CHAPTER

Method development in liquid chromatography 14

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CHAPTER OUTLINE

There are many different ways to develop a new high-performance liquid chromatography (HPLC) method, and one is not necessarily better than another as long as it attains the goals of the developer in a timely manner. Here, we share one approach based on our experience in a method development environment, years of interacting with clients in the pharmaceutical industry, and working with widely accepted scientific principles.

It should be noted that method development for liquid chromatography is a complex process that involves a number of steps, so this discussion should be considered as an overview rather than a step-by-step instruction manual. For in-depth information, refer to Refs. [1–3], as well as the scientific literature and column manufacturer's technical notes.

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14.1 INTRODUCTION

In recent years, the pharmaceutical industry has been applying quality by design (QbD) to various tasks in the laboratory and manufacturing environment. QbD is based on an ICH (International Committee on Harmonization) document [4], which states that to have a high-quality product (e.g., method), quality must be *designed* into the product, not tested into it. Another concept of QbD is the *design space*, which is the multidimensional space of operational variables within which a method is valid and does not require revalidation. In practical terms, changes in conditions are allowed within the design space to meet system suitability requirements (without revalidating the method). For HPLC, the design space encompasses the range of allowed values of various conditions (%organic solvent, pH, °C, etc.) where changes in any combination of these variables are allowed. This approach provides the flexibility to adjust a method to restore performance, if necessary. A requirement of QbD is that the effects of various conditions on the separation must be defined, so that the design space limits can be identified. QbD, although new in name, is not a new concept to experienced chromatographers. It does, however, provide a practical organizational structure for method development, which we apply in this chapter.

The method development process comprises six consecutive steps:

1. Define the goals of the method (Section 14.2).
2. Determine the method development approach (Section 14.3).
3. Develop the method (Section 14.4).
4. Perform prevalidation experiments (Section 14.5).
5. Validate the method (Section 14.6).
6. Document the process (Section 14.7).

We emphasize step 2 but also examine each step in more or less detail. Reversed-phase separation is assumed unless otherwise noted.

A survey of the scientific literature, existing in-house methods, or other resources may provide leads on how to proceed with a particular sample. Such information can be helpful at the outset, but be careful, because information about method robustness is rarely available; also, starting method development from a poorly developed existing method seldom is a good approach. It is better to use available information for choosing starting conditions, such as the initial column, organic solvent, and (maybe) mobile-phase pH.

14.2 GOALS

Before method development can start, the goals (or practical application) of the method must be delineated. Related questions may include the following:

- How will the method be used (research, production, quality control, random generic samples, high throughput, etc.)?
- Who will use the method (location, training, special communication problems, etc.)?

- What are the chromatographic goals (resolution, run time, number of samples to analyze per batch, detection and quantification limits, linearity, range, etc.)?
- Are any restrictions or limitations placed on the method (laboratory environment, isocratic only, UV detection only, etc.)?
- What level of validation is required (R&D method, regulatory approval, etc.)?
- Are sufficient resources available for adequate method development (time, personnel, budget, equipment, etc.)?

For example, a method for the content assay of a pharmaceutical product for regulatory purposes has different requirements than a method used to support a synthetic chemist or one used for in-process monitoring. Once the goals are established, the method development process can proceed.

14.3 A STRUCTURED APPROACH TO METHOD DEVELOPMENT

Adequate resolution, R_s, between adjacent peaks of interest is a primary goal of most HPLC methods. For method development, a fundamental resolution equation for isocratic separation can serve as a useful guide:

$$R_s = \underset{i}{0.25}\underset{ii}{N^{0.5}}\underset{iii}{\left[k_1/(1+k_1)\right](\alpha-1)}, \tag{14.1}$$

where N is the column plate number, k_1 is the retention factor, k, for the first peak, and α is the separation factor (selectivity):

$$\alpha = k_2/k_1, \tag{14.2}$$

where k_2 is the retention factor of the second peak.

We recommend using Eq. (14.1) as a guide for method development. First, start with a column that has an adequate value of N. The value $N \approx 10{,}000$ is recommended unless other factors suggest larger or smaller N values. Usually a C_8 or C_{18} column is chosen at the start, because these columns often can provide a successful separation (see Section 14.4.2 for column type screening). Next, use a gradient scouting run to determine whether isocratic or gradient conditions should be used (Section 15.3 and the discussion of Fig. 15.7 in Chapter 15). The adjustment of either the isocratic percent of organic solvent, $\%B$, or gradient time, t_G, may be sufficient to obtain the desired separation. If greater resolution is needed, explore each of the various factors that influence α. The simplest approach is to use a combination of t_G (or $\%B$) and temperature (°C) (e.g., Fig. 12.8), then change solvent or column type if necessary. It is usually prudent to select a pH (e.g., pH 2.5) for initial experiments and reserve changes in pH for later. After changes in α have been explored, the value of N can be revisited. If there is excess resolution, the run time (and N) can be reduced by using a shorter column and increased flow rate. Conversely, a limited increase (generally, no more than 25%–40%) in R_s can be gained by using a longer or smaller particle column. Additional choices are discussed in Section 14.4 and Chapter 2.

14.3.1 COLUMN PLATE NUMBER, *N*: TERM *I* OF EQ. (14.1)

For most separations, values of N fall within a range of $5000 \geq N \geq 20{,}000$, corresponding to a maximum twofold change in resolution. Larger values of N require longer run times, so changes in α are often preferable. N increases for longer columns, smaller particles, and lower flow rates—but flow rate usually has a relatively small effect on plate number and resolution. For conventional HPLC operation with a maximum pressure of 400 bar (6000 psi), a 100×4.6-mm column packed with 3-μm particles represents a good starting point ($N = 10{,}000$). For ultrahigh–performance liquid chromatography (UHPLC) operation and a maximum pressure of 1000 bar, shorter columns with smaller particles (<2 μm) are used. For more details on the dependence of N and column pressure drop on separation conditions, see Chapter 2.

14.3.2 RETENTION FACTOR, *K*: TERM *II* OF EQ. (14.1)

Examination of a plot of term ii of Eq. (14.1) vs. R_s leads to the conclusion that $2 < k < 10$ is a favorable retention range. Retention times are not conveniently long, and R_s is not strongly affected by small changes in k. For practical purposes, however, $2 < k < 10$ may not be possible, so a range of $1 < k < 20$ is usually acceptable. For $k < 2$, R_s can be affected strongly by changes in k, and interference with nonretained materials may create problems; for $k > 10$, excessive run times and undesirable peak broadening can occur. When the range of k values exceeds $0.5 < k < 20$, gradient elution is usually recommended (Chapter 12).

The retention factor is controlled most easily by adjustment of the mobile-phase strength (% B solvent). For isocratic conditions, this can be achieved by progressively reducing $\%B$ in a sequence of 90% B, 80% B, 70% B, and so on until the desired k range is reached. An alternative approach is to use gradient scouting runs (Sections 14.3.4 and 12.3). Fine-tuning k often provides additional benefits (Section 14.3.3).

14.3.3 SELECTIVITY, α: TERM *III* OF EQ. (14.1)

Selectivity, which defines the spacing of two peaks, is influenced by different chromatographic variables. Unfortunately, without prior knowledge (experimental data, sample-structure information, etc.), it is not possible to predict the influence of a particular variable on α for a given pair of peaks. It is possible, however, to make general statements about the influence of different variables on α. One such study examined 67 chemically diverse solutes in this regard, with the results summarized in Table 14.1. The study determined the average change in α ($|\delta \log \alpha|$) for the sample set for a defined change in a variable; we refer to this as the *orthogonal power* (OP) for that variable. If $\text{OP} \geq 0.1$, it is likely that a significant change in selectivity will occur. This, of course, does not guarantee the separation of any particular peak pair, but it is a good starting point.

We can approximately rank values of OP:

$$[\text{buffer}](\text{least effective}) \ll \%B \approx t_G \approx {}^{\circ}\text{C} < \text{solvent type} \approx \text{column type} \ll \text{pH}(\text{most effective})$$

Table 14.1 Comparison of Orthogonal Power of Chromatographic Variables

Variable[a]	Change	Example	Orthogonal Power (OP)[b]
%*B*	10%	50% ACN to 60% ACN	0.08
t_G	3×	10–30 min	0.07
°C	20°C	35–45°C	0.07
ACN (MeOH)	To MeOH (ACN)	Replace ACN by MeOH (or vice versa)	0.20
Column	$F_s > 65$[c]; $F_s > 100$[d]		0.19
pH	5 units	pH 2.5–7.5	$\gg 0.7$[e]
[Buffer]	2×	25–50 mM	0.02

[a] *%B, %-organic solvent; t_G, gradient time; °C, column temperature; [buffer], concentration of buffer.*
[b] *Average $|\delta \log \alpha|$; OP ≥ 0.1 needed for "orthogonal" conditions.*
[c] *$F_s = F$ value in Ref. [5]; for ionic or ionizable compounds.*
[d] *For nonionized compounds.*
[e] *Ionic samples only.*

where t_G is gradient time (Chapter 12). The OP values of different variables will be examined next (in the order presented in Table 14.1).

- *%B*, t_G. According to the linear-solvent-strength model [3], *%B* and t_G (or gradient steepness) are equivalent variables for controlling a separation. A change of 10% *B* (e.g., from 50% ACN to 60% ACN) changes *k* values by about 2.5-fold (Ref. [2, p. 58]). Similarly, a 2.5-fold change in t_G (e.g., from a 10-min to a 25-min gradient) changes retention about 2.5-fold. Either such change has OP ≈ 0.07–0.08 (Table 14.1), slightly less than the target minimum of OP ≥ 0.1. However, these variables are easy to change while maintaining *k* values in an acceptable range (Section 14.3.2). Furthermore, changes in *%B* or t_G may provide sufficient changes in α to obtain adequate R_s. For these reasons, we recommend that *%B* or t_G should be investigated early in the method development process, despite their relatively lower OP values.
- °C. The value of OP = 0.07 for a 20°C change in column temperature (Table 14.1) suggests that temperature is somewhat limited in its ability to increase resolution. However, for partially ionized solutes, a change in column temperature can have a dramatic effect on selectivity [6]. Furthermore, the nature of the selectivity change for °C may be different than that of *%B* or t_G, so that a combined change of °C and *%B* or t_G may be especially effective. The convenience of temperature changes leads us to recommend simultaneous changes in °C and either *%B* or t_G at an early stage in method development (in this connection, see also Fig. 15.8 in Chapter 15). The column temperature should be controlled in all cases (usually slightly above room temperature, e.g., 30–35°C).

- *Solvent type*. A change in the *B* solvent (e.g., methanol (MeOH) vs. acetonitrile (ACN)) can be effective for changing α during method development. According to Table 14.1, replacing ACN with MeOH (or vice versa) has OP = 0.2, double the minimum desired OP ≥ 0.1. Any of the three popular organic solvents (ACN, MeOH, and tetrahydrofuran (THF)) can be blended for improved control of selectivity [6]. One approach to method development is to screen two or more solvents early in the development process to see which one separates more peaks. Then, the chosen solvent can be fine tuned (as previously) by adjusting *%B* or t_G; the use of mixtures of two or more *B* solvents can also be considered.
- *Column type*. For years it has been known that changing from one column type to another (e.g., C_{18} to cyano) can result in a significant change in α; however, changing from one C_{18} column to another can sometimes also provide an adequate change in selectivity. Recent developments (the hydrophobic-subtraction model [7]) have led to a better understanding of column selectivity, as well as its implementation by means of free column-comparison software (USP-PQRI database [5]). Using the latter software, we can identify similar (equivalent) or different ("orthogonal") columns by means of a derived comparison function, F_s. Two columns with $F_s \leq 3$ can be assumed to be equivalent. As F_s increases, the columns become more different. For maximum change in column selectivity (OP ≥ 0.19 in Table 14.1), a value of $F_s > 65$ is sufficient for ionizable solutes (acids or bases), while $F_s > 100$ is adequate for neutral or nonionized compounds.
- *pH*. A change in mobile-phase pH can be one of the most powerful ways to change α if the analytes are ionizable. For such samples, a 5-unit change in pH (e.g., pH 2.5–7.5) can have OP > 0.7 (Table 14.1); an operating range of 2 < pH < 8 generally is advised for silica-based columns. At pH < 2, the bonded phase hydrolyzes and is lost; at pH > 8, the silica dissolves. For most columns, a low pH buffer of 2.5 ≤ pH ≤ 3 is a good starting place. Low pH suppresses the ionization of column silanols and acidic analytes, providing better peak shape. Many basic analytes have sufficiently high pKa values that they remain ionized at pH < 8. For work at pH > 8, several manufacturers offer silica-based columns that are stable at pH > 8.
- *Buffer concentration*. For most reversed-phase separations, a change in mobile-phase buffer concentration has little effect on selectivity (OP = 0.02, Table 14.1). Exceptions exist for mixed mode or HILIC separations (Chapter 5), where ionic or electrostatic interactions play a significant role in the separation. A buffer concentration of 5–10 mM (measured in the total mobile-phase) is recommended. Higher buffer concentrations (e.g., >50 mM) can result in buffer solubility problems.

14.3.4 GRADIENT ELUTION

Many samples have a sufficiently wide polarity range that $1 < k < 20$ is not possible for any isocratic condition. Furthermore, even when isocratic separation is possible, identifying those conditions by stepwise changes in *%B* can be time consuming. An

initial gradient separation is instead recommended prior to method development to determine whether isocratic separation is possible—and if so, what *%B* provides $1 < k < 20$ for the sample. A free calculator [8] can use the results of this initial gradient to determine approximate isocratic separation conditions. If only gradient elution is feasible, the calculator also can be used to trim "wasted" time off the beginning or end of the gradient. Resolution-modeling software (Section 14.4.1) can further increase the information content of a limited number of experimental runs. We recommend starting method development with gradient runs that can be used with resolution-modeling software.

14.4 METHOD DEVELOPMENT IN PRACTICE

Implementation of the method development approach of Section 14.3 involves several additional choices, as presented in this section. The method development process should represent a best compromise among the factors that affect method development for a given sample.

14.4.1 RESOLUTION-MODELING SOFTWARE

A linear relationship exists between retention (log *k*) and mobile-phase *%B*:

$$\log k = a + b\%B \tag{14.3}$$

where *a* and *b* are constants for a given solute and separation conditions. Similar relationships exist between values of *k* and °C; other curve fits can be used to describe the relationship between *k* and other variables (pH, ion-pair-reagent concentration, etc.). These relationships allow accurate prediction of retention as a function of separation conditions, based on two or more experimental measurements for changes in each condition. This in turn allows predictions of R_s for simultaneous changes in one to three variables, such as temperature and gradient time. It is convenient to display the results of such calculations as *resolution maps*, where R_s is plotted vs. one to three conditions, using resolution-modeling ("computer-simulation") software (e.g., DryLab, Molnar Institute, Berlin). Using data from the initial "calibration runs," resolution maps allow optimum conditions for a separation to be determined quickly. So, for example, 12 experimental runs (2 t_G values × 2°C values × 3 pH values) can give a three-dimensional model (cube) allowing prediction of R_s under any combination of these three variables, as well as any isocratic *%B*–°C–pH combination. Thus, just a few runs can answer the following questions:

- Can an adequate separation be obtained using the tested variables?
- If so, what conditions should be used?
- How sensitive is the separation to each (or a combination of) variables?
- What conditions should be tested to demonstrate robustness in QbD (Sections 14.5–14.6)?

A further benefit of resolution-modeling software is that it requires high-quality input data for accurate predictions. This adds discipline to the method development process, so that, even if the software is not used, the quality of the experimental data—and the results of method development—tend to be better. We strongly recommend using resolution-modeling software during method development for both improved productivity and higher quality methods.

14.4.2 PRIORITY OF COLUMN SCREENING

All the variables listed in Table 14.1 can be varied in a continuous manner—except column selectivity. Optimization of these "continuous" variables can be achieved by incrementally changing the variable or by using resolution-modeling software (Section 14.4.1). Column selection, on the other hand, requires a choice between one column and another—columns cannot be blended conveniently for intermediate results. Historically, predictability of differences in column selectivity was poor, so successfully changing a separation by changing columns was often more luck than skill. Today, column selectivity differences can be predicted (Section 14.3.3), improving the chance of changing a separation by using a different column. This leads to two general approaches:

- *Screen continuous variables first*. This is the traditional approach, where a single column is chosen and then a systematic investigation of other variables (e.g., t_G, and °C, pH) is performed. This approach is easily automated with modern HPLC equipment, and the number of experiments can be reduced if resolution-modeling software is used. This can be an efficient way to conduct method development.
- *Column screening first*. An alternative approach is to screen two or more columns of different selectivity at the beginning of the method development process, to pick a column for further method development. The problem with this approach historically is that candidate columns were chosen for reasons that may not have reflected the orthogonal nature of the column; each lab had a favorite column set but often could not offer a solid rationale for the selection. With recent advances in the understanding of column selectivity [7], and the availability of a free database for selecting orthogonal columns [5], column screening now makes more sense. A simple switching valve system can facilitate screening several columns in an unattended manner. Visual inspection or peak counting can facilitate choosing the most promising column for further method development.

14.4.3 HPLC VS. UHPLC

A thorough investigation of several variables can be time consuming. Consider first a conventional HPLC system (<400 bar) and a 100×4.6 mm, 3-µm column operated at 2 mL/min, as summarized at the top of Table 14.2. Data are gathered such that they can be used in resolution-modeling software. Two gradient times are used,

Table 14.2 Comparison of HPLC Versus UHPLC Method Development Time

$k*$	t_G (min)	Equilibration (min)	Runs (2 Temperatures)	Columns	pHs	Solvents	Time (h)
HPLC[a]							
6 12	15 30	5 5	3 3	3 3	3 3	2 2 Total	18 31.5 50
UHPLC[b]							
6 12	4.5 9	1.5 1.5	3 3	3 3	3 3	2 2 Total	5.4 9.5 15
Intermediate pressure, shell particles[c]							
6 12	5.5 11	2 2	3 3	3 3	3 3	2 2 Total	6.7 11.7 19

[a] *100 × 4.6 mm, 3.0-μm column, 2 mL/min, 200 bar (3000 psi).*
[b] *75 × 2.1 mm, 1.8-μm column, 1 mL/min, 1000 bar (15,000 psi).*
[c] *75 × 2.1 mm, 2.7-μm shell-particle column, 0.8 mL/min, 370 bar (5300 psi).*

15 and 30 min, which generate gradient k^* values, Eq. (12.1), equivalent to isocratic $1 < k < 20$, with a 5-min equilibration inserted between each run. Two temperatures are used (e.g., 35°C and 60°C), making four total runs at this point (two t_G values × two °C values). It is wise to perform a blank gradient occasionally to ensure that spurious peaks do not confuse the data interpretation, so two additional blanks make six runs total. If we investigate three columns at three pH values, and with two solvents, we have a total of 108 runs. This would take a minimum of 50 h (Table 14.2).

By using UHPLC (generally 400–1000 bar-operating pressure), equivalent experiments take about one-third the time. In Table 14.2 (middle set of data), a 75 × 2.1 mm, 1.8-µm column is operated at 1.0 mL/min to generate gradients equivalent to those of the conventional HPLC example. A total time of 15 h is required, which could be completed in a single overnight batch.

The value of UHPLC for comprehensive method development is obvious from this example. However, additional care must be taken to avoid problems with such systems, which tend to be less fault tolerant than conventional HPLCs. One viable option being taken by some laboratories is to develop the method on a UHPLC system, because of the speed advantages and skill sets available in the R&D lab, then convert the method to conventional HPLC conditions for validation and routine use, where the instrumentation or skill sets may be more limited. An alternative approach is to use superficially porous particle columns (Section 1.3.1 and Ref. [9]), which have a packing that comprises a solid core covered with a porous layer of silica. The column pressure is determined by the overall particle size (e.g., 2.7 µm), but the efficiency is equivalent to smaller particles (e.g., 1.8 µm); the columns are relatively flow insensitive and can tolerate pressures to ≈ 600 bar (9000 psi). This combination of characteristics allows comprehensive method development screens in 19 h on conventional HPLC equipment (bottom section of Table 14.2).

14.4.4 A SYSTEMATIC PLAN

Whatever combination of these approaches is chosen, it is imperative that method development proceed in a systematic manner. Plan out the experiments, be careful to document all the conditions tested, make a sufficient number of duplicate runs to ensure that results are repeatable, and preserve all the experimental data. This approach adds credibility to the results and provides confidence that an adequate method is achieved. Two examples of method development follow:

Example: Continuous-Variable Screening

System: HPLC or UHPLC
Gradient scouting runs (add at least one blank run; equilibrate between runs).

Example: Column Screening First
Columns: Primary column, type-*B* C_{18} with equivalent available [5].

If the separation is not successful after investigating all the variables just discussed, a different chromatography mode may be required. For example, normal phase (Chapter 8), HILIC (Chapter 5), mixed mode, or another technique may be

needed. More detailed instructions can be found in general references, such as Refs. [1–3], the scientific literature, or column manufacturers' technical notes. Before moving on to prevalidation, check the goals of the method to ensure there is no more development work to do.

14.5 PREVALIDATION

At this stage, the method should be ready to validate, but it is prudent to perform some prevalidation experiments first, especially if a formal validation with submission to a regulatory agency is planned. A formal validation must be performed under an approved protocol; any changes, failures, or deviations from that plan require additional documentation and proof statements that are not required before formal validation. Prevalidation can minimize such problems by making a "dry run" of some or all of the validation tests; if additional adjustment is needed, it can be done under method development rules. In other words, you want to be reasonably certain that formal validation will be successful before you start that process.

Prevalidation is an ideal time to establish limits for each selectivity variable that determines the boundaries of the QbD design space. Robustness—the ability of a method to withstand small, intentional changes in the values of different variables—is a method characteristic that must be confirmed during validation. The design space and robustness can be estimated by using resolution-modeling software to simulate runs based on data gathered during method development. For example, experiments based on a full factorial design can be used to test the limits (high and low) for each of five variables (e.g., *%B*, °C, pH, flow rate, and equivalent column). This would require $2^5 = 32$ experiments. At 30 min per experiment, 16 h would be required for this work, often with the result that one or more of the variables is not as robust as anticipated. This would require repeating the matrix of experiments with different values. With computer-simulated runs using resolution-modeling software, these 32 "runs" could be made and evaluated in <1 h. Once the limit tests using simulated data are acceptable, the actual experiments to confirm the expected results can be run with more confidence.

Computer-simulation also can be used to test the effects of variables that are difficult to change experimentally, such as peak tailing and column plate number. By modeling the effect of changes in peak tailing and plate number, appropriate system suitability limits for these variables can be chosen with more confidence [10].

A draft of the method document and validation protocol should be written and used during prevalidation. This helps debug both documents and ensures that the results will be successful when formal validation is undertaken. At a minimum, the prevalidation experiments should confirm the following (if they will be part of validation):

- Method range and linearity (or curve-fit choice)
- Adequate precision and accuracy
- Tests of lower limits of detection (LOD) and quantification (LLOQ)
- A full batch of samples can be run successfully (e.g., 96 injections)

In some companies, method development is done by a development group, but validation is performed by another group in the company. If this is the case, prevalidation is an ideal stage to have the validation team to take over the process but with close involvement of the development group. Once prevalidation is completed, formal validation can proceed.

14.6 VALIDATION

If a method is to be submitted for regulatory approval, the guidelines of the regulatory agency (e.g., Refs. [11–13]) define the validation criteria. Additional validation information can be found in books specializing in this topic (e.g., Ref. [13]). In most validated methods, the following components will be included:

- *Method document*. The method must be written as a controlled document (i.e., approved by the quality unit with a control process for any changes).
- *Validation protocol*. The validation protocol, also a controlled document, contains specific instructions for the validation process. These include descriptions of the validation tests (e.g., for precision, how many injections and at what concentration) and acceptance limits (e.g., ±2%, 1 standard deviation).
- *Core performance tests*. Nearly every method includes a test of precision and accuracy (often at different sample concentrations), a test of linearity, range tests, a demonstration of specificity (the ability to measure the analyte in the presence of potential interferences), and method limits (LOD, LLOQ).
- *Repeatability*. This can test (at least) three aspects of the method. The ICH [11] distinguishes between repeatability, intermediate precision, and reproducibility. *Repeatability*, also called *intra-assay precision*, is the precision of the method over a short time under the same operating conditions, usually one batch of samples (e.g., 50–200). *Intermediate precision* tests the within-laboratory variability of the method, such as different operators, different instruments, or different days. *Reproducibility* tests the method precision between laboratories and may not be appropriate for a method that will be used in only one laboratory.
- *Robustness*. Robustness tests the method performance against small, intentional changes in method conditions. If the QbD approach is being used, prevalidation should have identified the limits of the design space. These limiting conditions, or a sufficient subset of them, should be tested to demonstrate robustness.
- *System suitability tests*. System suitability is a set of tests performed before running a batch of actual samples. Its purpose is to verify that the method is capable of producing valid results under the present operating conditions. Specific tests to include in system suitability vary from one method to another, but most such tests include a retention time (or window) for the analyte, a check of resolution between difficult-to-separate peaks, some measure of peak tailing and peak width (or plate number), and a determination of precision (e.g., for $n=6$ injections). System suitability tests are often finalized based on data gathered during prevalidation and validation experiments, then are included in the final method document.

14.7 DOCUMENTATION

The proof of the performance of an HPLC method is in the documentation. A final documentation package usually will be approved by the quality unit of the organization and will be archived for future review by regulatory authorities. The documentation for a method usually includes

- *Laboratory notebook*. This is the primary record of the method development process.
- *Electronic records*. All data collected during method development should be stored for future access. Usually, this will be in electronic archives, although some groups may use paper records.
- *Method document*. This may require minor revision after validation is completed but then should be an unchanging document. It should contain sufficient information to allow use of the method without other documentation (of course, other laboratory standard operating procedures may be referenced). For a QbD method, a section should be included that defines the design space within which the method can be adjusted without revalidation. It should also define what documentation is required when a method is adjusted (e.g., record the adjustment and demonstrate that system suitability passes under the revised conditions).
- *Validation protocol*. This document is used to guide the validation process.
- *Validation report*. This document describes the validation results, including pass-fail statements, statistical data, representative chromatograms, and references to other documentation.
- *Method development report*. Although it may not be formally required, a method development report is strongly recommended. This may be as simple as an outline of the major steps taken during method development, including successful and failed experiments. A cross-referenced table that organizes electronic data and laboratory notebook pages will be useful for future revisions of the method. Many methods require updating as new formulations or new regulations are introduced; the method development report is a roadmap to help identify the important experiments that need to be made and ones to avoid, speeding method updates.

14.8 SUMMARY

Regardless of which method development components are incorporated in the final method development process, it is important that the entire process should be carefully planned and documented. It is inevitable that initial plans will require changes and unexpected events will occur, but if the plan has a good scientific basis and is well documented, it should lead to a successful method with a minimum of wasted time.

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