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Professional paper

Comparative overview of European Pharmacopoeia and United States Pharmacopoeia requirements for validation of alternative microbiological methods

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Abstract

Pharmacopoeial microbiological methods, foundational to the functioning of the pharmaceutical facilities, are limited by the time required for analysis, which is associated with significant costs. To overcome this limitation, alternative (rapid) microbiological methods (ARMMs) are encouraged due to faster execution, easier monitoring and automation while improving accuracy, specificity, sensitivity, and precision. This article presents an overview of the requirements for demonstrating equivalency or superiority of ARMMs to the established Pharmacopoeia methods, as recommended by Ph. Eur. and USP. Steps that should be taken to evaluate candidate alternative methods, from selection of analytical technology to qualification of the method with actual product are described. Special emphasis is given on the validation process for the ARMMs and the validation criteria that need to be evaluated in order to prove method suitability and equivalency to Pharmacopoeia methods.

Key words: alternative (rapid) microbiological methods**,** Pharmacopoeia requirements, suitability, implementation

Introduction

The presence of microorganisms (microbial contamination) in pharmaceutical products that can occur at any stage of the drug manufacturing, distribution, or dispensing process, compromises quality and poses health risks for the patients (Eissa, 2016; Myemba et al., 2022). Mesophilic bacteria and fungi that may grow under aerobic conditions can alter the chemical composition of the product which may result in adverse or toxic effects due to the formation of toxic degradation products (Larmené-Beld et al., 2019; Smith et al., 2013). Additionally, microbial contaminants, particularly on antimicrobial products, may give rise to resistant strains, contributing to antimicrobial resistance (Kamil and Lupuliasa, 2011). Therefore, all pharmaceutical products must meet stringent quality standards to ensure their safety and efficacy. Regulatory agencies worldwide, such as the Food and Drug administration (FDA) and the European Medicines Agency (EMA), have established strict guidelines for microbiological control in pharmaceutical production.

Official Pharmacopoeias provide microbiological methods (compendial methods) that are inexpensive, easy to perform, and do not require specialized equipment. These tests usually last several days, which makes the

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release of the results slower. Also, they cannot detect certain microorganisms which may be present in the sample, such as the viable but nonculturable microorganisms (VBNC) (Lemos et al., 2019; Miller, 2010). The growing need for more rapid results led to the development of the so-called alternative (rapid) microbiological methods (ARMMs) (Henriques et al., 2019; Lemos et al., 2019; Prada-Ramirez, 2023).

ARMMs encompass broad group of analytical methods that can be classified based on the type of determination and the detection principle. According to the type of determination, they can be qualitative, quantitative or identification tests. Qualitative ARMMs evaluate the presence or absence of viable bacteria, quantitative ARMMs assess the number of microorganisms present in a sample, and the identification tests permit classification of a specific group of microorganisms. The three detection methods in ARMMs are detection of growth, direct analysis or analysis of cell components (indirect analysis) (Henriques et al., 2019). Compared to the Pharmacopeial methods, ARMMs offer the advantages of faster execution, monitoring and automation while improving accuracy, specificity, sensitivity and precision (Johnson, 2023; Prada-Ramirez, 2023, Prada-Ramírez et al., 2023). However, their implementation in pharmaceutical industry has slow progress because they require expensive equipment and the regulatory agencies tend to be overly cautious in endorsing these methods as an integral part of the product release process (Henriques et al., 2019; Prada-Ramírez et al., 2023).

Despite the obvious advantages, prior implementation ARMMs must be subjected to robust validation in which all essential method parameters are tested: sufficient specificity, accuracy, precision, limit of qualification (LOQ), and other. Utilizing alternative methods can assist pharmaceutical manufacturers in monitoring their entire process to ensure that it is free of microbial contamination faster than Pharmacopeial methods. Additional benefits include reduced time to obtain the test results and no need to perform repetitive work. (Provenzano, 2024).

The aim of this article is to present an overview of the main requirements of Ph. Eur and USP chapters for validation of ARMMs, how the applicability of the method should be evaluated as well as the compatibility of the tests with the products to be examined.

New technologies available to replace Pharmacopoeial microbiological methods – short summary

The technologies that could potentially replace Pharmacopeial microbiological tests have been categorized by the Parenteral Drug Associations (PDA's) Technical Report Number 33 (PDA, 2000) as growth-based methods, viability-based methods, both direct measurement methods, and cellular component or artefact-based technologies, as well as nucleic acid-based technologies. The growth-based methods detect a signal after a short incubation period in liquid or on a solid media (for example, detection of CO_2 production by colorimetric methods, or measurement of changes in headspace pressures, impedance and biochemical assays) (Moldenhauer, 2003; Moldenhauer, 2013). The third type of ARMMs is cell component analysis or indirect measurement; expression of certain cell components correlates to microbial presence (for example, amplification of DNA or RNA by polymerase chain reaction (PCR)). The application of Maldi-TOF can be used for identification of microbial species while Raman spectroscopy can be used for detection, enumeration, and identification (Moldenhauer, 2013; Ronningen and Barko, 2009; Shelep, 2011). Having in mind the potential of the new technologies, it becomes obvious why it is important to perform a rigorous microbiological method validation (Sandle, 2021).

Guidelines for validation of ARMMs

The process of introducing an alternative method does not differ significantly when compared to introduction of a Pharmacopoeia method. The key aspects that must be answered with the validation are: does the method work and is the method appropriate for the specific sample(s) to be tested? The key validation criteria, acceptable recovery rates for the examined microorganisms as well as the fact that the alternative method must provide accurate identification, are the same for both alternative or Pharmacopoeia methods. Thus, ARMMs must be compared to the Pharmacopeial methods to demonstrate they are equivalent or even that they provide less uncertainty (ICH *Q2* R1, 2005; Peris-Vicente et al., 2015; Riley, 2006).

The first information provided as a guidance for validation of ARMMs was The Parenteral Drug Association (PDA) Technical Report No. 33 (PDA, 2000). This document provides guidance for the successful evaluation, validation, and implementation of ARMMs needed by the pharmaceutical, biotechnology and medical device industries to assure product quality. It was written to establish industry-wide criteria on what constitutes an acceptable alternative or rapid microbiology test to the Pharmacopeial or classical method according to fulfill the criteria of quality organizations and regulatory agencies (PDA, 2013).

The general European Pharmacopoeia (Ph. Eur.) chapter 5.1.6 "Alternative methods for control of microbiological quality" was first introduced in 2006 aiming to facilitate the implementation and use of ARMMs where this can lead to cost-effective microbiological control and improved assurance for pharmaceutical product's quality. The principle of detection, enumeration, isolation, and identification of methods that have successfully been used in the quality control (QC) of

pharmaceuticals are described and a guidance on how to validate alternative microbiological methods is provided (Ph. Eur. 11.5 07/2017:50106).

The United States Pharmacopoeia (USP) Chapter <1223>, "Validation of alternative microbiological methods", provides detailed guidance on the selection, evaluation, and use of ARMMs. These include tests to determine whether microorganisms are present in a sample, tests to quantify the number of microorganisms and tests designed to identify microorganisms. To properly implement alternative methods, one must consider a number of important issues before selecting the analytical technology and qualifying the method with the actual product. These issues include, but are not limited to, identification of suitable alternative methodology, development of user specifications for equipment selection, demonstration of the applicability of the method as a replacement for a standard compendial method, and qualification of the method in the work environment where it is meant to be used. The limitations of the use of CFU (Colony Forming Unit) as a standard signal for microbiological methods are also described (Miller, 2015; Provenzano, 2024).

Overview and comparison of the Ph. Eur. and USP requirements for validation of ARMMs: key parameters and considerations

Despite the unique nature of microbiological tests, the validation of a microbiological method is a process where it is experimentally established that method's performance characteristics fulfill the requirements for the intended application, when compared to the compendial method. For ARMMs, the validation must prove that the alternative method provides reliable and consistent results (Moldenhauer, 2013; Riley, 2006).

The Pharmacopeial chapters (Ph. Eur., USP) and the guidelines provided in PDA TR33 describe important steps that should be taken to evaluate candidate alternative methods, to select the analytical technology, and ultimately to qualify the method with actual product. These steps include, but are not limited to:

1. Identification of a potentially suitable alternative methodology;
2. Demonstration that the method is equivalent and applicable as a replacement for a standard compendial method;
3. Development of user specifications for equipment selection;
4. Qualification of the method in the laboratory.

Based on their characteristics, the ARMMs described in the Ph. Eur. 11.5 and USP <1223> are qualitative methods (not enumerative), a category that includes assays intended to demonstrate the absence of microorganism and quantitative methods that give a numerical (enumerative) result in terms of the microbial content. There are inherent analytical factors that must be considered in the implementation of the candidate ARMMs and in the comparison with Pharmacopoeial method. With respect to qualitative ("absence of") analysis, it is critical to consider that in microbiology, the finding of "no microorganisms present" does not mean in absolute terms that zero cells are actually present. A result of "no growth" in a current Pharmacopoeial method is properly interpreted as "no growth was detected under the specified conditions" (Dalmaso and Denoya, 2016; USP 1223).

The Ph. Eur. chapter 5.1.6 "Alternative methods for control of microbial quality" explains that the validation process should be performed at two levels, namely primary validation (performed by the supplier of the alternative technology) and validation for the intended use, a responsibility of the method user (Ph. Eur. 11.5). USP <1223> explains alternative microbiological methods will generally provide a cell count signal or unit of measurement that is not a CFU. Therefore, the validation of such methods should involve the following phases: qualification of the instrumentation or equipment, qualification of the analytical method using standardized microorganisms (in the absence of product) and finally, demonstration of equivalence to the Pharmacopoeia method using standardized microorganisms (in the absence of product). This is followed by method suitability testing, in which the actual product or test samples are introduced (USP <1223>).

The first phase in the validation is to qualify the instruments or the suitable equipment that will be associated with the alternative method (Miller, 2015). The Ph. Eur. 5.1.6, in Validation for the intended use recommends the following phases: user requirement specification (URS), design specification (DS), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). Extended outline is provided only on how to perform the PQ. USP <1223> directs the user to the guidance provided in USP <1058>, *Analytical Instrument Qualification*, and briefly lists the following phases to be addressed: the development of the URS, (IQ, OQ, and PQ). Both guidelines agree that the PQ is the phase of validation, which demonstrates that the alternative method consistently performs in accordance with predetermined criteria (i.e., as specified in the user requirements) and thereby provides correct and appropriate results. Hence, the PQ can comprise qualifying the analytical method, demonstrating equivalence to the compendial or existing method and verifying the product or test sample does not significantly impact the performance of the alternative method (Ph. Eur. 11.5; Provenzano, 2024; USP <1223>).

In the next phase of the USP's validation design (qualification of the analytical method), it should be demonstrated that the alternative method will detect or enumerate a relevant panel of microorganisms. During this phase, there is no comparison to the compendial method (studies are conducted in the absence of actual product or

test samples). A few minor differences exist and these may be due to how each validation phase is identified and when the phase is performed (e.i., when a comparison to the Pharmacopeial method is made). However, there is a significant difference between USP <1223> and the other two documents relating to the criterion "repeatability." Repeatability is a subset of precision, and USP <1223> defines repeatability precision as follows: "The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of the same suspension of microorganisms and uses different suspensions across the range of the test". This definition is essentially identical to the definition of precision in PDA TR33 and very similar to what is defined in Ph. Eur. 5.1.6. However, precision (including repeatability) is not identified in either PDA TR33 or Ph. Eur. 5.1.6 as being a recommended validation parameter for a qualitative method. Additionally, ruggedness does not appear in Ph. Eur. 5.1.6 as a validation criterion. This is not an issue because ruggedness (as described in the USP and TR33) is synonymous to intermediate precision within Ph. Eur. 5.1.6. Also, Ph. Eur. 5.1.6 proposes that supplier of the method/technology perform a *primary validation* using Pharmacopoeia test strains appropriate for the intended use and in the absence of product. This would be followed by the end-user verification of some of the supplier's primary validation data and critical parameters of the method (in the absence of product) using similar Pharmacopeial test strains, in-house isolates, or, stressed/slow-growing microorganisms or, a comparison of the response between the alternative and the compendial methods, which would be analogous to what is described in the USP <1223> equivalency section.

A comparison of the parameters required for validation of ARMMs according to the test type and according to different guidelines is presented in Table 1.

As it can be seen from Table 1, some minor differences in the criteria across the documents can be noticed. As such it is important to clearly identify the requirements and the criteria that must be met at the specific cite. Within these documents there are differences upon whether it is a qualitative, quantitative or an identification test method (Moldenhauer, 2013).

Table 1. Overview of validation requirements for ARMMs according to type of test and different guidelines

	Ph. Eur. 5.1.6		USP		PDA Technical report 33	
	Test type		Test type		Test type	
Parameter	Qualitative	Quantitative	Qualitative	Quantitative	Qualitative	Quantitative
Accuracy	Yes(1)	Yes	No	Yes	No	Yes
Precision	No	Yes	No	Yes	No	Yes
Specificity	Yes	Yes	Yes	Yes	Yes	Yes
LOD*	Yes	Yes(2)	Yes	Yes	Yes	Yes
LOQ**	No	Yes	No	Yes	No	Yes
Linearity	No	Yes	No	Yes	No	Yes
Range	No	Yes	No	Yes	No	Yes
Robustness	Yes	Yes	Yes	Yes	Yes	Yes
Ruggedness	***	Yes	Yes	Yes	Yes	Yes
Repeatability	***	Yes	Yes	Yes	***	***
Suitability testing***	Yes	Yes	***	***	***	***
Equivalence testing	Yes	Yes	Yes	Yes	Yes	Yes

*LOD= limit of detection; **LOQ=quantitation limit; ***parameter is not mentioned in reference; (1) – detection limit test can be performed instead of accuracy test; (2) – may be needed in certain cases

Specificity

Ph. Eur. 5.1.6 defines specificity for alternative qualitative and for alternative quantitative method as the ability to detect only the required microorganisms and as the ability to quantify only the required microorganisms (Ph. Eur. 11.5). This is similar to the definition provided in PDA TR33 and USP <1223>. For growth-based methods, USP recommends detection of around 100 CFU of each microorganism for both types of methods. This is similar to what Ph. Eur. 5.1.6 recommends for qualitative methods that rely on growth (i.e., to demonstrate presence and absence of microorganisms, "specificity is adequately addressed by demonstrating the growth promotion properties of the media"). For non-growth-based methods, both Pharmacopoeias recommend use of negative and positive controls to demonstrate that extraneous matter does not interfere with the ability of the alternative method to detect the panel of microorganisms. PDA TR33 and Ph. Eur. 5.1.6 provide additional guidance that is not specifically mentioned within USP <1223>. For example, TR33 and chapter 5.1.6 both recommend to use mixed cultures and stressed microorganisms, when appropriate, and depending on the method and/or application (e.i., using low levels of stressed microorganisms for alternative sterility test methods).

Detection limit (DL)

Due the unique nature of microbiological tests, DL is a parameter that reflects the number of microorganisms present in the original sample before any dilution or incubation steps. Therefore, DL of an ARMM must not be a greater than DL of the compendial method. Ph. Eur. 5.1.6 defines DL of a qualitative ARMM as the lowest number of microorganisms in a sample that can be detected under the stated analytical conditions, which is very similar to the definition provided by USP <1223>.

USP gives two options (methods) for assessment of the limit of detection (LOD). In the first method, microorganisms are diluted to a concentration where the Pharmacopeial assay will show growth in 50% of the test samples. The diluted samples are then tested in the Pharmacopeial and alternative methods using a sufficient number of replicates and the following statistically significant risks are employed: an alpha risk of 0.05 and a beta risk of 0.20. A Chi-Square test or other appropriate statistical model is used to demonstrate equivalent recovery of microorganisms. The second method employs the so called Most Probable Number (MPN) strategy where a series of 10-fold dilutions (e.i., from 10^{-1} CFU to 10^{-2} CFU) or 5-fold dilutions (e.i., from 5 CFU to 10^{-1} CFU) are analyzed by both, the compendial and the alternative method. Five replicates from each dilution are assayed and the MPN is determined from three dilutions in series that provide both positive and negative results (i.e., growth in the compendial method and an appropriate signal in the alternative method). A Chi-Square test or other appropriate statistical model is used to demonstrate equivalent recovery. Ph. Eur. 5.1.6 recommends a similar strategy whereby three 10-fold dilutions are prepared (50 CFU to 0.05 CFU for each challenge microorganism) and ten replicates from each dilution are assayed in the compendial and alternative methods. Using an MPN table, the 95% confidence interval is determined. If the intervals between both methods overlap, no significant difference for the LOD of the methods is observed. The evaluation of LOD should be based on two independent test runs per microorganism and the results of the alternative method should be at least equivalent to those of the compendial method.

Robustness

Ph. Eur. 5.1.6 defines the robustness of a qualitative ARMM as the measure of its capacity to remain unaffected by small but deliberate variations in methods parameters (incubation time or incubation temperature range). Similar definition is provided in USP <1223> where robustness is explained as the limits of the operating parameters of the method. All three documents (Ph.Eur., USP, and PDA TR33) state that robustness is a validation parameter that may be addressed through data provided by the supplier of the alternative method. However, if the end-user modifies a method's critical parameters, the effects on robustness will need to be evaluated. PDA TR33 and Ph. Eur. 5.1.6 provide additional guidance on how to conduct robustness studies while employing validation criteria appropriate for the type of method under investigation.

Ruggedness

USP <1223> defines ruggedness as the degree of precision of test results obtained by the analysis of the same samples under a variety of typical test conditions such as different analysts, instruments, and reagent lots. PDA TR33 defines ruggedness as the degree of intermediate precision or reproducibility of test results obtained by assessing the same samples under a variety of normal test conditions, such as different analysts, different instruments, different lots of reagents or on different days. According to TR33, intermediate precision is performed within the same laboratory, and reproducibility is performed between laboratories. Ph. Eur. 5.1.6 does not recognize the term ruggedness but instead refers to intermediate precision and reproducibility within its precision sections. USP <1223> and PDA TR33 state that ruggedness is a validation parameter that may be assessed through data supplied by the ARMMs supplier. However, TR33 provides additional guidance on how to conduct ruggedness testing (as does Ph. Eur. for intermediate precision and reproducibility) while using validation criteria suitable for the type of method under investigation.

Demonstration of equivalency

The implementation of the ARMMs implies that the alternative method is equivalent or non-inferior to the Pharmacopeial method, thus method equivalency must be proven. It is performed using standardized microorganisms, generally in the absence of actual product.

The USP states that when comparing the compendial and the candidate ARMM to show equivalent or better performance statistical evidence must be provided to prove equivalence or in statistical terms, non-inferiority. For example, equivalency may be demonstrated amongst two quantitative methods if there is no statistical difference between the mean cell counts. Yet, USP <1223> claims this may not be possible when the two methods provide different signals.

USP <1223> provides four different options when demonstrating equivalence where only three of them require direct comparison with the compendial method. A comparison of the four equivalence options is given in Table 2.

As it can be seen from Table 2, four options are available to establish the equivalence of a candidate ARMMs:

- Option 1: The "acceptable procedure" option, in which the alternative method only needs to meet a minimum performance or acceptance requirement and is not compared with the compendial method.
- Option 2: The "performance equivalence" option, where multiple characteristics of the alternative and compendial methods are compared and an equivalence determination is made based on the results of the comparisons.
- Option 3: The "results equivalence" option, wherein a single characteristic of the alternative and compendial methods are compared and an equivalence determination is made based on the results of the comparison.
- Option 4: The "decision equivalence" option, in which a single characteristic of the alternative and compendial methods are compared and an equivalence determination is made based on the conclusions of the analysis.

Only three of them require direct comparison with the compendial method. Additionally, it is inferred that equivalency is generally demonstrated in the absence of actual product (i.e., product is used during method suitability studies).

The "acceptable procedure" option does not require a direct comparison with the compendial method. Instead, a reference material with known properties might be used (standard inoculum of a specific microorganism, highly purified nucleic acid material, or another appropriate signal specific to the method). Under certain conditions, it may be required that the candidate method measures the signal in the presence of the test sample, using validation criteria that are appropriate for the technology, as described in relevant literature (Delmaso and Denoya, 2016; Miller 2015).

The second option "performance equivalence" requires a comparison of multiple validation criteria with the Pharmacopeial method. For example, "equivalency or better results" should be shown with respect to validation criteria that are relevant to the alternative method, such as accuracy, precision, specificity, DL, LOQ, robustness, and ruggedness. USP also states that although an alternative method may not meet certain validation parameters it may still be acceptable for use because of other advantages (e.i., reduced time to obtain a result). This equivalency option

Table 2. A comparison of four available equivalence options

Option	Demonstration	Comparison to official compendial method	Based on numerical results or conclusions	Number of characteristics
1. Acceptable procedures	Acceptable	No	Results	Multiple
2. Performance Equivalence	Equivalent or better	Yes	Results	Multiple
3. Results Equivalence	Equivalent	Yes	Results	Single
4. Decision Equivalence	Equivalent	Yes	Conclusions	Single

appears to be the closest approach to the validation strategies presented in Ph. Eur. 5.1.6 and PDA TR33, which is why many multinational companies have used this option. PDA TR33 also gives explanation when it may be possible for an alternative method to be acceptable for use even if a specific validation may not be met. Specifically, TR33 states an alternative method should not be significantly worse than an existing method except when a "clear rationale or justification" exists why the difference can be tolerated (PDA, 2013).

The "results equivalence" option demonstrates that an alternative and Pharmacopeial method give an equivalent numerical result. A tolerance interval is established when comparing the two methods and the alternative method is shown to be numerically superior or non-inferior. USP discusses the potential for alternative, non-growth-based methods to produce a significantly higher cell count as compared with growth-based methods that produce a CFU. Therefore, when using this equivalency option, a calibration curve can be used to show a correlation between the two methods and within an appropriate product or test sample specification range (Delmaso and Denoya, 2016; Miller, 2015).

A "Decision equivalence" is similar to the results equivalence except that numerical result is not generated but a pass/fail outcome is obtained. Using this option, the incidence of pass outcome to fail outcome for an alternative method should be no worse equivalent or better (i.e., the method is non-inferior) than to the results obtained with the Pharmacopeial method. For the purposes of qualification, laboratory studies involving low levels of microorganisms may be used (Delmaso and Denoya, 2016). This option would probably be acceptable for alternative methods that have been previously and comprehensively validated (Miller, 2015).

Demonstration of equivalence for an alternative qualitative method and for an alternative quantitative method

Ph. Eur. 5.1.6 and PDA TR33 provide guidance on how to test against specific validation criteria for a qualitative ARMM but instead of employing a single characteristic as is described in USP's Option 4, both documents generally evaluate all the relevant validation criteria as presented in Table 1. For example, the Ph. Eur. 5.1.6 chapter considers specificity, DL and robustness, as validation criteria. Method suitability and equivalency testing with actual product is also identified. In the case of quantitative ARMMs, the validation criteria are presented in Table 1. For example, the Ph. Eur. 5.1.6 chapter specifies the following validation criteria for a quantitative method: accuracy, precision, specificity, LOQ, linearity, range, and robustness. Method suitability and equivalency testing with actual product is also required. The presented two phases generally utilize standardized microbial suspensions in the absence of product or test samples.

PDA TR33 states if the coefficient of variation (CV) of an alternative method is less than 35% (this corresponds to a %CV for the traditional plate count method when at least 10 CFU is recovered) there is no need to compare the result to the %CV for a Pharmacopeial method. However, both TR33 and Ph. Eur. 5.1.6 state that regardless of the calculated %CV, the alternative method must have a variance lower than the variance of the Pharmacopeial method. If a statistical comparison of %CV's is required, TR33 recommends to use the McKay approximation (where confidence intervals are compared), a test for equal variance (Bartlett's test for normal distributed data or Levene's test for data that is not normally distributed) or a paired t-test.

To demonstrate that an alternative method is highly correlated with the numerical results of a Pharmacopeial method, it is necessary to confirm the acceptance criteria expressed as CFU are calibrated to acceptance criteria expressed as the alternative method's signal or unit of measurement (Miller, 2015). To do this, USP recommends to challenge both the alternative and the Pharmacopoeial methods with at least duplicate samples at each of four different bioburden levels covering a range that starts close to the LOQ and ends one log higher than a specification limit relevant to the appropriate Pharmacopeial assay. The correlation (in terms of linearity) is determined by plotting the log values of the recovered microorganisms (e.i., the alternative method on the y-axis and the Pharmacopeial method on the x-axis). A correlation of at least 0.95 or a R^2 value of at least 0.9025 is required to demonstrate linearity between the two methods. If a nonlinear relationship is obtained, a Spearman (nonparametric) correlation can be used. If the required correlation is not met, USP states it may be possible to use Option 3 (Decision Equivalence) as an alternative. In this case, the user would have to establish a qualitative acceptance criterion for the alternative method that would match the quantitative specification in the Pharmacopeial test.

Method suitability and a comparison between method suitability testing strategies

Method suitability is defined as demonstration of lack of positive or a negative influence of the product on the signal generated by the method. Due to the differences in when and how product is used within each validation guidance document, it is necessary to briefly explore how each document refers to method suitability and equivalence.

The Ph. Eur. uses actual product during suitability testing and the demonstration of *equivalence*. Suitability Testing (5.1.6): "The alternative method must be applied according to the specified procedure and with the samples to be analyzed under the responsibility of the user". The method must be shown to give comparable results as

characterized in the model system used by the supplier. Compatibility of the response with the product prepared as needed by the user, evaluated using Pharmacopeial test strains. The same chapter defines equivalence testing as a direct approach for equivalence demonstration of two qualitative methods. To determine the degree to which the method under evaluation leads to the same pass or fail result as the compendial method, simultaneous testing of the two qualitative methods should be performed. This parallel testing shall be performed based on a prespecified period of time or number of samples (Ph. Eur. 11.5, ch: (5.1.6).

In the USP, the method suitability for each new product is to be evaluated with a validated alternative method. Suitability testing as prescribed in USP <51>, <61>, <62>, <63> and <71> should be performed using the same sample preparation, quantity, and number of units appropriate for the product and the required level of assay sensitivity. This testing should demonstrate the alternative signal is not quenched or increased in the presence of the product being evaluated. PDA TR33 and Ph. Eur. 5.1.6 also describe the use of actual product or test samples for this purpose. However, there may be some confusion over the terminologies being used in each document. For example, while USP identifies actual product to be used to demonstrate *method suitability,* TR33 uses actual product during *method suitability* and *equivalency testing:* Method suitability (TR33): "To demonstrate that the new method is compatible with specific product or sample matrices that will be routinely assayed, each material should be evaluated for the potential to produce interfering or abnormal results, such as false positives (e.i., a positive result when no viable microorganisms are present in the test sample) or false negatives (e.g., a negative result when microorganisms are present in the test sample)". This may also include evaluating whether cellular debris, dead microorganisms, or mammalian cell cultures have any impact on the ability of the new method and accompanying system to operate as it is intended to." Equivalence/comparative testing (TR33): "Equivalence or comparative testing involves the use of actual product and other sample matrices that will be routinely tested using the alternative or rapid method once it is validated and implemented."

USP <1223> recommends three independent tests to be conducted to demonstrate method suitability. For the quantitative methods only accuracy and precision are required to be evaluated. For qualitative tests, the recovery of microorganisms is the only parameter to be assessed. PDA TR33 similarly states it is the end-user's responsibility to ensure that all test samples will be compatible with the new or alternative method. However, neither TR33 nor Ph. Eur. 5.1.6 specifically restricts the assessment of the relevant validation criteria to a single product. Separately, TR33 describes a reduced validation strategy when transferring a validated, like-for-like alternative method to a secondary testing site. This may include testing a few reference microorganisms, identical to what was used during the original qualification, as well as some local facility isolates to confirm basic functionality and demonstrate that key qualification requirements are met (e.i., accuracy and precision). Nevertheless, even though the original equivalency testing may assess the same type of test sample as what the secondary site will be evaluating routinely, the microbial load (number and type of microorganisms) may not be the same. Therefore, equivalency testing may need to be conducted using the actual test material at the secondary site.

When speaking of the potential for false positive and false negative results, PDA TR33 explains a product or test sample could cause an improper indication of the presence of microorganisms if it contains material that produces background noise or interfering signals. Furthermore, a product or sample matrix containing material that may quench, mask or otherwise prevent the detection or enumeration of microorganisms present could cause an improper indication that microorganisms are absent. When testing for false positives, sterile test samples should be utilized, when possible. When testing for false negatives, samples with a known quantity and type of microorganisms should be used. Although no specific criteria to be evaluated are identified during these studies, similar strategies as recommended by USP may be employed (e.i., evaluating accuracy and precision for quantitative methods; LOD or inclusivity/exclusivity for qualitative methods). For suitability testing, the Ph. Eur. 5.1.6 recommends assessing the DL for qualitative methods and accuracy, LOQ, and linearity for quantitative methods. Acceptance criteria for the method in routine use will need to be defined as a function of the application and the validation data.

A comparison between method equivalence testing strategies

Equivalence testing, according to Ph. Eur. 5.1.6 is performed by running the alternative and the Pharmacopeial methods side-by-side on a predefined period of time and/or number of samples. For a qualitative method, the degree to which the method under evaluation leads to the same pass/fail result as the Pharmacopeial method is determined. However, this approach may not be suitable for an alternative sterility test, because the number of positive samples will be extremely low. In this instance, samples of representative drug products are inoculated with a very low number (e.i., less than 5 CFU) of at least three different strains of test microorganisms. For each test method, product and test microorganism, a minimum of three test runs with a sufficient number of replicates are performed. A comparison of the pass/fail results shows the alternative method to be at least equivalent to the Pharmacopoeia method.

For quantitative tests, equivalency again is demonstrated by performing the alternative and compendial methods side-by-side. The enumerative results (CFU and cell counts) are statistically evaluated to demonstrate the alternative method is at least equivalent to the compendial method.

USP <1223> recommends performing equivalency testing using standardized microorganism challenges and applicable validation criteria, depending on the equivalency option utilized. The chapter infers this testing is generally performed in the absence of actual product or test samples. Conversely, PDA TR33 and Ph. Eur. 5.1.6 use actual product or test samples during the demonstration of equivalency. TR33 specifies product or test samples are assessed in parallel for a specified period of time or number of product batches. Similar procedures and data analyses previously utilized for the validation criteria with standardized cultures in a suitable diluent can be used (e.g., accuracy, precision, LOQ, LOD, linearity, or range).

Based on this comparison, it appears that the USP's use of actual product during the demonstration of *method suitability* is included in both *method suitability* and *equivalence* within PDA TR33 and Ph. Eur. 5.1.6. Additionally, USP's use of standardized microorganisms during the demonstration of equivalency is incorporated in *validation criteria testing* and the *verification of primary validation* as described in PDA TR33 and Ph. Eur. 5.1.6, respectively.

Conclusion

The adoption of ARMMs is likely to increase, offering potential benefits in terms of speed, automation, and sensitivity. The validation of these methods is a critical step in ensuring the safety and quality of pharmaceutical products. It involves a thorough evaluation of the method's performance, robustness, and suitability for the intended product or matrix. Proper validation documentation and ongoing monitoring are essential to maintain the ARMMs integrity and reliability. Utilizing alternative methods can assist pharmaceutical manufacturers in monitoring the manufacturing process to ensure that it is free of microbial contamination. Ph. Eur. and USP chapters provide a comprehensive framework for the validation of ARMMs used in the pharmaceutical industry. By following the principles and requirements outlined here, manufacturers can ensure that alternative methods are fit for their intended purpose, provide reliable and accurate results, and meet regulatory requirements.

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Резиме

Компаративен преглед на барањата на Европската Фармакопеја и на Американската Фармакопеја за валидација на алтернативни микробиолошки методи

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Клучни зборови: алтернативни (брзи) микробиолошки методи, фармакопејски барања, соодветност, имплементација

Микробиолошките методи достапни во Фармакопеите имаат ограничувања како што се долго време потребно за изведување на анализата, поврзано со значителни трошоци. Со цел надминување на овој недостаток, се промовираат т.н. алтернативни (брзи) микробиолошки методи (АБММ) кои се автоматизирани и можат постојано да се следат, се изведуваат многу побрзо и нудат подобра точност, специфичност, осетливост и прецизност. Во овој труд е направен компаративен преглед на фармакопејските барања на Ph. Eur. и USP за докажување на еквивалентност и/или супериорност на АБММ споредено со фармакопејските методи. Опишани се најважните чекори што треба да се преземат при процена на потенцијален АБММ, како да се избере аналитичката технологија и како да се квалификува потенцијалниот метод со фармацевтскиот производ за кој е наменет. Посебно внимание е посветено на процесот на валидација на АБММ, како и критериумите што треба да ги задоволат испитуваните параметри со цел да се докаже соодветноста на методот и дека истиот е еквивалентен со фармакопејските методи.