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Perspective: What constitutes a quality paper in drug analysis?

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Drug analysis

Drug analysis is a part of analytical chemistry focused on pharmaceuticals [1]. It encompasses raw material (excipients and drug substances) and medicines (drug products). Development of drugs is a long-term process from drug discovery to approved drug patient administration through marketing authorization. Each step of the process involved analytical chemistry from generic method for drugs screening to quality control of medicines. In the context of marketed pharmaceuticals, the finished product is already fully characterized with defined qualitative and quantitative composition. Consequently, the main objective of the analytical method devoted to drug analysis is then the specific accurate quantification of the active principal component and/or related impurities in a defined matrix.

What constitutes a quality paper?

The question addressed today is focused on paper quality in the field of drug analysis. Next to the form aspects (context of the study, figures quality, grammar, spelling, etc.), the scientific novelty and relevance are the main topic assessed by the reviewing process. In the present discussion, we would like to focus the debate on the method lifecycle and, in particular, on reliability of generated results using the published analytical methods. Indeed, we can suggest that a quality paper provides consistent results. It means that the method described in the publication is able to provide reliable results in the context of its intended purpose. Results consistency is a concept often used in literature but not fully described and sometimes not well understood.

Analytical method must be considered through its own lifecycle as illustrated in Fig. 1. The cycle concept means that it is not an ended process with routine analysis at the final step. Indeed, medicines routine analysis is the final goal but some further steps could happen. If we consider a drug marketed for several years or several decades, we can easily imagine that the original developed method is no longer 'up-to-date'. The recent progress of analytical technologies (i.e. column geometry and stationary phase, instrumentations, etc.) could led to the development of an optimized method regarding its fastness, easiness or efficiency. Consequently, the analytical method will continue through this cycle for development and validation of an optimized method. Another usual situation is method transfer, for example to another production site or an external quality control laboratory [2]. Depending of the transfer protocol, method revalidation could be required before going to routine analysis. To summarize, analytical method lifecycle is a continuous process with the objective of constant quality improvement.

Besides the efforts to optimize the best analytical method (i.e., specificity, efficiency, sensitivity, etc.), the usual way to evaluate method reliability is to perform method validation. Indeed, method validation evaluates the quantitative performance of analytical method but performing method validation is not an absolute guarantee of results consistency. The following discussion aimed to propose a way to reach them.

Analytical target profile (ATP)

Before selecting and developing a suitable analytical method, it is important to pay attention to the analytical instrumentation. Indeed, equipment performance has a direct impact on the overall performance of the analytical method. The US Pharmacopeia recently introduced a chapter dedicated to analytical instrument qualification [3]. Actually, instrument qualification is the keystone of data quality [4], before method validation. Unfortunately, equipment qualification is too often neglected prior to method development in unregulated laboratories (e.g., academia). The use of fully maintained and qualified instruments is the only way to guarantee data quality of measurements and results.

In addition to this basic aspect, the development of an analytical method should always start with an accurate and exhaustive description of the needs. This major step is now commonly linked to the definition of the analytical target profile (ATP). The ATP specifies the purpose of the method and must be as large as possible with a global vision throughout the analytical method lifecycle. Regarding objectives, all conceivable analytical techniques or instruments as well as various procedures should be investigated. Consequently, precise context, sample

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characteristics, regulatory constraints [2], laboratory resources and desired performances are critical elements to determine. All these elements help the scientist to investigate method parameters that potentially affect both qualitative and quantitative method performances. For instance, in the context of chromatographic applications, method parameters could have a huge impact on the separation of two critical components, such as impurities or matrix compounds, or even on total run time, a key aspect in high-throughput applications. Depending on the detection technique, method parameters could also have influence on analytical response but also on targeted dosing range.

Prior scientific knowledge is a reliable base to gather such information and must always be involved in the development process. Anyway, a broader vision of the intended purpose of the analytical method should be taken into account. Nowadays, such an approach is increasingly considered and tackled by a risk-based and integrate approach of the method development, namely the analytical quality by design strategy (AQbD) [5]. AQbD strategy is a multivariate approach supported by design of experiments (DoE) allowing the definition of a wisely defined working space. This method operability design region (MODR) gathers a set of experimental conditions where qualitative performances of the analytical method is guaranteed with a defined risk.

However, method knowledge and flexibility in terms of efficient operable conditions offered by such a strategy should not cover up the need for accurate quantitative performance that essentially contributes to an adequate analytical method in drug analysis.

Error sources

Systematic and random error are mainly used and described in the literature. In drug analysis context, the first critical point to mention is that these errors have to be estimated on the method results (i.e., measured concentrations) and not on the responses (*i.e.*, signal). Moreover, to be representative of the future routine use of the method, the validation should be performed using a suitable matrix to mimic the samples. Consequently, the first step of method validation is to define the best response function considering its ability to properly estimate the results. The response function depends on the analytical method used, the concentration range and the matrix. Mathematical models such as linear, quadratic, linear after logarithmic transformation, etc., should be considered. The use of a response function is a first error source as the results will be affected by a prediction error. This prediction error is minimized if a proper model is used. In addition, determination coefficient (R^2) is largely used to evaluate the suitability of a linear regression model, and often wrongly associated with method linearity (see below). As illustrated in Fig. 2, the selection of an adequate response function decreases the prediction error, i.e., the error associated to the model. To summarize, the objective of this first step is to define the best calibration model considering all analytical aspects. The evaluation of method linearity is never related to its calibration model as discussed in the next section.

Following the selection of response function, other validation criteria could be then estimated on the results (i.e., back-calculated concentrations) [6-7]. Analytical method errors could be compared to archery as illustrated in Fig. 3. Considering the objective of this sport, which is to reach the center of the target, the archer plays with two different types of errors: (i) the mean of his assay results is not centered on the target, it is the systematic error (or bias); (ii) his assay results are randomly distributed around the mean, it is the random error. In archery and in analytical chemistry, the systematic error can be deeply assessed and

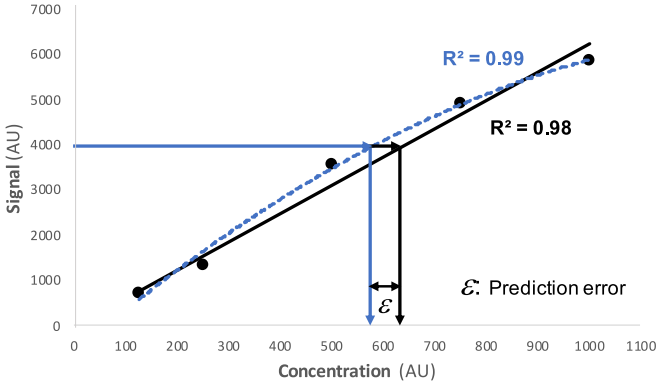

Fig. 2. Response function – *AU arbitraty unit.*

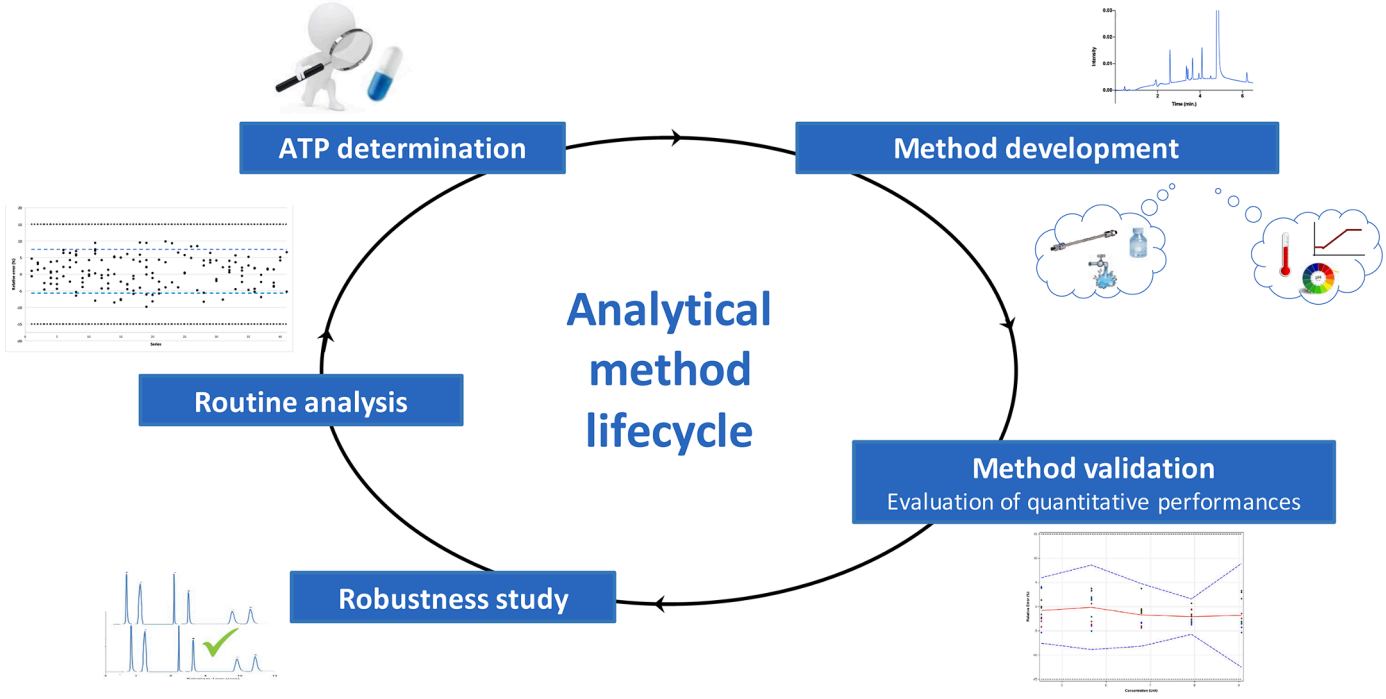

Fig. 1. Analytical method lifecycle for drug analysis.

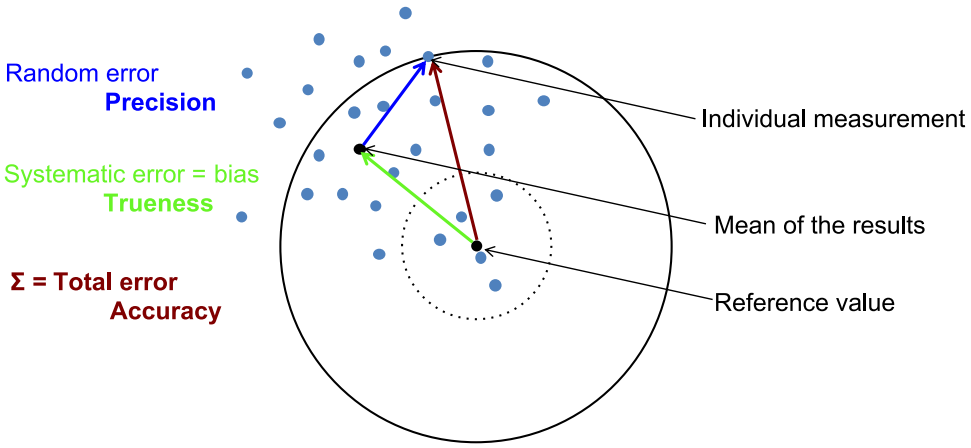

Fig. 3. Analytical method error source.

controlled. In our sport example, the correction of arrow direction could cancel the bias. In drug analysis, bias could be explained by matrix effect and could be then canceled (e.g., by performing the calibration within matrix or by performing blank correction). On the contrary, random error could not be canceled but it is possible to minimize it. Again, in our sport example, intensive practice could help to reduce the shoot variability and then observe all of them in a narrower area of the target. In the same way, the multiplication of the assays will enhance statistics leading to better prediction and then reduced the overall variability when all significant sources of variability are properly identified and further well managed.

Indeed, precision (random error) encompasses repeatability, intermediate precision and reproducibility. Attention should be paid to the terminology used in the publications. Indeed, reproducibility is sometimes wrongly used to mention repeatability or intermediate precision. It evaluates method precision at the interlaboratory step [8], this criterion is then not applicable when method validation is only performed at the laboratory scale. To summarize, repeatability represents the replicate effect while intermediate precision represents the series/day effect. To evaluate these criteria, it means that the same protocol including several replicates should be repeated during several series (often associated to days). These series effects could also involve different operators, standards or reagents batches, etc. to mimic the future use of the method during routine analysis. Suitable variance analysis should be performed to properly evaluate these two criteria [8]. Repeatability estimates the replicate effect while the intermediate precision criterion combines the replicate (intra-series) and series effects (inter-series). It is out of scope of the present paper but we would like to remind that calculating the overall RSD for the whole measurement of different series do not provide a consistent evaluation of method intermediate precision.

Both trueness (systematic error) and precision (random error) determine the accuracy criterion (total error) [9-11]. The question to answer at this stage is the following: is the error budget (*i.e.*, total error) in accordance with previously defined method specifications (ATP)? If a positive answer is drawn, it means that accurate results will be obtained using this analytical method. It is important to keep in mind that the specifications, and consequently the error budget, could differ depending the method objectives (e.g., API, impurities, large molecules, matrices, etc.). Accurate results indicate that results consistency is demonstrated and highlight method reliability in accordance with the ATP definition.

Robustness

We also would like to briefly discuss about robustness. Method robustness is an important criterion to evaluate with different strategies proposed in the literature [5;12-13]. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters [4]. To extrapolate, it means that a robust method provides unaffected results even with some parameters changes. Nevertheless, method robustness is not enough to demonstrate results consistency, and its quantitative performances should be evaluated by means of validation as described above. Thenceforth, method robustness is an important step on the analytical method lifecycle that should be evaluated prior or post validation step, depending in the method development strategy [5;12-13].

Conclusion

Accurate results can be only be obtained with a “good” method but a “good” method does not necessarily provide accurate results. Consistent, accurate and reliable results are obtained if the total error estimated during method validation is acceptable considering the specifications defined within the ATP. It means that the method is able to provide reliable results in the context of its intended purpose. Regarding the last validation criterion, i.e., linearity, we can also mention that method linearity is guaranteed if accurate results are obtained. Indeed, method linearity evaluates the linearity between measured concentration (the results) and introduced concentration. Such relation is only demonstrated during validation step.

From a publication point of view, a good paper in the field of drug analysis must demonstrate the ability to obtain accurate results. It means that the reader must also generate accurate results when using the published method. Scientific paper combining hot topic research area and demonstration of results consistency should be largely considered by other researchers. In conclusion, advice is provided for the preparation of quality papers in the field of drug analysis. A particular attention should be paid to the ATP definition. Both method objective and requirements will impact the whole analytical method lifecycle and then indirectly impact results consistency. The second important aspect is the evaluation of error budget by means of method validation. Thanks to this evaluation, we can answer to the question: is my method able to provide accurate results considering the specifications?

Declaration of Competing Interest

None.

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References

[1] A.-.M. Tivert, J. Vessman, Pharmaceutical analysis – overview, in: P. Worshlod, A. Townshend, C. Poole (Eds.), Encyclopedia of Analytical Science, Elsevier, Amsterdam, 2005, pp. 78–89.

[2] Eudralex volume 4 GMP part 1 chapter 6 quality control https://ec.europa.eu/health/sites/default/files/files/eudralex/vol-4/2014-11_vol4_chapter_6.pdf 2021.

[3] USP, Chapter 1058 – analytical instrument qualification 2021.

[4] C. Hubert, J. Widart, E. Ziemons, Ph. Hubert, Process analysis | maintenance, reliability, and training☆, in: P. Worsfold, C. Poole, A. Townshend, M. Miró (Eds.), Encyclopedia of Analytical Science (Third Edition), Academic Press, 2019, pp. 389–395.

[5] A. Dispas, H.T. Avohou, P. Lebrun, Ph. Hubert, C. Hubert, Quality by design' approach for the analysis of impurities in pharmaceutical drug products and drug substances, TrAC 101 (2018) 24–33.

[6] ICH Q2 R1 – validation of analytical procedures: text and methodology 2021.

[7] A. Bouabidi, E. Rozet, M. Fillet, E. Ziemons, E. Chapuzet, B. Mertens, R. Klinkenberg, A. Ceccato, M. Talbi, B. Streel, A. Bouklouze, B. Boulanger, Ph. Hubert, Critical analysis of several analytical method validation strategies in the framework of the fit for purpose concept, J. Chromatogr.A 1217 (2010) 3180–3192.

[8] ISO5725-2:2019 accuracy (trueness and precision of measurement methods and results 2021.

[9] Ph. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P. A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal – part I, J. Pharm. Biomed. Anal. 36 (2004) 579–586.

[10] Ph. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P. A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, E. Rozet, Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal – part II, J. Pharm. Biomed. Anal. 45 (2007) 70–81.

[11] R.K. Burdick, D.J. LeBlond, D. Sandell, H. Yang, H. Pappa, Statistical methods for validation of procedure accuracy and precision, Pharmacopeial Forum 39 (2013).

[12] B. Dejaegher, Y. Vander Heyden, Ruggedness and robustness testing, J. Chromatogr.A 1158 (2007) 138–157.

[13] Y. Vander Heyden, A. Nijhuis, J. Smeyers-Verbeke, B.G.M. Vandeginste, D. L. Massart, Guidance for robustness/ruggedness tests in method validation, J. Pharm. Biomed. Anal. 24 (2001) 723–753.

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