



Editorial

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Dear Colleagues,

In the fifth issue of *Journal of Planar Chromatography (JPC)* 2022, eleven topics on high-performance thin-layer chromatography (HPTLC) are presented by leading scientists in the field of planar chromatography. Six of the papers employ HPTLC for quantification. Five papers use absorption measurements and one paper uses fluorescence as quantification method. HPTLC is easy to perform and usually requires little or no sample pretreatment because separation and quantification are done on disposable plates. I strongly believe that HPTLC is a versatile analytical technique for the analysis of drug formulations that can meet all required standards according to ICH guidelines.

Each month, JPC receives dozens of submissions for the analysis of chemical substances (drugs, produced by pharmaceutical companies), all with a similar experimental design. All of these submissions claim to describe a method, in accordance with ICH guidelines, to “accurately quantify the drug content of the tablet formulation” [1]. All these submissions use the same text structure, similar phrases, and all cite the guidelines Q2(R1) of the International Council for Harmonisation (ICH). All of these contributions to various single- or multicomponent formulations describe an HPTLC separation in conjunction with a scanner quantification (nearly always in absorption) and an evaluation of usually three measured tracks ($n=3$) from which the peak areas (y_i) are taken and their variance (S^2) and mean (\bar{Y}) are calculated according to the following expressions:

$$\bar{Y} = \frac{(y_1 + \dots + y_n)}{n} \text{ and variance } S^2 = \frac{1}{n-1} \sum_{i=1}^n (y_i - \bar{Y})^2$$

The relative standard deviation (%RSD) is calculated from the variance and mean as follows:

$$\%RSD = \frac{\sqrt{S^2}}{\bar{Y}} 100\%$$

Typically reported %RSD values for two pharmaceutical active drugs, for example, are %RSD = 0.98% and %RSD = 1.20% [1]. A typical conclusion of such a paper is “The developed method was found to be precise as the %RSD values for repeatability and intermediate precision studies were < 2%, respectively, as recommended by ICH guidelines” [1].

What is the problem with all these submissions? In all these papers, it is emphasized that one works according to the guideline ICH Q2(R1), which deals with the validation of analytical procedures. The introduction to these guidelines clearly describes their goal: “**The objective of validation of an analytical procedure is to demonstrate that the analytical procedure is suitable for the intended purpose**” [2]. In all these contributions, the “purpose,” the reason for the generation of the method is missing. Thus, the justification of the submitted work is missing as well, because the proof that the intended purpose was achieved is the justification of the work. No one reads a paper that shows it does not work. But that is exactly what all these papers show: HPTLC does not work for drug analysis! As a former control manager in the European pharmaceutical industry, I am sorry to say that such works are simply unnecessary because the results are based on false premises. I do not know the origin of the decision to consider a %RSD value < 2% as precise, but this practice is **not** recommended in the ICH guidelines. The current 2005 ICH Q2(R1) guidelines for validation of analytical methods for drug substances and drug products require that **confidence intervals** should be reported for both trueness and all types of precision. A specific value is not given.

The intended purpose of all global drug analyses with the goal of selling the product (as I learnt in the industry) is to scientifically prove that the true content of the active pharmaceutical ingredient is within a $\pm 5\%$ range of the labeled value, calculated for a 95% level of significance. This can be done by calculating a confidence interval from

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the variance and mean of the analytical measurement. A confidence interval with a significance level of 95% can be interpreted as containing the true value with a probability of 95%. Commonly, analytics and production shared the remaining $\pm 5\%$ uncertainty equally, leading to the analytical objective of quantifying the real content of the tablet formulation with a confidence interval of $\{-2.5\% \text{ of the labeled content}\} \leq \text{labeled content} \leq \{2.5\% \text{ of the labeled content}\}$. As far as I know, this is also routinely used in today's industry and should be the usual goal when developing an analytical method that claims to "accurately quantify the active ingredient content of the tablet formulation" [1]. The task is not done by simply expressing the confidence interval as $-\%RSD \leq \bar{X} \leq \%RSD$, as is often done in such publications!

What do scientists have to do to successfully submit their publications on quantitative HPTLC drug analysis? They simply need to state their goals (the "intended purposes") and then prove with their work that they can achieve them! What to do to prove the claim that the content of the pharmaceutical formulation is within a $\pm 2.5\%$ range of the labeled content? First, they must ensure that their measured HPTLC peak areas $\{y_1, \dots, y_n\}$ are an **independent**, normally distributed set of data (i.e., have the shape of a Gauss distribution). This data set has the unknown parameters true mean μ and true variance σ^2 , which are unknown because we cannot measure an infinite number of data. Often a publication already fails here because a single tablet sample was applied on the plate multiple times and measured after separation, which does not result in an independent data set, not to mention submitters measuring a single track several times to generate their data set. Correctly, the estimation of mean (\bar{Y}) and variance (S^2) must be performed using a set of independently worked up samples, individually applied on plate and measured track by track after separation. We have to consider that the results of our measurements are estimates of the true variance and mean and not the true variance and mean of the Gaussian distribution because we have a limited number of data ($n < \infty$). Both values belong to a Student's t distribution with $f = n - 1$ degrees of freedom and not to a Gauss distribution!

In the second step, we have to calculate the content (e.g., in mg/tablet) from the peak area data (which do not have the unit mg/tablet). For this purpose, a reference measurement (with a certified standard) should be used. Once we have proved the linearity (without intercept!) between the measurement data (y values) and the content data (x values), we can apply the rule of proportion to calculate the sample content from sample and standard measurements (calculated from n data each). The relative standard deviation of our content (S_c/\bar{X}) is the sum of the relative mean variances of the analyte in sample and reference measurement.

$$\frac{S_c}{\bar{X}} = \sqrt{\frac{S_s^2}{n\bar{Y}_s^2} + \frac{S_r^2}{n\bar{Y}_r^2}}$$

If both relative variances are identical, the standard deviation of the final result is by the factor $\sqrt{2/n}$ larger than the standard deviation of a single measurement.

The area of the Gauss function (bell-curve) is 100% when the integration limits are set from $-\infty$ to $+\infty$. The integration in the $\pm \sigma$ limits covers an area of only 68.3%. If we wanted to calculate a 95% confidence interval ($cnf_{95\%}$) for the real mean μ , one would have to integrate the Gauss function in $\pm 2\sigma$ limits (correct: $\pm 1.96 \sigma$)! In this case, there is a 2.5% probability that the value is smaller and a 2.5% probability that it is larger than the 95% confidence interval. Unfortunately, we do not know the value of either μ nor σ (see above), but we can estimate it from S^2 and \bar{Y} using Student's t distribution. The Student factor t depends on n and f (and α) and is the factor between Gaussian and Student's distribution.

$$cnf_{95\%} = 1.96 \frac{\sigma}{\mu} 100\% = t_{\alpha, f} \frac{S_c}{\bar{X}} 100\%$$

This result can be interpreted that the confidence interval with the limits $\bar{X} - cnf_{95\%}$ and $\bar{X} + cnf_{95\%}$ contains the real mean μ with a probability of 95%. For eight measurements ($n = 8$), the Student factor for a 95% confidence interval (i.e., a significant level of $\alpha = 0.05$) is $t_{(\alpha = 0.05, f = 7)} = 2.365$, for three measurements $t_{(\alpha = 0.05, f = 2)} = 4.303$. The confidence interval for three measurements in the case that the variances for reference and analyte are identical is then

$$\pm cnf = \pm \left(4.303 * \sqrt{2/3} * \%RSD \right) = \pm 3.513 * \%RSD$$

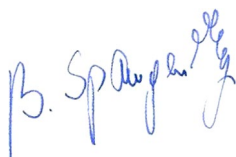
The conclusion "The developed method was found to be precise as the %RSD values for repeatability and intermediate precision studies were $< 2\%$ " misses the true value by a factor of 3.5!

In this issue of JPC, there are two papers on the quantification of chemically produced drugs and four papers on the quantification of drugs in plant samples. In all of them, the term "accuracy" is tested by adding a certain amount of a standard substance and then calculating the recovery rate. The percentage recovery is often misunderstood as the relative error ($Er\%$) of the method. For example, a typical statement is "The percentage relative standard deviation (RSD%) and percentage relative error ($Er\%$) did not exceed 2.0% proving the high repeatability and accuracy of the developed method for the estimation of the analytes in their bulk form." I must emphasize again that there is no 2% threshold that distinguishes between acceptable and unacceptable results. The aim of the ICH guidelines is to "demonstrate that the

analytical procedure is suitable for the intended purpose” and to do this, the intended purpose must be stated prior to the analytical demonstration. On the subject of “accuracy,” the ICH guidelines state “The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness [2].” ICH guidelines recommend the use of confidence intervals for reporting accuracy results. If one claims to work according to ICH guidelines, one should use confidence intervals (with a significant level of $\alpha = 0.05!$), for example, in the form that the average percentage recovery should be within a defined range. After that, it should be demonstrated in the paper that this is achievable with the described method. Tolerance intervals can be used to specify appropriate accuracy specifications, e.g., that no percent recovery should be less than 80% or greater than 120% [3].

According to the current ICH guideline, it is necessary to specify confidence intervals for both trueness and all types of precision [2]. If one claims to work according to ICH guidelines, one should do so!

By the way, all articles in this JPC issue are worth reading.



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