Bioanalytical method validation: How much should we do and how should we document?

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Scientists need analytical methods to measure elements, low molecular weight compounds, and macromolecules in various matrices to support pharmaceuticals, environmental, biological, medical, or food sciences. For that purpose, quantitative methods are developed and characterized demonstrating that the analytical procedure employed for a specific test is suitable for its intended use. This process is often referred to as the method validation [1]. The intention of method validation is to determine the key characteristics on an assay such as sample volume, limit of quantification, linearity, precision, accuracy, and selectivity, and to address potential issues such as analyte stability or sample storage to name only a few. Out of the 843 published papers in 2018 in *Analytical and Bioanalytical Chemistry*, 49 contributions used the term quantification and quantitation determination in the title, and 110 the term validation in the manuscript. Some manuscripts describe only linearity, limit of quantification (LOQ), and limit of detection (LOD) while others refer to published documents as for example “the validation was performed according to Commission Decision 2002/657/EC” [2] or “the validation procedures per the Food and Drug Administration guidelines on validation of bioanalytical methods were appropriately followed in this study” [3] and provide more details. As guidelines are not standard operation procedures (SOP), it is often difficult to follow what the authors really did for method validation and what their acceptance criteria were.

On Web of Sciences, many papers/reviews can be found describing various method validation procedures. Also, many scientific societies (e.g., Eurachem [4]) and agencies (e.g., European Medical Agency (EMA) [5]) have issued method validation documents. Fundamentally, the extent of validation and the

formalism are needed depending on the fit for purpose of the assay. Method validation can be considered as a time-consuming procedure but not doing it properly may result in a waste of money, resources, and in wrong scientific knowledge. In the field of bioanalysis where various players are involved including universities, governmental agencies, contract research organization (CRO), hospital, and the pharmaceutical industry, assay methods are developed to quantify pharmaceutical and their metabolites, endogenous compounds, peptides, and proteins in various biological matrices. In an academic environment, novel analytical approaches and workflows are investigated to improve sensitivity, to reduce cost, and to improve throughput of analysis or analytical methods that are developed to support research programs. Pharmaceutical industries and CRO validate assays for their use in the drug discovery and drug development process while in hospital analytical methods are applied to optimize drug treatment. Governmental agencies need assay methods to control quality of drugs and to monitor abuse of drug or exposure in the environment. The purpose of the FDA guidance is to ensure sound scientific principles and with strict attention to quality assurance and quality control and to help sponsors of investigational new drug applications (INDs) or applicants of new drug applications (NDAs) to validate bioanalytical methods used in human or animal studies, or biomarker concentration evaluation. Many of these studies are performed in laboratories performing work under good laboratory practice (GLP) or good clinical practice (GCP), which are regularly audited. With the emergence of multiple guidance’s from different health authorities, the pharmaceutical industry has also express its concerns and calls for towards a global harmonization of the guidelines on bioanalytical method validation and sample analysis for pre-clinical and clinical studies [6]. Guidance from FDA or EMA is binding for the industry but not for academic research laboratories. The question which finally arises is in the frame of a publication what is the appropriate way to document the validation a novel assay/methodology or assays which were used to address important investigations. A scientific publication should not be written as a standard operation procedure but present novel

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methodology, workflows, or applications. Method validation should support the analytical claims made by the authors and the following fundamental parameters should be discussed: pre-analytical conditions, accuracy, precision, dynamic range, sensitivity, and selectivity. Acceptance criteria defined by the authors should be clearly presented and can vary depending on the fit of purpose. A very important point to keep in mind is that bioanalytical method validation is generally performed with analyte(s) spiked in blank urine, plasma, or tissues, and even a fully validated assay can fail when applied to real samples [7]. Therefore, the performance of an assay should be illustrated with the analyses of real samples which are sometimes problematic for academic research laboratories. Also, when claims are made for better performances compared with published methods, cross-validation results should be presented.

Method validation is an important topic in analytical sciences for quantitative analysis and subject of controversial views in particular for publications. Validation data are of high importance to the reviewers and readers to build an opinion about the applicability and performance of a novel assay or an innovative analytical methodology. The extent of validation is clearly dependent on the fit of purpose and therefore should be clearly defined and discussed.

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