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Chromatographic system suitability tests — what should we be using?*

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Abstract: System suitability tests are applied to chromatographic analytical methods to confirm the methods' continuing suitability for use on different occasions following their initial validation. The paper discusses the checks and controls which have traditionally been applied, questions their appropriateness and makes recommendations for others that should be considered.

Keywords: *Chromatographic methods; system suitability tests; validation.*

Introduction

The validation of an analytical method consists of a series of experiments to confirm that the method produces results which have the requisite accuracy and precision for the method's intended use.

The validation exercise confirms that the method was suitable for use on a particular occasion with a particular system. To confirm the method's continuing suitability for use with different systems, checks or system suitability tests (SSTs) should be run each time the method is used.

SSTs were first proposed by workers from the FDA in 1974 [1]. The 1990 edition of the USP [2] describes three SSTs for chromatographic methods: reproducibility of replicate injections, peak tailing factor (or peak asymmetry) and resolution. Earlier, Roman [3] had suggested that SSTs controlling peak retention and chromatographic column efficiency should also be included. A survey by Wilson and Fogarty [4] suggested that the majority of new USP monographs appearing between 1987 and 1988 specified SSTs for reproducibility and resolution only. This bears out an earlier view [5] which suggested that it was inappropriate to include peak tailing and efficiency among SSTs.

It is the authors' opinion that SSTs should be considered for each of the parameters which are checked during the validation of the method. Table 1 describes current practice and shows that there are a number of gaps in what is presently being done. Suggestions are made on how these gaps might be filled. In addition, as suggested by other authors [5], the usefulness of SSTs for peak tailing and column efficiency is questioned.

Method Validation Parameters and System Suitability Tests

Ruggedness and robustness

A series of experiments, conducted during the validation of the method, perhaps using the Plackett and Burman approach [6], will have confirmed the effect of operational parameters on the method's suitability (ruggedness) or its capacity to be transferred to another laboratory (robustness). The experiments will have identified critical parameters. In the main, the compliance of the method with the SSTs described below (for accuracy, precision, etc.) will be confirmation that these parameters are operating within their defined ranges. However, it may be appropriate to confirm this by the inclusion of a specific check, for example on the flow rate or column temperature or time of shaking during the sample preparation or on any other parameter identified as being critical.

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Table 1
Method validation parameters and system suitability tests applied

Method validation parameter	Traditional SSTs	Recommended SSTs
Ruggedness/robustness	None	Check on critical method parameters
Accuracy	None	Control samples Re-extraction Mass balance
Precision	RSD of replicate injections	RSD of replicate injections RSD of replicate sample preparations
Selectivity	Resolution check	Resolution check (using impure standards or samples of the impurities)
Stability of measurement solutions/system	None	Comparison of standards at start and end of run
Linearity	None	Use of standards at different concentrations
Limit of detection/quantitation (signal-to-noise ratio)	None	Calculation of H/s_B ratio
General acceptability	None	Chromatogram compared to reference chromatogram
None	Tailing factor/peak asymmetry	None
None	Column efficiency/plate count	None

Accuracy

It is an essential feature of an analytical method that it be accurate. This will be confirmed during the validation experiments by spiking (recovery) experiments or by a cross comparison with a separately validated method.

The suitability of a system for use with the method may be confirmed by:

Control samples. Such samples have an accurately defined content and, if stable, can be analysed alongside the test samples. The method is suitable for use if results are obtained for the control samples which fall within defined acceptance criteria.

Mass balance. This approach compares the values obtained for assay and related impurities and requires that a drop in assay is matched by an increase in impurities before the results from either method can be accepted as suitable.

Sample re-extraction. This approach requires that, for methods involving extraction from a solid matrix, the residue is re-extracted to confirm that no more drug can be got out. A limit might be put on the maximum amount of drug which it is acceptable to obtain during the re-extraction.

Precision

Precision is confirmed by SSTs which monitor the relative standard deviation of replicate sample or standard applications to the chromatographic system. It is usually unnecessary to perform these SSTs prior to the commencement of the analyses if using an autosampler of proven reliability and precision. Consequently the SST can be performed based on a series of standards interspersed throughout the chromatographic run. Failure to comply with the acceptance criterion (typically RSD $\nmid 1\%$) may be indicative of instrumental drift and can be overcome by using standard bracketting to divide the run into "compliant" portions. (In this circumstance the RSD for all standard injections might be 1–2% with that of the standards used for bracketting $\nmid 1\%$.)

The precision of the method as a whole can be monitored by performing replicate determinations on each sample, provided the sample is homogeneous.

Selectivity

The principle reason for choosing a chromatographic method is its ability to resolve the analyte(s) from other interfering species. It is therefore essential that the method continues to fulfil this objective. An SST for selectivity will require that a certain resolution is achieved between the analyte and the closest running interfering species.

This interfering species can take a number of forms: separately available degradation products or synthetic impurities; samples of non-analyte related interfering species (e.g. pharmaceutical excipients, blank biological matrices etc.); mixtures of components with the analyte(s) (e.g. from forced degradation

studies or specific batches of the analyte known to contain the interfering species).

If separately available, the interfering species can be added to the analyte to produce a resolution check solution. A limit for the resolution of $\nless 1.2–1.5$ is usually taken as representing baseline resolution. Alternatively, in the absence of a sample of the interfering species, the test may be applied to one of the mixtures described above. It may also be possible to incorporate the resolution check into the normal analytical standard and thereby confirm the method's suitability for use and determine an assay value simultaneously. On occasion it may be necessary to specify a resolution criterion >1.5. This will be likely if the closest running interfering species is not available. In this situation a criterion will be set for the available impurity such that there is "room" within which the closest running species might fit were it to occur.

The calculation of a resolution term may be inappropriate when a method is used to quantify a small impurity in the presence of a much larger component. (Consider Fig. 1.) In such cases a pragmatic approach might be taken and resolution confirmed by visual inspection or by the correctness of the baseline integrator codes.

An alternative approach is to consider a resolution requirement based on the ratio of the peak height of the impurity measured to the "baseline" on each side of the peak (*a* and *b*). A resolution criterion might then be set such that $a/b \nless \chi$.

The advent of photodiode array detectors has introduced the possibility of determining the selectivity of the method by evaluating the homogeneity of the analyte peak. This may be achieved by comparing the spectra of the upslope, apex and downslope of the peak or by comparison of other spectroscopic features of the peak, e.g. ratiograms or peak purity parameters.

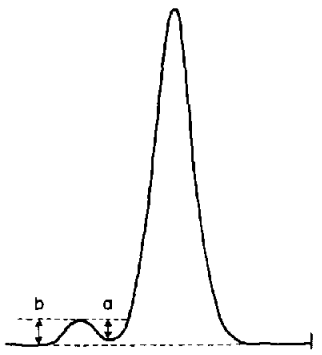

Figure 1
Resolution criterion for use where a minor component is to be resolved from a major component. The resolution requirement should fulfil: $a/b \nless x$.

Although this would appear to be a useful approach, in practice its value is somewhat limited. The level of an impurity which might typically be expected in a pharmaceutical product is low (usually below 1%). In addition, it is to be expected that any impurities which are not resolved are likely to be structurally similar and hence have very similar UV spectra. Both these probabilities place considerable demands on the detector's sensitivity and on the algorithms employed for determining peak homogeneity.

Stability of the measurement system

With the advent of modern autosamplers it has become more important that measurement solutions remain stable during the course of the analysis.

An SST should be applied to confirm the similarity of a standard injection made at the start and at the end of the chromatographic run. This test might compare peak areas (differences being attributable to solution instability of the analyte — with or without the formation of detectable degradation products, or to drift in the measurement system), peak shape (tailing factor and plate count) (differences suggesting a change in chromatographic column performance during the run) and peak retention time (again indicative of a drift in instrumental parameters). An acceptance criterion for each of these factors might specify the amount by which the factor (peak area, shape or retention time) might differ from the start to the end of the run.

In TLC, the instability of the analyte on the chromatographic plate is a common problem. An SST to monitor this might be a comparison of the impurity profile of a sample or standard spotted as the first application compared with the same solution spotted as the last application.

Linearity

Once validated, the linearity of a method is rarely checked in everyday used even though there are a number of factors which may cause the method to be non-linear (e.g. incorrectly set integration parameters or particularly sharp

peaks with maximum signals outside of the detector's linear range).

A check on the linearity of the method can be readily achieved [7] by the use of three or more standard solutions covering the range of say 80–120% of the nominal analyte concentration.

The calculation of results can utilize the mean response factor calculated for all of the standards. The SST for linearity would check the slope, intercept and linearity of the linear regression line. Acceptance criteria might be: slope — equivalent to that found during validation; intercept — <2% of the nominal analyte response; linearity — regression line confirmed linear.

Limit of detection and quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) determinations should form part of the validation of any chromatographic method for the determination of trace components. To ensure that a method is performing to these limits during normal usage signal-to-noise (S/N) ratios should be determined.

In order to calculate this parameter, a noise dependent factor such as the standard deviation of the baseline (s_B) is determined [8]. This term is derived from the variation in the baseline noise over a region of a blank chromatogram situated where the analyte peak is expected and covering 20 times the width of the analyte peak. The response of a specified standard (H) then allows calculation of the S/N ratio, i.e. $S/N = H/s_B$. The SST protocol will then determine a minimum acceptable value for this term.

Recognition of the importance of this parameter has been demonstrated by European Pharmacopoeial Authorities in recent PhEur monographs on GC and HPLC. In situations where impurities are to be determined by comparison with dilute standards then a minimum S/N ratio requirement may be assigned to the most dilute standard to ensure that results are obtained with appropriate precision and accuracy. As a general rule a requirement for S/N ≮5 might be selected.

For a TLC method, a spot can be applied to the chromatoplate at a level just above the LOD. The method is valid if the spot is detectable. A refinement of this approach, when developing the plate by staining, would be to apply a spot to each end of the chromatoplate. Visualization of both spots then confirms that the plate has been uniformly stained.

Tailing factor and column efficiency

It is questionable whether in absolute terms either of these SSTs add anything to the suitability for use of a method.

Consider the two peaks in Fig. 2. Peak A has a tailing factor of approximately 4 while peak B has a tailing factor of about 1. Normal SST requirements would regard peak B as "acceptable" while rejecting peak A. Logic, however, suggests that in terms of (1) LOD and LOQ, and (2) selectivity, — peak A is much superior.

From the authors' experience the criterion for an SST using column efficiency depends on the values which happened to be obtained during the course of method validation. Little attempt is usually made to determine if failure to comply with the criterion means that the method is any less valid.

The column efficiency and tailing factor values will depend heavily upon the peak that is used for their calculation. In a method for trace components, the error in the determination of these terms on a small analyte peak will be very large. Hence the value of the terms under these circumstances is even more questionable.

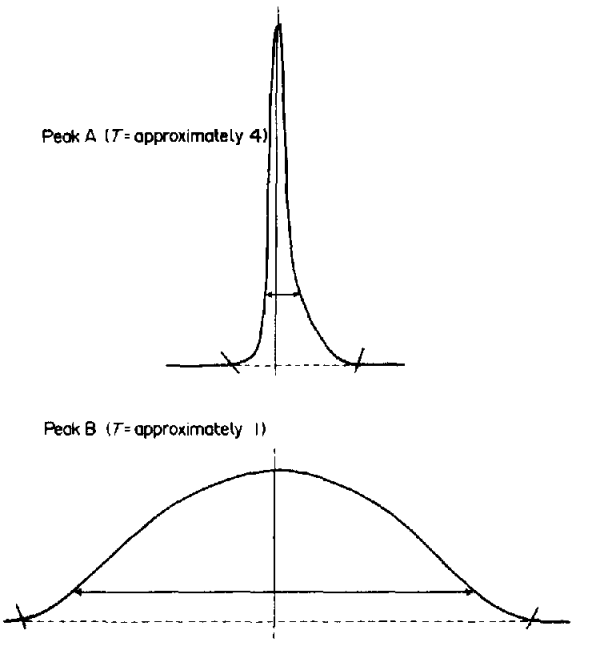

Figure 2
Comparison of the tailing factor values obtained for a sharp asymmetrical peak and a broad symmetrical peak. Peak A (T = approximately 4), peak B (T = approximately 1).

An inefficient column will be characterized by a low column efficiency, but also by an inability to separate the components of interest. It is this latter consideration which should be paramount and which can be adequately monitored by the selectivity SST.

One use of tailing factor and column efficiency is in a relative rather than absolute way. In this approach it is the change which occurs in the parameters throughout a run which is important rather than their absolute values.

Another use of tailing factor might be to control peak integration (asymmetric peaks being harder to accurately and precisely integrate) [9]. However, both of these factors should be effectively controlled by previously discussed SSTs. A further point to make considering Fig. 2 again, is that a distinction could be made between peaks A and B if a factor combining both tailing (T) and efficiency (E) is proposed. For example a factor based on E/T would allow peaks A and B to be distinguished providing peak B's efficiency was <0.25 peak A's.

Other system suitability tests

Before any method is deemed suitable for use it is essential that a chromatogram of a standard, sample or text mix is compared with a reference chromatogram. This serves a number of purposes: allows identification of the peaks or spots of interest; illustrates what is regarded as acceptable integration; illustrates what is regarded as acceptable peak shape/appearance; removes the need to apply limits to peak retention times or capacity factors.

The accurate integration of the chromatographic peaks will be essential if the method is used quantitatively. It is difficult to envisage an SST which specifically covers this concern. The SSTs suggested for accuracy, precision, linearity, LOD/LOQ and the comparison of the chromatogram with a reference chromatogram will all indirectly check on the accuracy and precision of integration.

Conclusion

The value of some of the currently performed SSTs is questioned and alternatives are proposed. When considering SSTs it is important that the method is looked at as a whole. Table 1 summarizes the suggested SSTs which could be used to confirm a system's suitability for use with respect to all of the parameters examined during validation.

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