Plate Number Requirements for Establishing Method Suitability

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Abstract

Establishing the suitability of an analytical system has become a routine requirement in the testing of modern pharmaceuticals. Acceptable parameters that illustrate the system is performing as intended and in an equivalent manner to the original validation are often set at the time of method validation and transferred with the method to the production laboratory. For chromatographic methods, these parameters include—but are not limited to—resolution, tailing, and plate number specifications. Transferring methods is often a seamless transition from research to quality control. However, far too often the quality group receives arguably "overzealous" and strict requirements for the method. More specifically, chromatographic methods get issued with plate number specifications that far exceed the minimum number required to achieve sufficient resolution of the analytes. Presented here is a discussion of the setting of realistic plate number specifications that still maintain the minimum resolution of the chromatographic critical pair.

Introduction

Bridging distillation theory to modern compendial abstracts, the use of plate number and rate theory has a well-established role in the history and application of column chromatography (1–6). Gauging the effectiveness of separations in gas chromatography using plate heights or in liquid chromatography (LC) using plate numbers has continually withstood the test of time. In pursuit of reproducible separations, chromatographers routinely track and establish the suitability of the analytical system by comparison with established chromatographic parameters such as plate numbers, resolution, peak tailing, and peak asymmetry.

Chromatographic systems with electronic collection/integration of data easily calculate these experimentally determined chromatographic parameters. The suitability parameter(s) that are issued and tracked varies with company policy and practice. Sadly, this process has become so routine that some analysts simply "check the box" on these numbers rather than considering what these parameters are telling them about the separation. If such data is simply being passed along, how much is tracking all these numbers still warranted or business justified? Do we still need symmetry, tailing, or plate number results printed out with every run when, arguably, the most important aspect for our separation is that we have adequate resolution?

In the pharmaceutical industry, the validation requirements for an analytical method have become quite standard (7–13). With governance from the International Conference on Harmonization (ICH), it's fair to say that chromatographic emphasis has changed to the critical pair theory (14). Right or wrong, the emphasis in method development is currently more focused on defining the resolution of our most important (or "critical") pair rather than setting appropriate system suitability parameters for plate number or capacity factor. Recent articles on method development and column characterization studies tend to monitor column efficiency in terms of plate number, but do not address an expected minimum number of plates required for a suitable separation (15–19).

Undergraduate and graduate texts abound with definitions for resolution and plate numbers. They adequately define the theory and determination of these parameters, yet fail to go further in detailing how these numbers interact. When establishing system suitability criteria, the literature is quite vague regarding how specifically to set the plate number criteria for a method specification. ICH and United States Pharmacopeia guidelines require suitability parameters be established to "ensure the validity of the analytical procedure is maintained wherever used", but provide little detail as to how this should be done (7,10). In our experience, laboratories have used the 95% confidence interval of the average plate number found during validation, 80% of the average plate number found during validation, three standard deviations (3σ) below the average plate number found during validation, or simply a suspected minimum found from robustness studies.

Others have proposed, and we agree that the minimum plate number is that number of plates determined from the minimum acceptable resolution of the chromatographic system (21,22). We often find that this is not the plate number we are typically

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targeting when addressing system suitability criteria received with a chromatographic method. Defining where this minimum acceptable resolution exists is still not clear. In their guidance for robustness/ruggedness tests, Vander Heyden et al. proposed that suitability parameters, including plate number, be defined during the robustness testing of the method validation and proceed to use an intensive Plackett-Burman design for optimization (21). The suitability limit is then based on a one-sided 95% confidence interval or worst-case result. In a follow-up study, Hund et al. proposed using the ICH robustness requirement to assess the acceptable limits for the suitability parameters, but they also suggest that this approach can lead to "very strict" suitability limits (22).

The goal of setting suitability limits should not be to restrict the bench chromatographer from applying the method, but rather practical in nature. Strict limits fail or are frequently violated during method transfer (21). Analysts who set arbitrary suitability limits may be getting their methods approved by regulatory agencies, but in the long run they are hurting the company by unnecessarily handicapping the laboratories that receive the method down the line. The true goal for setting suitability limits is to establish: (*i*) the minimum acceptable conditions for a valid separation and (*ii*) confirm that the current system in use replicates the minimum requirement established with the validated method.

Analysts spend a great deal of time and cost delaying the posting of analytical results or shipping product while trying to hit an elusive plate number that may or may not be "critical." Presented here is a formal attempt to review and address the current usage of plate number theory in the pharmaceutical industry and to review the determination of the minimum number of plates suitable for a column separation.

The frustration of poorly defined plate number specifications

During a recent review of our validation standard operating procedure, a chemist in our department challenged, "Why are we still monitoring plate numbers as part of system suitability?" The crux of the question comes from the familiar experience of failed suitability runs in which all the criteria for a method's suitability (such as critical pair resolution, tailing, asymmetry, etc.) were met except for the required number of theoretical plates. In a good manufacturing practice environment, such a suitability failure halts further testing on the system because the suspect result has to be investigated prior to moving forward. These delays are time consuming and, too often, costly barriers in posting results and shipping product. Inevitably, "Murphy's Law" applies when releasing product under critical timelines.

Of those whose curiosity was raised in this debate, there seemed to be two factions of thought: (*a*) those who agree that plate numbers caused too many type 1 errors (in which system suitability failures were not justified) and (*b*) those who felt plate numbers were still vital in establishing the suitability of the method. The first argument against plate numbers looks at the concept as a historical artifact left over from days when analysts packed their own columns and did not have chromatographic data systems that routinely output every chromatographic parameter of interest. Arguing this line, many practicing chromatographers would tell you that as long as the critical pair resolution (14) is being maintained with the associated tailing/asymmetry specifications, plate number values are of little concern. Although great advances have been made in the robust manufacturing of columns, it is still common to see plate number values vary with column lots while valid chromatographic profiles are maintained. The second group of chemists held that during method development it is vital to characterize the "least acceptable" chromatographic performance and to set suitability parameters to ensure that this criterion is being met. They argued that plate numbers are invaluable in defining the chromatographic condition at the time. Advancing the chromatographic method forward into production, where all the validation articles are no longer routinely available, the plate number specification helps ensure that later use of the method is properly mimicking the original validated state of the method.

Perhaps the arguments are not all that different. The question is not so much whether plate numbers are critical but whether alternative ways exist to represent the critical number of plates required to achieve the desired chromatographic separation. Equations for the chromatographic parameters of interest are all interrelated; a critical resolution determination has a plate number term in the equation. Perhaps equally important, is this disconnect between chemists over plate numbers simply a result of poor specification setting?

Examples of plate number specification issues

Case 1: common quality control frustration—N set too high.

The first example of a plate number specification issue is one

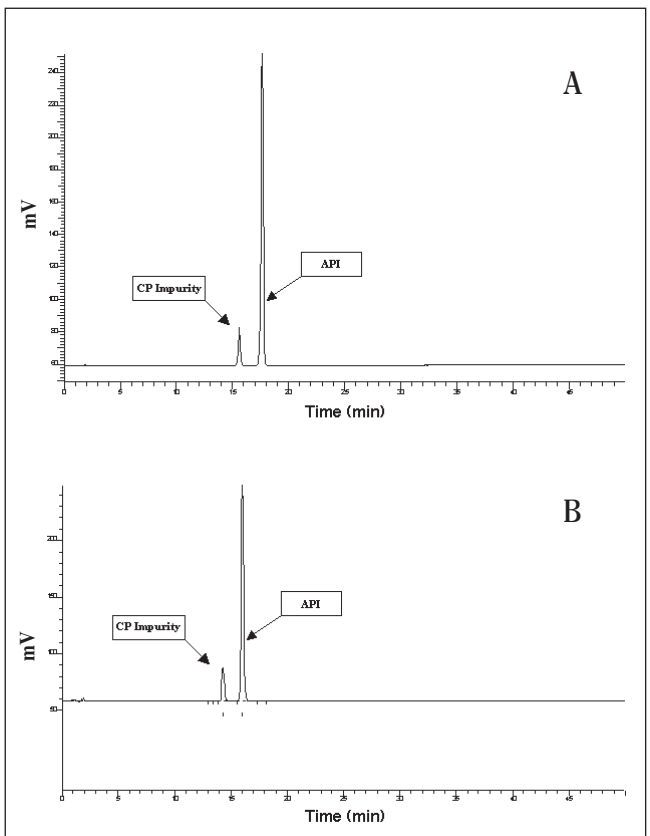

Figure 1. Example with failing plate number suitability. (A) Validated drug substance profile meeting specified method conditions: *N* = 33 and 99 and *Rs,cp* = 5.5. (B) The same drug substance method failing specified method conditions: *N* = 23,598 and *Rs,cp* = 4.2

familiar to those experienced in quality control settings: receiving a method in which the plate number specification was apparently set too high. In Figure 1A, the analytical method was transferred with a plate number specification of $N \geq 30{,}000$ for the active pharmaceutical ingredient (API) peak and a resolution specification of $R \geq 3.8$ between the API and the critical pair impurity. When the method was used by another group in the same company to release some new clinical batches and stability time points, the second laboratory (Figure 1B) could not release their data because their system met all suitability requirements except for the plate number specification. This time the method recorded 23,598 plates, thus failing the method specification. The system met the resolution criteria ($R = 4.2$), posted suitable tailing and yielded the equivalent relative retention times. Chromatographically, the system and profile were acceptable, but because the plate number specification was set higher than what was needed to achieve the validated separation, the results in this case were not usable. The company had to wait for additional data runs and investigations prior to overriding this specification in accordance to regulated procedures.

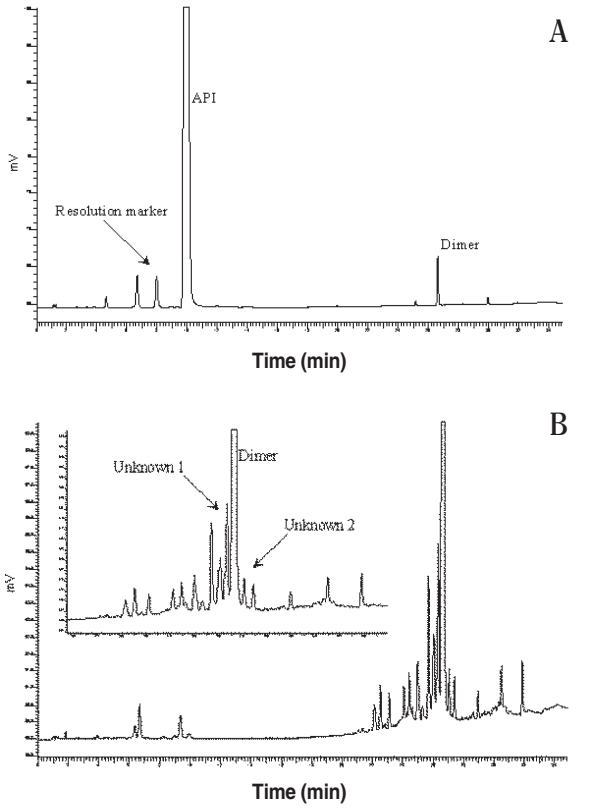

Figure 2. Case 2 illustration: (A) API separation, 150-mm column, $R_s = 6.2$, $N = 12{,}585$; and (B) dimer separation, $R_s = 2.7$ (between dimer and unknown 2).

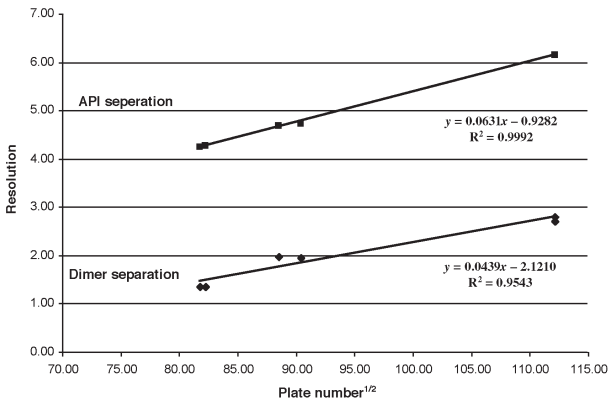

Figure 3. Plot of resolution versus the square root of plate number.

Case 2: N and R seem arbitrary or unrelated

A second case in which a receiving laboratory could not achieve the required number of theoretical plates is represented in Figure 2A. Based upon data generated during validation and release of early API lots, the specification was originally set at ≥ 7,500 plates and later amended to ≥ 10,000 plates. The requirement for resolution between the parent peak and the nearest impurity was reduced from 6.0 to 5.0 at the same time. The large resolution value indicates that the resolution marker for the method is not a true critical pair (i.e., the separation capacity of the column would need to be compromised severely to fail to resolve these components).

Initially developed and validated for evaluating potency and purity of API (Figure 2A), this method has also been used for, but not specifically optimized for, purity assessment of a dimer of the parent compound (Figure 2B). Low resolution of peaks adjacent to the dimer peak lead to the expectation that tight control over the number of theoretical plates is necessary to maintain the separation.

The required resolution and plate numbers for the parent compound and dimer separations were investigated using three columns packed with the same stationary phase but differing in length (150, 100, and 75 mm). Figure 3 is a plot of resolution for a "critical pair" in each separation versus the square root of the number of theoretical plates evaluated for the parent peak. Linear regression produces equations that can be used to relate these two parameters and evaluate the given specifications. For example, when resolution between the parent and the resolution marker is set to the minimum acceptable by specification ($R_s = 5.0$), the system generates 8826 theoretical plates, which corresponds to a resolution of 2.0 between the dimer and Unknown 2*. Likewise, setting the resolution between the dimer and Unknown 2 to the minimum baseline resolution of 1.5, the theoretical plate count is 6803, which equates to a resolution of 4.3 between the parent and the resolution marker. When considering the dimer separation, it is counter-intuitive that the minimally acceptable (but by no means ideal) separation of the dimer and Unknown 2 can be achieved with only a modest number of theoretical plates. The use of a pair of peaks in a parallel separation to assess one or more parameters may be practical in the absence of a reasonable resolution marker for a given separation.

Close examination of these results in light of the stated specifications underscores the fact that the original theoretical plate specification of ≥ 7,500 for the parent separation was set unnecessarily high. Furthermore, the theoretical plate number and resolution specifications do not appear to be coordinated (i.e.,

* Unknown 2 preferred over Unknown 1. Unknown 1 is actually two coeluters that make peak width measurements difficult.

according to Figure 3, a region exists where resolution is not acceptable but the minimum number of theoretical plates has been met). In adjusting these minimum requirements, one problem may have been exchanged for another here—resolution is clearly adequate, although failing the specification, but the number of theoretical plates is sufficiently high to ensure adequate separation capacity.

Setting the plate number specification

In case 1, the required plate number specification was $N >$ 30,000. Although the details of how this number was originally derived are not open to disclosure, it is readily apparent that this specification is not the minimum number of plates required to achieve an adequate separation (benchmarked to be defined as $R_s > 1.5$) of the critical pair.

(*i*) The plate number range reported on the "failing" runs was 23,598 to 29,636 plates. Robustness of analytical methods can be challenged by varying the method conditions that are likely to be encountered in post approvals application and transfer (5). Such challenges to the method commonly used include (12):

(*i*) A ± 2% relative change in the volume of the lesser component (organic or aqueous) of the mobile phase (the larger component volume remains unchanged); (*ii*) ± 2°C change in column temperature; (*iii*) ± 5% relative change in the mobile phase flow rate; and (*iv*) A ± 0.1 unit in mobile phase pH.

In agreement with Hund et al. (22), using these conditions did not greatly alter the expected plate number performance of the method, as seen in Table I. Thus, if we were using the robustness study to define the plate number for this separation, we would be reporting the actual plate number found on the system during these experiments, not the required minimum number of plates to yield a defined separation. It is more a "picture in time" than a reflection of the minimum specification. Some may argue that meeting this higher plate number requirement ensures the second lab is operating the method consistently with the validation conditions. While an interesting ideal, it is unrealistic to expect the method as it goes through its 15-year life cycle with varying LCs, column lots, and manufactures, to live up to this notion at a reasonable cost. Industry, while striving to maintain "ideals", must also meet the pragmatic aspects of maintaining quality, business productivity, and shipping product.

Table I. Plate Number Results Under Varying Method Conditions

Method challenge	Critical pair resolution	Plate number
Validated method condition	4.1	35,577
–2% relative change in the volume of the organic component in the mobile phase	4.9	35,701
+2% relative change in the volume of the organic component in the mobile phase	5.0	36,217
–2°C change in column temperature	4.4	36,097
+2°C change in column temperature	4.9	36,476
–5% relative change in the mobile phase flow rate	5.3	37,163
+5% relative change in the mobile phase flow rate	5.2	32,574

So where is the compromise? The compromise is requiring the method to operate above the minimum requirements needed to maintain acceptable resolution. The original method required the resolution for the API and the second eluting impurity to be greater than 3.8 ($R_s > 3.8$). The minimum resolution found for this critical pair was $R_s = 4.1$. Thus, at no time was the robustness approaching this least acceptable separation. Focusing in on the minimum number of plates to achieve the resolution of this critical pair can be accomplished theoretically or experimentally.

Theoretical determination of critical plate number

From our validated separation, we know the following parameters: (*i*) retention time of the void, $t_M = 2.3$ min; (*ii*) retention time of the API, $t_{R,1} = 15.55$ min; (*iii*) retention time of the critical pair impurity, $t_{R,2} = 17.62$ min; (*iv*) critical pair resolution requirement, $R_{s,cp} = 3.8$.

From the chromatogram we also know that: (*i*) relative retention (or separation factor) of the critical pair, $\alpha = (t_{R,2}/t_{R,1}) = 1.13$ and (*ii*) capacity factor of the critical pair impurity, $k_b = 6.66$.

Rearranging a classic equation for resolution (equation 1), the minimum number of theoretical plates required to achieve the method separation can be determined from equation 2.

$$R_{s,cp} = \frac{\sqrt{N}}{4}\left(\frac{a-1}{a}\right)\left(\frac{k_b}{k_{b+1}}\right) \qquad \text{Eq. 1}$$

$$N_c = \left[\frac{4R_{s,cp}}{\left(\frac{a-1}{a}\right)\left(\frac{k_b}{k_{b+1}}\right)}\right]^2 \qquad \text{Eq. 2}$$

[This equation works for isocratic methods and is an approximation for gradients. As suggested by Neue, gradient methods should take the steepness of the gradient into consideration (23). The greater the steepness of the gradient, the resulting number of plates increases as shown when equation 3 replaces equation 1.] Here:

$$R_{s,cp} = \frac{\sqrt{N}}{4}\left(\frac{1}{G(1+b_g)}\right)1n\left(\frac{k_2}{k_1}\right) \qquad \text{Eq. 3}$$

where G and b_g are the peak compression and gradient steepness factors, respectively. (23)

Inserting our values for this method into equation 2, the critical minimum number of plates (N_c) required is 13,196! That is less than half the specified plates the original method required and more than 30,000 less plates than found in the robustness study.

The obvious question is, does this theoretical N_c severely underestimate the number of plates our method needs? No. To illustrate, let us change the experimental parameters to a tighter profile: (*i*) retention time of the void, $t_M = 2.3$ min; (*ii*) retention time of the API, $t_{R,1} = 17.13$ min; (*iii*) retention time of the critical pair impurity, $t_{R,2} = 17.72$ min; (*iv*) critical pair resolution

requirement, $R_{s,cp}$ = 1.5; (v) relative retention (or separation factor) of the critical pair, a = ($t_{R,2}$ /$t_{R,1}$) = 1.04; and (v;) Capacity factor of the critical pair impurity, k_b = 6.70.

In this second example, the critical minimum number of plates (N_c) required is 32,143. It just so happens that the original separation was relatively simple to achieve, although the second example was much more stringent and, thus, the N_c requires a greater efficiency to be established. The resulting suitability in this case justifiably requires a much higher plate number.

The overriding issue is that pharmaceutical chemists traditionally (or more accurately "historically") report the number of plates typically found during the method validation, not the critical minimum number of plates required. The pharmaceutical chemist focuses on resolution as the first major achievement of the analytical separation. As long as the original separation profile is maintained on a second system resulting in corresponding relative retention times, resolution and tailing aspects of the original method, the method should run equivalently to the validated method on this second system. With the proper assessment of the critical minimum number of plates, this is relatively easy to achieve. It is when the plate number is set artificially high by using what was observed under ideal method development conditions that frustration and delays occur. Had the original development chemist set the plate number requirement at N_c = 13,210 our Case 1 example and its associated frustration would not have occurred. The system would have been suitable for analysis and the results released.

Table II. Plate Number Results Under Experimental Least Acceptable Separation Conditions

Condition	Flow (mL/min)	Mobile phase	Critical pair resolution	Plate number
Original	1.3	Gradient: 50–75% ACN in 50 min	5.4	32,676
2	1.1	Gradient: 50–75% ACN in 50 min	5.5	37,346
3	1.5	Gradient: 50–75% ACN in 50 min	5.2	29,080
4	1.7	Gradient: 50–75% ACN in 50 min	5.1	26,053
5	1.0	Gradient: 50–65% ACN in 50 min	5.5	26,930
6	1.0	Gradient: 50–95% ACN in 50 min	5.0	40,957
7	1.0	Gradient: 50–75% ACN in 50 min	5.0	23,440
8	1.0	Gradient: 60–75% ACN in 50 min	4.4	18,834
9	1.7	Gradient: 60–75% ACN in 50 min	4.1	15,814
10	1.7	Gradient: 65–75% ACN in 50 min	3.5	14,115
11	1.0	Gradient: 65–75% ACN in 50 min	3.8	16,395
12	1.0	Isocratic: 65% ACN	3.8	15,117
13	1.0	Isocratic: 60% ACN	4.5	15,422
14	1.0	Isocratic: 55% ACN	5.2	15,720
15	1.7	Isocratic: 50% ACN	5.0	13,694

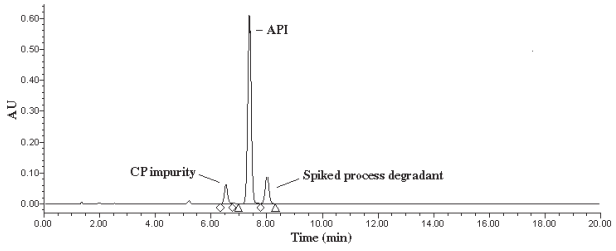

Figure 4. Experimental "least acceptable" profile.

Experimental determination of critical plate number

Returning to the notion that system suitability parameters establish the minimum acceptable separation or system performance for the analytical method, let us review whether comparable results for plate number can be experimentally found in the same realm of the theoretical determination. During validation, the method employed in Case 1 established a resolution of 3.8 between the impurity and the parent peaks. Thus, the least acceptable separation maintains this resolution of the critical pair, and maintains these analytes as the critical pair. A profile change or change in the primary critical pair with changing analytical conditions is a violation of the least acceptable separation norm.

Method conditions were varied to reduce the efficiency of the current system down to the least acceptable separation. For this method, the organic content was increased. As shown in Table II, as the resolution decreases in the critical pair, so does the number of plates. From equation 1, this should be expected. It is interesting to note that the critical pair resolution is maintained even well below the original 30,000 plate method specification. Figure 4 illustrates the least acceptable resolution experimentally achieved for this study. It is clear the experimentally derived plate number more closely matches the theoretical N_c determination rather than the original method specifications or the plate numbers posted in Table I during the ICH robustness runs. It is again clear to see that specifications for plate number were set well above what was actually needed.

Conclusion

The need for sufficient resolution of any chromatographic system is achieved by the analyte of interest experiencing a critical minimum number of theoretical plates. This is as true today as it was for the pioneers of chromatography. However, in the quest for regulatory acceptance, many chromatographers require methods to achieve more than the required numbered of plates. A great deal of frustration can be avoided in these instances by going back and reviewing the chromatographic profile and establishing the proper critical number of theoretical plates required to achieve the least acceptable analytical separation. This is easily established by first developing and validating the method profile, establishing the critical pair resolution needed, and then calculating the equivalent number of theoretical plates.

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