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Journal of Chromatography A, 917 (2001) 23–27

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Setting system suitability criteria for detectability in high-performance liquid chromatography methods using signal-to-noise ratio statistical tolerance intervals

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Received 25 August 2000; received in revised form 19 January 2001; accepted 16 February 2001

Abstract

For pharmaceutical products, one approach developed to assure that different chromatographic systems are capable of generating valid results is the system suitability test. Typically, a system suitability test involves numerical limits for predefined chromatographic parameters such as theoretical plates, tailing factor, injector reproducibility, etc. An estimation of the ratio of signal compared to baseline is one way to measure system performance, according to a valid method, independent of the instrument. However, since this comparison relates the height of the signal to the height of the noise, it is difficult to relate to the peak area measurements that are typically used for quantification of samples. Additionally, although peak area and peak height may be highly correlated over a wide region, peak area at very low concentrations can be more sensitive to all components of noise due to peak shape. To establish a system suitability criterion, one can use the ratio of the area signal to the baseline noise for replicate injections for samples prepared at concentration equal to the limit of quantitation during the validation studies. A lower limit for this ratio can be derived using statistical tolerance intervals. This lower limit can be applied as a system suitability criterion to measure that any system is performing adequately for measuring low level components in the sample for all future use of the method.

Keywords: Signal-to-noise ratio; System suitability criteria; Detection, LC; Prochlorperazine

1. Introduction

In developing methods to be used to monitor potential impurities, very low concentrations of these peaks are evaluated. Usually, the validation includes some measure of the degree of agreement at nominal concentration across a few instruments. However, it is impractical to test every type of available instrument. Once the method is developed and validated there is no guarantee that the same instrument and conditions will be used to perform the method each time it is executed. Some independent check should be incorporated into the method that assures peaks at low concentrations can be detected and quantitated with performance not worse than that claimed in the validation of the method. One way to satisfy the analyst that the system can measure low concentration samples as specified in the original method validation is to put a limit on the signal-to-noise ratio (S/N) as a measure of system performance indepen-

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)00669-0

dent of the instrument. It is commonly accepted that a S/N of 10 is one definition of the limit of quantitation (LOQ), CPMP/ICH/281/95 (ICH Topic Q2B). However, the (S/N) is a ratio of the peak height to the height of the noise. Most typically, peak area rather than peak height is used to quantitate the sample and there are no published procedures for linking noise and peak area. This paper describes a procedure for using actual data gathered in a method validation to set a meaningful lower limit on peak area S/N. This number is a one-sided lower tolerance limit for peak area to noise. The calculated lower tolerance limit would be valuable for the lifetime of the method assuring that potentially hundreds of the thousands of measured impurities can be adequately measured.

2. Experimental

In this experiment, a stability indicating HPLC assay for Prochlorperazine maleate was used to illustrate the development of a tolerance interval (Fig. 1). Appendix A lists the conditions under which this assay, a slightly modified compendial method [1], is normally run. The previously determined limit of quantitation or LOQ for this method was not reassessed for the purpose of this paper, but the data generated is intended to serve as a validation of the previously determined LOQ. A single sample was prepared at the LOQ, 0.05% of the nominal concentration. This single sample was injected 10 times. Both the area of the sample peak and baseline noise were determined for each individual injection. The area response for the LOQ sample was found to have an acceptable RSD of 6%. Table 1 is the raw data of signal and noise found for each injection. A maximum excursion from an assumed baseline was used to calculate noise. The S/N calculated for this method is higher than 10 and considered acceptable. From this data, we could assume that quantitation of impurities at the LOQ would be accurate and reasonably precise. If this method were to run on a different instrument, would it be possible to make a single injection of a sample prepared at 0.05% of the nominal concentration and use the ratio between peak area signal and noise as an indication of comparable accuracy and precision? Described below is a way to link peak area and noise.

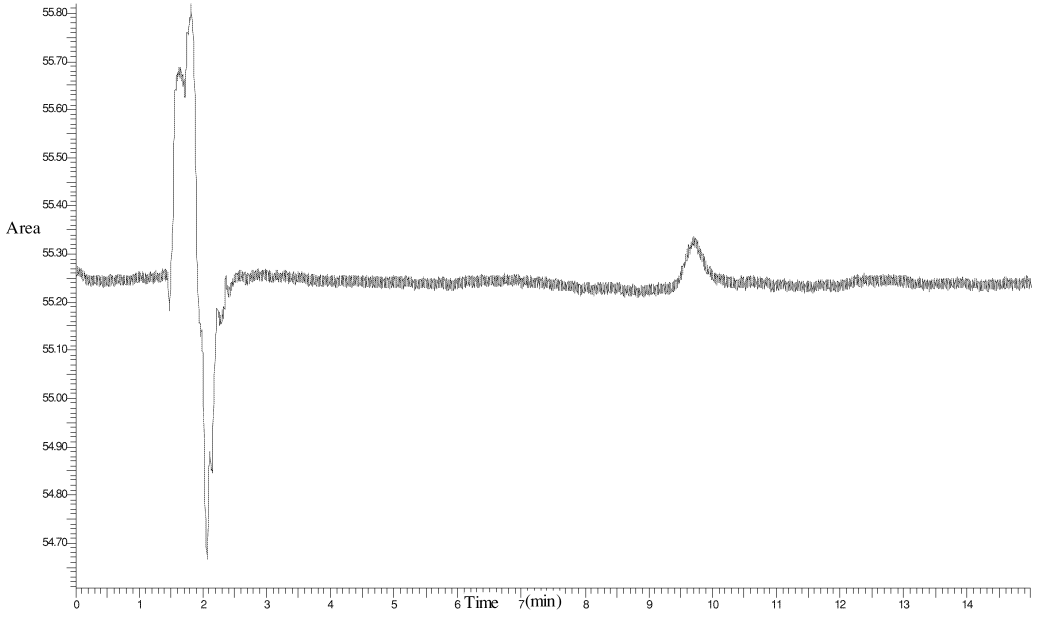

Fig. 1. Typical LOQ chromatogram for prochlorperazine.

Table 1
Area response, height and baseline noise data for LOQ injections of prochlorperazine maleate

Injection	Area (uV·s)	Height uV	Noise uV
1	2184	102	6.066384
2	2108	103	6.672515
3	1981	101	6.358132
4	2026	98	5.950226
5	2175	100	6.113855
6	2341	106	6.05049
7	1931	97	6.205814
8	2214	106	6.475164
9	2225	103	6.301487
10	2252	104	5.992058
Average	2144	102	6.218613
% RSD	6.1	3.1	3.7
Average signal-to-noise		16.4	

These area readings (Table 1) appear to follow a normal distribution, examination of a larger sample of baseline noise readings revealed that this distribution was not normal, but more closely log normal. The proposed S/N ratio would then involve a normally distributed numerator and a log normally distributed denominator. The distribution of that ratio is not well categorized. However, if a distribution (of positive values) with the characteristic bell-shape of a normal distribution has a standard deviation small in comparison to its mean (as in this case) it can be adequately modeled by a log-normal distribution [2]. Thus the S/N ratio can be treated as a ratio of log-normal distributions.

In dealing with log-normal distributions, which are not symmetric, the relationship of interest would be the ratio of their medians or equivalently the ratio of their geometric means. Let the median of a lognormal distribution be e^{μ}, where μ equals the mean of the logs.

$$\text{Then, ratio of two log normals} \Rightarrow e^{\mu_{\text{signal}}}/e^{\mu_{\text{noise}}} \quad (1)$$

By taking a log transformation of the data the ratio can be expressed as a difference between means:

$$\ln(e^{\mu_{\text{signal}}}/e^{\mu_{\text{noise}}}) = \mu_{\text{signal}} - \mu_{\text{noise}} \quad (2)$$

Because we would like to use this ratio to set a system suitability criterion independent of the instrument, we wish to draw conclusions about a relatively large number of future values of the ratio, based upon this small set of data from the population of interest [3]. This leads to the definition of a tolerance interval.

Specifically, a tolerance interval is an interval that one can claim to contain at least a specified proportion, p, of the population with a specified degree of confidence, $100(1-\alpha)\%$. Because we are only interested in controlling values that are too small we are interested in a one-sided lower tolerance limit, $\underset{\sim p}{T}$, such that:

$$\underset{\sim p}{T} = (\bar{x}_{\log\ \text{signal}} - \bar{x}_{\log\ \text{noise}}) - k_{(1-\alpha;p)}s_{\text{diff}} \quad (3)$$

where $k_{(1-\alpha,p)}$ is the one-sided tolerance factor and $s^2_{\text{diff}} = s^2_{\log\ \text{signal}} + s^2_{\log\ \text{noise}}$

For the difference between two normal populations the mathematical problem is to solve for the lower tolerance factor, k, such that:

$$\Pr[(\bar{x}_{\text{signal}} - \bar{x}_{\text{noise}}) - k_{(1-\alpha;p)}s_{\text{diff}} \leq \mu_{\text{signal}} - \mu_{\text{noise}} - K_p] = \alpha \quad (4)$$

where K_p is the p percentile of the normal distribution. In other words, the constant, $k_{(1-\alpha;p)}$, can be determined, such that, at least a proportion, p, of the differences of the logs of the signal and noise means will be above the tolerance limit calculated with probability of α. The expression can be manipulated into a form resembling a noncentral t distribution [4]. The expression is not exactly noncentral t distributed because s^2_{diff} is not a chi-square (f) divided by its degrees of freedom (except in the case where $\sigma^2_{\text{signal}} = \sigma^2_{\text{noise}}$). However, it is not unreasonable to use, as an approximation, the noncentral t distribution with a noncentrality parameter $K_p\sqrt{n'}$ and equivalent degrees of freedom (df) f' [5]. The equivalent sample size, n', is:

$$n' = \frac{\sigma^2_{\text{signal}} + \sigma^2_{\text{noise}}}{\dfrac{\sigma^2_{\text{signal}}}{n} + \dfrac{\sigma^2_{\text{noise}}}{m}} = \frac{n(R+1)}{R+c} \quad (5)$$

where $R = \sigma^2_{\text{signal}}/\sigma^2_{\text{noise}}$, $c = n/m$, and m = number of signal samples, n = number of noise samples and the equivalent df f' is:

$$f' = \frac{(\sigma^2_{\text{signal}} + \sigma^2_{\text{noise}})^2}{\frac{(\sigma^2_{\text{signal}})^2}{n-1} + \frac{(\sigma^2_{\text{noise}})^2}{m-1}} = \frac{(n-1)(R+1)^2}{R^2 + (n-1)/(m-1)} \quad (6)$$

Since the ratio R is not known it is replaced by the unbiased estimate [5]:

$$\hat{R} = (s^2_{\text{signal}}/s^2_{\text{noise}})(m-3)/(m-1) \quad (7)$$

For this application, where signal and noise are measured from the same chromatogram, $m = n = n'$, and is independent of R. A table of one-sided tolerance factors, $k_{(1-\alpha;p)}$, was generated for $n = m = 10$, $P = 0.99$, and $\alpha = 0.05$ using the inverse central t-function in SAS® (Table 2) [6]. For this example, the noncentrality parameter $K_p\sqrt{n'}$ is equal to 7.359.

The lower tolerance specification can be left as the difference between the log values of the signal and noise or the antilog of the limit can be taken to translate it back into a tolerance limit on the ratio of the original units.

3. Results

Table 3 illustrates a lower tolerance limit for S/N ratio for the sample data given in Table 1 using Eq. (3). In the natural log scale, the lower limit is 5.592. Table 4 provides an example of how the criterion would be applied to a new instrument. A single injection is made on the new instrument and the natural log of the area to noise ratio is calculated as 5.421. Since this value is less than the lower tolerance limit of 5.592, the system suitability test fails, although, it appears suitable by a 10:1 criteria for signal to noise measured conventionally. It is a strong indication that with this new instrument it may be difficult to quantitate degradation products, with the same degree of accuracy and precision, close to 0.05% of the nominal concentration of the assay.

Table 2
One sided tolerance factors ($P = 0.99$, alpha = 0.05, $n = \text{m} = \text{n}' = 10$)

Degrees of freedom	$k(1-\alpha;\ p)$
9	3.982
10	3.867
11	3.774
12	3.697
13	3.632
14	3.577
15	3.529
16	3.488
17	3.451
18	3.419
19	3.390
20	3.363

Table 3
Log transformed data from Table 1

Log area	Log noise	
7.688913	1.802763	
7.653495	1.897997	
7.591357	1.849735	
7.613819	1.783429	
7.684784	1.810558	
7.758333	1.800139	
7.565793	1.825487	
7.702556	1.867974	
7.707512	1.840786	
7.719574	1.790435	
7.668614	1.82693	Average
0.003751	0.00136	Variance
	5.841684	(Eq. (2)) Difference between averages
	0.005111	Variance of the difference
	0.071492	Standard deviation of the difference
	16	(Eq. (6)) Degrees of freedom
	3.488	Tolerance factor
	5.592	(Eq. (3)) Lower tolerance limit

Table 4
System suitability test injection

Area	Height	Noise
2218.65	101.48	9.811128
Log area		Log noise
7.70465418		2.283517
Difference of logs	Signal to noise ratio (height ratio)	
5.42113693	10.34	

4. Conclusions

The ratio of signal compared to baseline noise of a sample prepared at the limit of quantitation is one way to measure system performance independent of the instrument. The approach described in this paper illustrates how to formulate a system suitability check based on this ratio. Differences between instruments can be overcome by adjustments to the injection volume, however effects of injection volume were outside the scope of the paper. The system suitability criterion is established as part of method validation. It is less arbitrary and more sensitive than conventional measurements of signal-to-noise ratios. It allows the use of peak area rather than peak height, a measure more relevant to sample quantitation. The test is executed by preparing a single sample at LOQ for every run. The signal-to-noise ratio based on peak area is compared to the lower tolerance limit established during validation to determine whether or not any given instrument is performing acceptably. Finally, the FDA does not yet require method developers to include a check of LOQ in a stability indicating method. However, many method developers are including repeat injections of LOQ samples in order to prove that quantitation is appropriately accurate and precise at the impurity level. The method proposed in this paper would produce a means to assure this with a single injection, a significant resource savings over the lifetime of the method.

Appendix A

Conditions[a] Instrument/Chemical	Supplier	Location
HP1050	Hewlett-Packard	Palo Alto, CA
PE Turbochrome v6.1.0	Perkin-Elmer	Norwalk, CT
μBondapak 3.9×300 mm, 5 um	Waters	Milford, MA
Sodium octane sulfonate (SOS)	Kodak	Rochester NY
Acetonitrile (ACN)	EM Science	Gibbstown NJ
Methanol (MeOH)	J.T. Baker	Phillipsburg, NJ

[a] Flow-rate: 1.5 ml/min; Temperature: Ambient; Mobile Phase: 0.02 *M* SOS:ACN:MeOH::47:48:5 (v:v:v); Injection volume: 15 uL; Run time: 15 min; Peak elution time: ~9.5 min; Wavelength: 254 nm; Nominal concentration: 0.4 mg/ml.

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