System Suitability Tests in Regulatory Liquid and Gas Chromatographic Methods: Adjustments Versus Modifications

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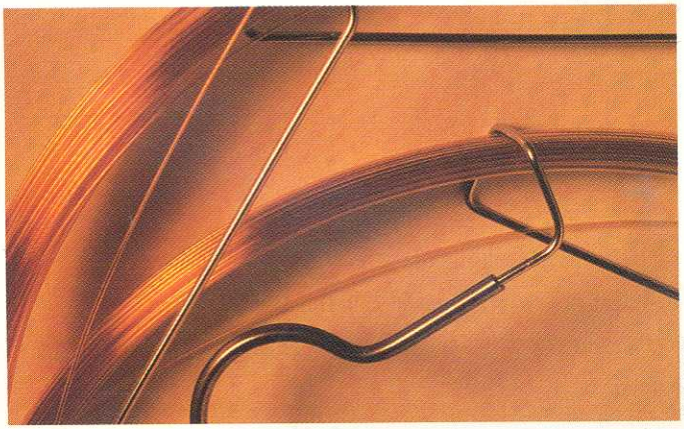

SUPELCO

Regulatory chromatographic methods, such as those described in the *United States Pharmacopeia,* may need adjustments in operating conditions to meet the requirements of system suitability tests. This article suggests ranges within which such a method may be adjusted and still be considered the regulatory method, i.e., not a "modified" method that requires complete revalidation. Note: This article does not represent FDA policy.

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Regulatory methods include those in the *United States Pharmacopeia/National Formulary (USP/NF)*, in *Official Methods of Analysis* published by the Association of Official Analytical Chemists International (AOACI), and in approved new drug applications and abbreviated new drug applications. Such methods are recognized in the Food, Drug, and Cosmetic Act and by the current good manufacturing practice regulations as having special significance in the process of drug regulation in the United States. The *Code of Federal Regulations* states (1):

> If the method employed is in the current revision of the *United States Pharmacopeia, National Formulary, Association of Official Analytical Chemists, Book of Methods,* or in other recognized standard references, or is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice.

Thus, if a laboratory intends simply to reference a USP method, it must use the method as written ("not modified") to avoid performing a full validation of a "modified" regulatory method.

Most regulatory gas chromatographic (GC) and liquid chromatographic (LC) methods provide tests for system suitability, which may include resolution, tailing, column efficiency, precision, and others. Although there is often little difficulty getting satisfactory values for a regulatory chromatographic method, one must sometimes adjust conditions to meet system suitability requirements.

One point of view within FDA holds that no adjustments are to be permitted in regulatory chromatographic procedures. Yet *USP 23,* a major source of regulatory chromatographic procedures, states in its general chapter on chromatography, "Adjustments of operating conditions to meet system suitability requirements may be necessary" (2). Given that adjustments are permitted, if necessary, it becomes of interest to the regulatory agency and the regulated

industry to provide answers to these questions: At what point does one cross over from *adjusting* to *modifying* a regulatory chromatographic method? At what point should a laboratory conduct revalidation because the procedure is no longer the regulatory method?

The proposals presented in this article are intended to initiate discussion among all parties. It would be impossible to address all topics here, so we are not covering, for example, recommendations for gradient LC and certain parameters of GC such as adjustments in coating thickness in capillary columns.

WHY ADJUST CONDITIONS?

Perhaps the most common reason for a system to fail system suitability requirements is failure of the column, which can often be remedied by column replacement and a repeat of the system suitability tests. Column failure is suggested by plate numbers that are too low, asymmetry (or tailing) factors that are too high, or overpressuring of the system.

Columns are sometimes changed for other purposes such as for greater mass sensitivity or reduced solvent consumption. For example, the column diameter, length, or particle size may be reduced. Such changes may also require a change in flow rate to meet system-pressure and run-time requirements. If limits of detection are satisfactory with the original column, the change in injection volume should be scaled with the square of the change in the radius. If this scaling is not done, caution should be exercised to prevent overloading the column.

If column replacement fails to produce acceptable system suitability results, one should consider possible batch-to-batch differences in column characteristics. In most USP methods, column manufacturers are not specified; instead, the type of packing is given. Although manufacturers take considerable pains to provide columns that will give a similar separation for a particular procedure, it is not uncommon to encounter significant changes in retention from column to column. Small changes in relative retention can mean the difference between completely resolved and completely overlapped adjacent band pairs. The latter circumstance often requires adjustment of conditions to meet system suitability requirements.

Before making any adjustments in chromatographic conditions, one should consider correcting the following possible causes of unacceptable system suitability results.

Error in conditions. Possible errors in the preparation of the mobile phase or analyte solutions and errors in instrumental settings for flow rate, temperature, etc., should be considered before one attributes system suitability failures to other causes. One should not try to compensate for errors by further adjusting other conditions.

Uncontrolled conditions. Column temperature control is highly recommended. If a column is not thermostated and ambient temperature fluctuates, retention and resolution of analytes can change. A substantial fraction of all LC procedures are carried out without column thermostating, and this can lead to a failure during system suitability testing. In LC, if neither the mobile phase nor the analyte solvent is buffered, unexpected changes in pH of the solutions can lead to changes in the separation. It is undesirable to change chromatographic conditions in an attempt to compensate for short-term changes in the temperature of an unthermostated column or differences in pH among test solutions and standard solutions.

Change of column characteristics during use. As noted earlier, change of column characteristics is a common cause of system suitability failure. When a change in column characteristics results from column aging, the operator may be tempted to adjust conditions to compensate, but the best solution is often to replace the column. For procedures in which column life is short and frequent column changes would be costly, an argument could be made that one can adjust conditions to compensate for column aging. This position is defensible, perhaps, for older procedures that use columns that were available several years ago. But when the currently available, more stable columns are used, adjusting conditions as a response to column aging should be discouraged.

ADJUSTMENT VERSUS MODIFICATION

Following are suggested limits to differentiate adjustments, which do not require revalidation of a regulatory method, from modifications, which do. These concepts do not represent official FDA policy but are the opinions of the authors. The limits suggested here resulted from advice and input from FDA, USP, and industry chemists.

Adjustments to bring a given analytical system into compliance with system suitability requirements apply only to that system and cannot be transferred to another. With each analytical system, one should start with the regulatory method, as it was written, and adjust it only if necessary.

The suggestions that follow apply when an analyst is adjusting a system to pass system suitability and wishes simply to give a reference to the method and use it without validating it and without obtaining ruggedness data. If the limits suggested later must be exceeded for the chromatographic system to pass system suitability, one should no longer consider the method as the regulatory method; i.e., the method has been modified. In such instances, the methodology problems should be communicated to the appropriate institution such as USP, AOACI, or FDA.

If a laboratory wishes to use the modified regulatory method, its staff should revalidate the method to ensure it is still suitable for its intended use under the modified conditions. If a laboratory has verified that the method is rugged enough to permit wider adjustments than those suggested below, its validation data take precedence. For example, the limit recommended below for adjustment in the concentration of a salt in the buffer solution used as a component of the mobile phase is $\pm 10\%$. If a laboratory has data to show that the method is suitable for use with a 15% change in concentration of a salt in the buffer solution, the laboratory may ignore the $\pm 10\%$ limit suggested here, but the change could not be considered an adjustment and the modified method would require revalidation.

SUGGESTED LIMITS

The purpose of these adjustments is to bring a chromatographic system into compliance with system suitability requirements. All the materials cited in the system suitability test and all the reference standards cited in the method must be used to monitor the chromatography to ensure that the adjustments are improving the system's performance.

pH of mobile phase (LC). One should not put pH electrodes directly into the mobile phase. Aqueous-organic mixtures are extremely complex; it is difficult to apply pH theory to them, and pH measurements in mixed media are usually unreliable. In this article it is assumed that verification of pH by measurement is performed on the aqueous buffer before a proportion of it is mixed into the mobile phase. The pH of the buffer should be formulated and/or verified by measurement to fall as close to the desired value as possible. Unless a wider range is provided in the method, pH adjustments within ±0.2 unit of the value specified in the method are allowed. All reference standards must be available for all analytes, and they must be used to show that the chromatographic results are improved by the adjustment in pH.

Concentration of salts in buffer (LC). In the buffer to be used as a component of the mobile phase, the concentration of buffer salts may be adjusted as much as ±10% so long as the buffer pH remains within ±0.2 unit of the value (or range) specified in the method.

Ratio of components in mobile phase (LC). The amount of the minor component(s) may be adjusted by ±30% relative or ±2% absolute, whichever is larger. However, the change in any component should not exceed ±10% absolute, nor should the final concentration of any specified component be reduced to zero.

Examples for binary mixtures:

- The method specifies a ratio of 50:50. Thirty percent of 50 is 15% absolute, but this exceeds the maximum permitted change in either component of ±10% absolute. Therefore, the mobile phase ratio may be adjusted from 40:60 to 60:40.
- The method states 95:5. Thirty percent of 5 is 1.5% absolute. However, adjustments as much as ±2% absolute are allowed. Therefore, the ratio may be adjusted within the range 93:7 to 97:3.
- The method states 2:98. In this case, 30% of 2 is 0.6% absolute. A −2% absolute adjustment is not allowed because it would reduce the amount of the first component to zero. Thus, the maximum allowed adjustment is 1.4:98.6 to 2.6:97.4.

Example for ternary mixtures: The method specifies ratios of 60:35:5. For the second component: Thirty percent of 35 is 10.5% absolute, but this exceeds the maximum permitted change in any component of ±10% absolute; the second component may therefore be adjusted within the range 25–45% absolute. For the third component: Thirty percent of 5 is 1.5% absolute. Since ±2% absolute is permitted and offers more freedom, the third component may be adjusted within the range 3–7% absolute. In all cases, a sufficient quantity of the first component is used to total 100%.

Detector wavelength (LC). Deviations from the wavelength(s) designated in the method are not permitted. The procedure specified by the detector manufacturer should be used to verify that error in the detector wavelength is no greater than ±3 nm (less, if possible). When a method requires the use of area percent to estimate impurities based on the area of another compound or on total peak area, one must avoid inadvertently changing relative sensitivities through misadjustment or inaccuracy of detector wavelength.

Column length (LC and GC). Column length may be adjusted as much as ±70%. Example: The method says to use an LC column 250 mm in length. One may substitute a column between 75 and 425 mm in length.

Column inner diameter (LC and GC). Column inner diameter may be adjusted as much as ±25%. Example: The method says to use an LC column of 3.9 mm diameter. One may use a column between 3.0 and 4.9 mm i.d.

Changing the column's inner diameter will change the linear velocity of the mobile phase if a constant flow rate is maintained. If column i.d. is changed, similar retention times will be achieved if the flow rate is changed proportionally to the change in column cross-sectional area.

Particle size (LC). Particle size may be reduced as much as 50%. Example: The method specifies 5-µm particles. One may use 3.0- or 3.5-µm particles.

Flow rate (LC and GC). Flow rate may be adjusted as much as ±50%. Example: The LC method says "about 1.5 mL per min" but the system is overpressuring. One may decrease the flow rate to 1.0 mL per min or 0.75 mL per min. Faster is better for completing more assays each day, but slower is often better for improved resolution, peak shape, etc.

Injection volume (LC and GC). Changing injection volume must not adversely affect factors such as baseline, peak shapes, resolution, and retention times. Keeping this proviso in mind, injection volume may be increased up to twice the volume specified in the method. Injection volume may be decreased as much as one likes, consistent with acceptable precision and limit of detection.

Column temperature (LC). The use of a column oven will often markedly improve control and reproducibility of retention time. Column temperature may be adjusted as much as 20 °C to meet requirements of system suitability. Such a change may be combined with a change in the ratio of solvents in the mobile phase to hold run time constant and still allow modest changes in selectivity (3).

Column temperature (GC). Column temperature may be adjusted within ±2% in terms of absolute temperature: ±5 °C near −20 °C; ±6 °C near 50 °C; ±7 °C near 100 °C; ±10 °C near 200 °C; etc.

Oven temperature program (GC). Adjustment of temperatures is allowed as given directly above. For the times the temperatures are to be held or the times required to change from one temperature to the next, an adjustment of ±20% is permitted.

COMMENTS ON ADJUSTING THE CHROMATOGRAPHIC SYSTEM

These adjustments are made only if necessary and are allowed only while one is tuning the chromatographic system to meet system suitability requirements; thereafter, during analysis these conditions must be held as constant as possible.

If one must exceed these suggested ranges to satisfy the system suitability requirements, it amounts to modifying the method, which can then no longer be considered the regulatory method. One should conduct validation or ruggedness tests to ensure the method is still suitable for its intended use under the conditions chosen. The difficulties should be reported to the appropriate organization, such as USP, AOACI, or FDA, to stimulate discussion on whether a change in the regulatory method is needed.

CHANGES OF EQUIPMENT MODULES OR COLUMNS

If a module (injector, pump, detector, integrator, etc.) or a column is changed, how should one requalify the system? In general one should, as a minimum, repeat the system suitability tests to ensure the system meets requirements.

For the specific case in which an analytical unit is devoted to repetitive, routine testing of one type of product, one may consider the use of a quality control sample to monitor one aspect of system reliability. A QC sample can give additional assurance of the repeatability of the system. One possible scheme is to run the QC sample at regular intervals, e.g., daily. Plot the results on a QC chart. After sufficient results have been obtained, one may add two lines representing the desired tolerance, ±3 standard deviations, for example.

Results between the two lines do not guarantee total quality of sample results but do show the system is operating in a repeatable fashion. Results outside the two lines might, for example, be warnings that something has happened to the system. A sudden break suggests a critical system change has occurred. Quality assurance textbooks give many other ways to interpret these charts.

After a pump or column has been changed, one should again ensure the system passes the system suitability tests. In addition, good results should continue to appear on the QC chart with no breaks.

The use of QC samples and QC charts is a suggestion and has no legal status. As with most aspects of quality control, each laboratory must decide whether the additional time required to make the measurements will result in commensurate improvement in the quality of the laboratory results.

DISCUSSION

A commonly asked question is, How can we permit all these changes and still regard the method as the regulatory method? For example, adjustment of the ratios of components in the mobile phase may result in a large, unexpected, and perhaps undesirable change in the chromatography; the same question may be posed for many of the other proposed adjustment ranges.

We must focus on the goal of these adjustments: to bring the chromatographic system into compliance with the system suitability requirements. If the system passes those requirements with the method as written, we have no need to try any of these adjustments.

If an adjustment of column length or ratio of components in the mobile phase, for example, worsens the chromatography, then one should not make those adjustments but should look elsewhere for the solution to the problem. The adjustments discussed in this article are permitted only when reference standards for all analytes are available and are used to show the adjustments have improved the quality of the chromatography.

RECOMMENDATION

The authors recommend that these ranges of adjustments and this discussion of their intended use be included in the USP General Chapter on Chromatography ⟨621⟩.

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REFERENCE

1. *Code of Federal Regulations Title 21, Food and Drugs* (Office of the *Federal Register,* Washington, DC, 1990), part 211.194(a)(2).
2. *USP 23* (United States Pharmacopeial Convention, Rockville, MD, 1995), chapter ⟨621⟩ Chromatography, p. 1776.
3. L.R. Snyder et al., "Selectivity Control in Reversed-Phase HPLC Method Development — Varying Temperature and Solvent Strength to Optimize Separations," *LC•GC* **15** (2), 136–151 (1997).

NEWS BRIEFS

Conference to focus on industrial process and QA/QC spectroscopy

The Spectroscopy in Process and Quality Control 1998 conference will be held 26 October 1998 in London. The conference will focus on the complementary techniques of near-infrared (NIR), Fourier transform infrared (FT IR), and Raman spectroscopy. The capabilities of these techniques for on-line, at-line, near-line, remote, or in situ measurements will be covered. Speakers will show how the effective use of optical spectroscopy can reduce the cost and time of pharmaceutical manufacturing, minimize industrial waste and pollution, and help manufacturers maintain international product quality standards.

Dr. H.W. Siesler (University of Essen, Germany) will be the keynote speaker. Topics for the main conference will include the capabilities of NIR and FT NIR spectroscopy in pharmaceutical applications, regulatory and validation issues for on-line NIR in the pharmaceutical industry, and chemometric strategies for industrial QA/QC.

Call for abstracts

The Ninth Annual Frederick Conference on Capillary Electrophoresis will be held on 19–21 October 1998 at Hood College in Frederick, Maryland. Subject matter for abstracts includes novel CE, MEKC, and CEC methodology and basic studies. The proceedings will be published by the *Journal of Chromatography B, Biomedical Applications*. Abstracts of 200 words must be submitted by 17 July 1998 for consideration for oral presentations. Abstracts received after the due date will be considered for poster presentation. For information contact Margaret L. Fanning, Conference Coordinator, SAIC Frederick, NCI-FCRDC, PO Box B, Frederick, MD 21702, tel. (301) 846-5865, fax (301) 846-5866, e-mail (fanningm@mail.ncifcrf.gov), WWW (http://129.43.32.72/cze.htm) or (http://469csal7.ncifcrf.gov/cze.htm).