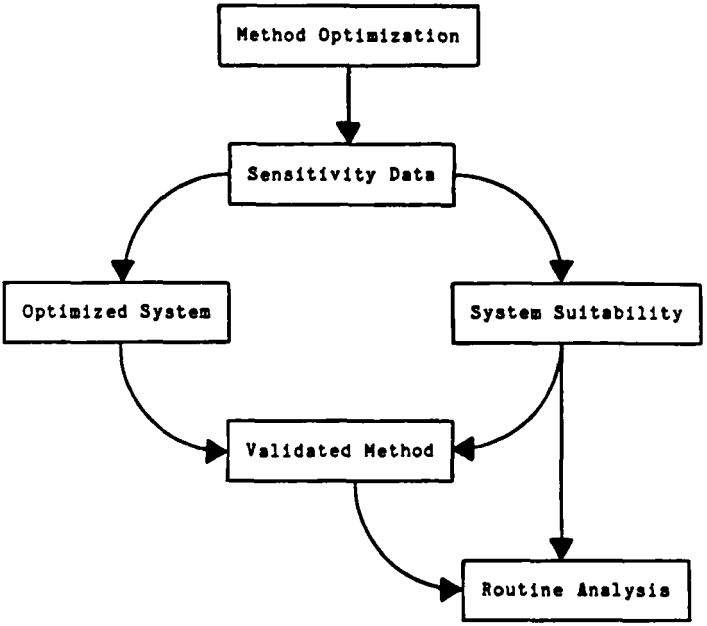

FIGURE 3 Flow Diagram from Method Optimization through Routine Analysis.

suitability criteria established, the assay is validated. Then, each time the assay is performed, system suitability parameters are calculated and compared against the minimum resolution criteria to verify continued performance of the assay as well as comparability to the originally validated method.

Figure 3 demonstrates that the key relating method optimization and system suitability is the sensitivity data. The use of this sensitivity data in maximizing resolution is widely known in mobile phase optimization studies. However, its use in system minimization is virtually unknown and might even appear

contradictory to method optimization. But by following through the process, this latter use is more easily understood and it becomes clear how both mobile phase optimization and system suitability criteria development use the same information in different ways.

Once the sensitivity of the method is determined, it is used to modify the chromatographic conditions to maximize resolution and minimize analysis time. With the optimized conditions set, the sensitivity data is then used in step #2 of the three-step process to temporarily make changes to the chromatographic system that will minimize the resolution. The purpose of this study is to determine the resolution value at which peak overlap begins to occur. As pointed out earlier, this resolution value will be greater than the theoretical value of 1.5 and will depend on the amount of peak tailing present. Once the minimum resolution is determined, the optimized conditions are restored so that validation of the method and the system suitability criteria can proceed.

Setting minimum resolution in this manner will prevent inaccurate quantitation due to peak overlap and avoid the establishment of an excessive resolution requirement. As was shown in a previous study (19), this strategy allowed the establishment of a realistic minimum resolution value that was one half of the original requirement.

TABLE I
Sensitivity Data

System Modification	Resolution
None*	12.30
pH @ 2.8	13.00
Flow @ 1.0 ml/min	13.99
pH @ 2.8 & Flow @ 1.0 ml/min	14.92

*Chromatographic conditions as given in reference 19.

An added benefit of the sensitivity data is that it provides the analyst with the information necessary to make improvements to a chromatographic system. Since it is known that aged columns lose efficiency (28), it is not surprising to find chromatographic systems that fail system suitability after repeated use. Armed with the sensitivity data from a method optimization, modifications can be easily made to the chromatographic system to improve the resolution above the minimum requirement. As Table I shows, this improvement is often greatly enhanced when conditions are modified simultaneously (19), information that could only feasibly have been obtained from an optimization study. Had this sensitivity data not been known beforehand, a lengthy trial-and-error approach would have been required to determine how the system could be improved.

However, it must be stressed that this approach will only work if system suitability criteria is set to reflect minimum rather than optimal conditions. If the resolution value set as the minimum for the assay is at the maximum for that local optima, then improvements can not be made to the system. The only choice is to replace the column and as mentioned earlier, the inherent variability of HPLC columns may even preclude this from being a solution. To allow improvements to unacceptable systems and avoid excessive resolution requirements, resolution criteria should be set at the minimum that will allow baseline resolution.

CONCLUSIONS

In conclusion, it can be seen that the system suitability test is a vital tool for the routine quality control of chromatographic assays. Additionally, it can be seen that the sensitivity data generated in a mobile phase optimization study can facilitate the setting of and compliance with these criteria. By judiciously setting system suitability parameters based on realistic minimum requirements, the test will not only ensure comparability to the original validated method, it will distinguish acceptable from unacceptable systems. If a system fails the system suitability criteria, the sensitivity information generated in the mobile phase optimization study can then be used to make improvements to the system.

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