Journal of Chromatography A, 1353 (2014) 10–27

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Bioanalytical chromatographic method validation according to current regulations, with a special focus on the non-well defined parameters limit of quantification, robustness and matrix effect

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ARTICLE INFO

Article history:
Received 3 December 2013
Received in revised form 21 March 2014
Accepted 27 March 2014
Available online 4 April 2014

Keywords:
Method validation
Bioanalysis
Limit of quantification
Robustness
Matrix effect

ABSTRACT

Method validation is a mandatory step in bioanalysis, to evaluate the ability of developed methods in providing reliable results for their routine application. Even if some organisations have developed guidelines to define the different parameters to be included in method validation (FDA, EMA); there are still some ambiguous concepts in validation criteria and methodology that need to be clarified. The methodology to calculate fundamental parameters such as the limit of quantification has been defined in several ways without reaching a harmonised definition, which can lead to very different values depending on the applied criterion. Other parameters such as robustness or ruggedness are usually omitted and when defined there is not an established approach to evaluate them. Especially significant is the case of the matrix effect evaluation which is one of the most critical points to be studied in LC-MS methods but has been traditionally overlooked. Due to the increasing importance of bioanalysis this scenario is no longer acceptable and harmonised criteria involving all the concerned parties should be arisen. The objective of this review is thus to discuss and highlight several essential aspects of method validation, focused in bioanalysis. The overall validation process including common validation parameters (selectivity, linearity range, precision, accuracy, stability. . .) will be reviewed. Furthermore, the most controversial parameters (limit of quantification, robustness and matrix effect) will be carefully studied and the definitions and methodology proposed by the different regulatory bodies will be compared. This review aims to clarify the methodology to be followed in bioanalytical method validation, facilitating this time consuming step.

Contents

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http://dx.doi.org/10.1016/j.chroma.2014.03.077
0021-9673/

1. Introduction

Method validation is a necessary process to demonstrate that an analytical method is suitable for its intended use, thus, that it can offer accurate, precise and reproducible results. These reliable results are essential for bioavailability, bioequivalence, pharmacokinetic, pharmacodynamics or toxicological studies where analytes must be quantified in biological matrices such as urine or plasma. Consequently, method validation is a crucial step in bioanalysis and essential for laboratories to adhere to current Good Manufacturing Processes (GMP), Good Laboratory Practices (GLP) or International Organisation for Standardisation (ISO) regulations, such as ISO17025 [1] and ISO15189 [2]. Nowadays several regulatory bodies deal with bioanalytical validation and even though there are still some divergences, a relative consensus has been reached by the scientific community. Nevertheless, the continuous advances in instrumentation and the emergence of more demanding analytical challenges make bioanalysis a field in permanent evolution. Therefore, method validation guidelines should keep up with this progression in order to cope with real requirements, and to this end the participation of all the concerned parties (pharmaceutical industry, statisticians, analytical chemists, academicians. . .) is imperative.

The first attempt to harmonise bioanalytical validation dates back to 1990, when the United States Food and Drug Administration (FDA) and the American Association of Pharmaceutical Scientists (AAPS) sponsored the first bioanalytical method validation workshop in Crystal City (Arlington, VA). The aim of this workshop was to reach a consensus on the requirements in validation for analytical methods focused on bioavailability, bioequivalence and pharmacokinetic studies. Parameters considered essential were set (stability, accuracy, precision, sensitivity, specificity, response and reproducibility) and the outcome of this workshop was well received by the scientific community eager of a harmonised policy to work with. In consequence, the report of this workshop published in 1992 [3] became the basis of nowadays bioanalytical validation and the starting point for a stimulating discussion. Soon, due to the importance of method validation and the great advances made in this field during the following years, the need for an official document became apparent, and finally, in 1999 the FDA issued the *"Draft Guidance for Industry: Bioanalytical Method Validation"* [4]. Shortly after, the FDA and AAPS organised the second Crystal City workshop in order to discuss the draft and give the scientific community a chance of sharing the experience accumulated over the 10 years elapse since the first workshop took place. New topics such as partial, full and cross-validation were discussed; stability experiments were studied more in depth and flourishing hyphenated mass spectrometry techniques (MS) were addressed. The summary of this workshop was published in 2000 [5] and it became the backbone for the official *"Guidance for Industry: Bioanalytical method validation"* issued in 2001 [6].

This document can be considered the cornerstone of bioanalytical validation since many laboratories standardised their validation procedures following this guideline and the later proposals for new guidelines used it as reference. Nevertheless, analytical chemistry and drug analysis are in an unceasing development and therefore new worries and problems are continuously emerging. This is so that after the publication of the guidance two new Crystal City meetings were organised in 2006 and 2008 and their respective white papers published in order to complete the initial guideline [7,8]. The first one dealt with some questions that remained unresolved such as metabolite and stability studies, carryover and especially, matrix effect (ME) [9]. In this workshop incurred sample reanalysis (ISR) was discussed for the first time [10] but it was not until 2008 workshop that this topic was carefully studied. The white papers arising from these workshops are widely accepted by the scientific community despite not being official documents. Nevertheless and after more than 10 years the FDA needed to release an update of the validation guideline. Therefore, the draft of the guideline update was published in September 2013 [11]. The AAPS called the scientific community for a new workshop held in Baltimore in December 2013 where the FDA draft was carefully studied. As expected, a vivid discussion about hot topics such as validation of biomarkers, endogenous compounds or anticoagulant change took place. Once the outcome of this conference is considered, the final guide will be released.

Besides FDA other regulatory agencies and organisations have dealt with bioanalytical method validation. In Europe, this field was to some extent covered by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) "*Validation of Analytical Procedures Q2 (R1)*" [12], developed between 1994 and 1996 by experts on method validation from European Union, Japan and USA. Nevertheless, this guideline is addressed to identification tests, control of impurities or active ingredient quantification. Obviously the European Union needed an official document to establish the regulation for bioanalytical validation and consequently, in December 2008 European Medicines Agency (EMA) released the "*Concept paper/recommendations on the need for a guideline on the validation of bioanalytical methods*" [13]. Immediately the European Bioanalysis Forum (EBF), an organisation comprised of bioanalytical scientists working within the pharmaceutical industry, expressed their concern about the possibility of contradiction between this new guideline and the world-widely recognised FDA guidance [14].