Unvalidated methods for medicine quality testing lead to misleading results:

Use of unvalidated methods can lead to unnecessary concern about drug quality and cause harmful consequences

Recently, media reports have highlighted the results of drug quality testing from some contract laboratories that are analyzing the quality attributes of human drug products using testing methods that are not validated. Test method validation is the process used to confirm that the analytical procedure employed in a specific test is suitable for its intended use. Test method validation is an integral part of any good analytical practice. Only results from validated methods should be used to judge the quality, reliability and consistency of analytical results for a specific drug product or any of its components.

Conversely, non-validated tests can, and often do, lead to misleading results. When those results concern the quality of a medicine—presented out of context—they can impact the behavior of health plans and patients, leading to drug shortages and can even reduce patient adherence to their treatment regimens, which can cause harm and may even be life threatening. Contract laboratories that report misleading results from unvalidated test methods may imply that medicines do not meet the quality specifications approved by the U.S. Food and Drug Administration (U.S. FDA) or articulated in a U.S. Pharmacopeia (USP) public quality standard. This paper outlines the requirements and benefits of using such methods and the consequences when they are not adhered to.

USP provides publicly available guidance for quality testing, including validated test methods and the quality specifications for specific drug products and their ingredients. All of USP's public quality standards are validated and demonstrated to be fit for their intended use.

Three types of standards are utilized to help ensure the quality of medicines:

- **Drug product monographs** articulate the quality expectations for a specific medicine, including its identity, strength, purity, and performance. They also describe the tests to validate that a medicine and its ingredients meet these criteria.
- **General Chapters** provide broadly applicable information to industry on accepted processes, tests and methods to support product development and manufacturing of medicines.
- **Reference Standards** are physical materials used in conjunction with monographs and general chapters to verify that a medicine and its ingredients can pass tests to ensure adherence to quality requirements.

Drug product monographs and General Chapters are documents published in the *United States Pharmacopeia–National Formulary* (*USP–NF*) online. Any prescription drug for human use, for which a USP monograph exists, is required to adhere to the quality specifications defined therein.

Method validation ensures a test is reliable and reproducible

The *USP–NF* contains monographs for thousands of drug products currently marketed in the U.S. and without exception, methods described therein have been rigorously validated using data from multiple, independent, ISO-certified laboratories. Examples include the correct and unequivocal identification of the active ingredient in a drug product or the accurate and precise quantification of impurities.

Orthogonal validation is comprised of two or more independent testing methods that use different techniques to confirm or refute the same results. An example of orthogonal techniques for detecting the presence of a compound are:

- ***Infrared spectroscopy*** **which measures for compounds by how they reflect or emit light in the infrared spectrum**
- ***Mass spectrometry*** **detects a relationship between the mass and electric charge of a given compound**

The *USP–NF* also contains many resources to help manufacturers and other stakeholders verify and validate these testing methods. In addition, *USP–NF* includes standards to verify that the analytical setup and procedures themselves are suitable for use (so-called system suitability standards). Whenever possible, "orthogonal" methods are used in the development of a standard, which involves using different tests to measure the same attribute to "double-check" results.

All USP standards and methods are validated through a documented and transparent process. The documentation process outlines the necessary procedures, equipment, and materials used in an analytical method to generate reliable and reproducible results. Method validation typically accounts for numerous variables and a range of analytical characteristics, such as its accuracy, precision, specificity, detection limit, quantitation limit, linearity, range, and robustness. An integral and important step in the standards development process is the involvement of almost 700 independent experts with extensive subject matter expertise and more than 200 liaisons from the U.S. FDA and other government agencies. They are organized in committees to help develop, review and decide on the establishment of USP standards.

- **Accuracy is the closeness of test results obtained by that procedure to the true value.**
- **Precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample.**
- **Specificity the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.**
- **Detection limit is a characteristic of limit tests. It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.**
- **Quantitation limit is the lowest amount of analyte in a sample that can be determined with acceptable Precision and Accuracy under the stated experimental conditions.**
- **Linearity is the ability of an analytical procedure to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.**
- **Range is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with a suitable level of Precision, Accuracy, and Linearity using the procedure as written.**
- **Robustness is a measure of an analytical procedure's capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and provides an indication of its suitability during normal usage.**

Source: USP General Chapter <1225> Validation of Compendial Procedures

Modern examples of inadequate method validation

A major contemporary development in regulations for method validation stemmed from the 1993 court case U.S. v. Barr Laboratories ("Barr"). Prior to the case, between 1989 and 1992, two different Barr sites were inspected numerous times by the U.S. FDA. Repeated instances of process validation failure and other inadequate laboratory practices resulted in the Agency taking regulatory action against Barr. In its report, the U.S. FDA concluded, "Your firm has no meaningful program for process validation since it fails to address many of the critical issues inherent in such a program."[1]

In response, Barr initiated a lawsuit against the U.S. FDA. The subsequent court case was favorable to the U.S. FDA and found that, "Pointing to its retrospective validation studies, Barr claims that it manufactures each of its 60 products in its current product-line under a validated process and, as a result, that it is in compliance with [Current Good Manufacturing Practice]. The government challenges this conclusion, pointing to numerous deficiencies in Barr's testing practice."[2] As a result, the Barr decision set a legal requirement for the entire drug development industry to engage in adequate method and process validation.[3]

Inadequate method validation risks releasing medicines of substandard quality into the supply chain. However, use of testing methods that have not been validated can also lead to misleading findings that inappropriately raise concerns about drug quality.

In 2020, a wave of medicine recalls occurred after Valisure, at that time an online pharmacy, tested medications for impurities and purported to detect the presence of potential carcinogens in a variety of commonly used medicines.[4,5,6] However, an inspection and untitled letter by the U.S. FDA highlighted "methodological deficiencies" and "analytic discrepancies" in the company's testing methods.[7] In particular, the letter noted that Valisure "inappropriately applied USP methods as part of its testing."[7] It is the responsibility of the user to correctly apply USP standards and methods for their own quality testing and quality assurance purposes. Changing, adapting or incorrectly applying USP methods disqualifies their validation and thereby forfeits any claim that USP methods have been used. And, as shown above, it can, and often does, lead to inaccurate results.

Valisure's methods were further called into question with the publication of peer-reviewed research that indicated that the medicines highlighted in Valisure's testing claims were incapable of being converted to the purported carcinogens under conditions simulating a normal population and use.[8,9]

In a subsequent analysis, FDA researchers followed Valisure's process of using gas chromatography/mass spectrometry (GC/MS) to gauge nitrosamine impurity levels in samples of acid blockers. The FDA researchers found that using Valisure's GC/MS process produced "charred remains" of the sample. Further testing showed that heating of the sample by CG/MS caused thermal degradation that resulted in the formation of nitrosamines.

The agency's researchers then evaluated the same samples using liquid chromatography-high resolution mass-spectrometry, which demonstrated much lower levels of nitrosamines, emphasizing the importance of orthogonal method validation as a built-in safeguard to ensure final results are cross-checked and independently verified.[10]

USP methods protect the medicines supply chain

When laboratories, such as in the case of Valisure, apply methodology that lacks rigorous and transparent validation, they are unable to claim that they use analytical techniques that are suitable for the substance they are trying to measure and/or detect. And when third-party contract labs purport to function as "consumer watchdogs," the findings from these labs become suspect when they use these testing methods.

In the Valisure case, the company responded to the FDA letter and stated its testing activities are only for, "informational and marketing use and not for regulatory purposes."[9] However, the FDA itself noted that many manufacturers may mistakenly use the results for regulatory purposes, and perhaps more importantly, media and society are largely unaware whether a contract lab is using unvalidated, non-transparent or non-rigorous test methods to arrive at their findings.

Conclusion

Quality testing that utilizes test methods that are not validated or transparent can have serious consequences. Unvalidated tests can be, and often are, misleading.

The consequences are serious and include:

1. Potential harm to patients if healthcare providers or patients' themselves change behavior based on the results of unvalidated and often inaccurate or deceptive results.

2. Undermining of public trust in the medicines supply chain by publicizing erroneous claims of quality deficits.

Analytical procedures must be supported by sufficiently rigorous and transparent laboratory data to document their validity, which is why the USP monograph should be utilized when testing medicines for quality.[11] USP has the expertise and resources, routinely works with contract laboratories that use validated methods, and stands ready to support government, industry, and other stakeholders in their application.

References

1. "Barr Labs shutdown sought by FDA due to GMP violations uncovered during agency inspections; firm claims FDA stance is retaliation for whistleblowing," Pink Sheet. November 11, 1991. Available at: https://pink.pharmaintelligence.informa.com/ps019974/barr-labs-shutdown-sought-by-fda-due-to-gmp-violations-uncovered-during-agency-inspections-firm-claims-fda-stance-is-retaliation-for-whistleblowing

2. "U.S. v. Barr Laboratories, Inc.," United States District Court, D. New Jersey. March 30, 1993. Available at: https://casetext.com/case/us-v-barr-laboratories-inc

3. "Introduction to science- and risk-based cleaning validation using ASTM E3106 & E3219," Pharmaceutical Online. May 6, 2020. Available at: https://www.pharmaceuticalonline.com/doc/introduction-to-science-and-risk-based-cleaning-validation-using-astm-e-e-0001

4. "Pfizer recalls some blood pressure drugs, citing cancer risk," New York Times. March 23, 2022. Available at: https://www.nytimes.com/2022/03/23/health/pfizer-recall-blood-pressure-drug-cancer.html

5. "Carcinogen found by pharmacy in popular U.S. diabetes drug," Bloomberg. March 2, 2020. Available at: https://www.bloomberg.com/news/articles/2020-03-02/carcinogen-found-by-pharmacy-in-popular-u-s-diabetes-drug#xj4y7vzkg

6. "Sanofi pulls Zantac from U.S. and Canada after carcinogen found," Reuters. October 18, 2019. Available at: https://www.reuters.com/article/us-sanofi-heartburn-zantac-idUSKBN1WX1WU

7. "Valisure LLC, New Haven, Connecticut," U.S. Food & Drug Administration. December 5, 2022. Available at: https://www.fda.gov/media/163682/download

8. "Effect of oral ranitidine on urinary excretion of N-nitrosodimethylamine (NDMA)," JAMA. June 28, 2021. Available at: https://jamanetwork.com/journals/jama/fullarticle/2781670

9. "In vitro analysis of N-nitrosodimethylamine (NDMA) formation from ranitidine under simulated gastrointestinal conditions," JAMA. June 28, 2021. Available at: https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2781455

10. "Nitrosamine detection tests should be 'fit for purpose,' says FDA," Regulatory Focus. November 1, 2021. Available at: https://www.raps.org/news-and-articles/news-articles/2021/11/nitrosamine-detection-tests-should-be-fit-for-purp

11. "Legal recognition – Standards categories," United States Pharmacopeia. Available at: https://www.usp.org/about/legal-recognition/standard-categories