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"Selection and Validation of Legal Reference Methods of Analysis for Pharmaceutical Products
in the U.S.A."

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In determining whether or not a drug product meets its purported quality characteristics, the U. S. Food and Drug Administration (FDA) must first determine which standard to apply. The applicable standard may derive from a compendium, e.g., the United States Pharmacopeia/National Formulary (USP/NF), invoked when the product's name is recognized in such a reference, an approved new-drug application (NDA) or abbreviated NDA (ANDA), or some other source, including a firm's individual in-house specifications.

Once the standard has been identified, the next step is to determine which analytical method to use in assessing conformance to that standard. Choosing the correct method prevents criticisms that the agency is using an "elastic yardstick" to take the measure of a drug's quality. The choice is especially critical when FDA must convince a court of law that a drug product suspected of being below par does, indeed, fail when tested by fair, scientifically sound, and clearly established analytical methods. Those methods, sometimes called "referee methods," are termed Legal Reference Methods. This paper describes how FDA determines exactly which method to apply and how the scientific validity of those methods is established.

Legal Reference Methods are those methods of analysis which are considered to be definitive in taking legal actions that could result in depriving individuals of property (seizure), livelihood (injunction), or freedom (incarceration). These actions are severe; thus, the methods of analysis used to decide whether to take such actions must be sound and conform to the applicable laws and regulations. To clarify which methods of analysis are appropriate to support regulatory actions, the FDA has established the following hierarchy as guidance to FDA personnel on the selection of methods of analysis for pharmaceutical products (1):

- (a) For official drugs (USP/NF) the official compendial analytical methods are to be used, unless the FDA has promulgated regulations under Section 501 (b) (or, for antibiotics, Section 507) of the Food, Drug and Cosmetic (FD&C) Act prescribing alternative appropriate tests or assay methods, in which case the promulgated regulations are to be followed.
- (b) A non-official drug that is the subject of an approved new-drug application is to be analyzed by the method in the NDA or ANDA.
- (c) A non-official drug that is not a new drug is to be analyzed by the method used by the manufacturer as part of its standard operating procedures.
- (d) When a drug is not covered by the above situations, the analyst may select an alternative appropriate method with which to analyze the product. In selecting the method, first consideration should be given to any existing AOAC International (AOAC) method because AOAC methods have withstood the rigors of collaborative study. Any method selected must have been properly validated.

A discussion of the FDA laws and regulations that provide the basis for the above hierarchy is given below.

Official, also called compendial, products are those covered by monographs in publications that are sanctioned by virtue of recognition by federal law. Among the definitions of "drug" set forth in the FD&C Act are "articles recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them." The USP was founded in 1820 to establish standards for pharmaceutical products. Since these standards were already in widespread use in the U.S.A., the framers of the 1906 Pure Food and Drug Act embodied them into law.

The Act currently states: Section 501. [351] A drug ... shall be deemed to be adulterated ... (b) If it purports to be or is represented as a drug the name of which is recognized in an official compendium, and its strength differs from, or its quality or purity falls below, the standards set forth in such compendium.

The law elaborates that in determining conformance to official compendial standards, "methods of assay set forth in such compendium" shall be used; a fair enough yardstick -- official drug meets official standard when measured by official methods.

The Act accounts for two exceptions for official products: when the analytical methods are absent or (in the judgment of the Secretary of the Department of Health and Human Services) insufficient. In these cases the law directs the Secretary to alert the compendial publishers to the problem and afford them the opportunity to establish suitable methods. If such methods are not forthcoming, in the Secretary's judgment, then the agency may promulgate regulations prescribing appropriate methods. However, it is rare that FDA must promulgate such regulations. The agency has a Compendial Operations Branch, which has the task of closely working with the USP to resolve any differences in the suitability of analytical methods and standards.

The second level in the hierarchy applies to products that are not covered by an official compendium but that are covered by approved NDAs. The new-drug regulations state what must be in an NDA. Within the chemistry, manufacturing, and controls section of the NDA, there must be a statement of the specifications for the product and its components, as well as a statement of each analytical method "necessary to assure the identity, strength, quality, purity and bioavailability of the drug product" (2). As part of the NDA review process, the agency will evaluate the suitability of the specifications and analytical methods proposed by the applicant. The applicant may propose to use alternate specifications and methods when appropriate. The specifications and analytical methods, including alternatives, become the legal references once the application is approved.

Sometimes the compendial and NDA specifications and analytical methods overlap. Some applicants will, upon NDA approval, petition the USP to establish a monograph for the product; a monograph that will typically include the firm's own specifications and analytical methods. The new-drug regulations noted above permit an NDA to incorporate by reference the applicable standards and methods contained in the current edition of the USP/NF. The applicant in such cases thus commits to keeping current with those references, and FDA will apply them as the legal reference methods/standards. Other firms may also make reference to that official

monograph or public standard in an ANDA and, when the patent or exclusivity license for that product expires and an approved ANDA is obtained, market that product.

The third level in the hierarchy (where no compendial or NDA/ANDA methods have been established) is a method of analysis used by the manufacturer; such a method has not undergone the rigors of standard setting reviews afforded by the new-drug review and/or compendial monograph establishment processes. However, this level shares a fundamental legal requirement common to all levels, namely that the specifications and analytical methods be scientifically sound. This fundamental requirement involves current good manufacturing practice and is based both on the Act and regulation. The Act states: Sec. 501.[351] A drug ... shall be deemed to be adulterated ... (a)(2)(B) if ... the methods used in ... its manufacture ... are not operated ... in conformity with current good manufacturing practice to assure that such drug ... has the identity and strength, and meets the quality and purity characteristics, which it purports or is represented to possess.

The Current Good Manufacturing Practices (CGMP) regulations state (3):
Laboratory controls shall include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling and drug products conform to appropriate standards of identity, strength, quality, and purity.

Generally, in the absence of an NDA/ANDA or official monograph, a firm will develop its own specifications and methods. However, that effort may be avoided if suitable specifications and methods have already been established by some other party (and possibly published). The CGMP regulations account for this by allowing a firm's laboratory records to cross-reference such third party accomplishments in addition to any work done by the firm itself to demonstrate the suitability of its standards and methods. Specifically, 21 CFR 211.194(a) states:
"Laboratory records shall include ... A statement of each method used in the testing of the sample. The statement shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested. If the method employed is in the current revision of the United States Pharmacopeia, National Formulary, Association of Official Analytical Chemists Book of Methods, or in other recognized standard references, or is detailed in an approved new-drug application and the referenced method is not modified, a statement indicating the method and reference will suffice."

The fourth level in the hierarchy is the use of a method of the AOAC as published in their "Official Methods of Analysis." The regulatory acceptance of such methods is contained not only in the CGMP regulations as cited above, but also in the agency's administrative procedures regulations. The latter regulations state (4): "Where the method of analysis is not prescribed in a regulation, it is the policy of the Food and Drug Administration in its enforcement programs to utilize the methods of analysis of the Association of Official Analytical Chemists (AOAC) as published in their latest edition of their publication "Official Methods of Analysis of the AOAC"

The above cited methods differ widely in the amounts and kinds of "validation" they receive and also in the philosophy behind the validation concepts. To discuss these issues as they relate to the Legal Reference Methods, it is important to introduce two terms used in method validation. First, "repeatability" -- the variance determined from repetitive testing of the same materials in the same laboratory (within laboratory variance). Second, "reproducibility" -- the variance determined from repetitive testing of the same materials at different laboratories (among laboratory variance). The determination of a method's "repeatability" is useful as an initial assessment of method performance. However, the "reproducibility" or among laboratory variance is critical from a regulatory perspective because this characteristic can be contentious in law enforcement. As has been noted by Taylor, "... Validation is the process of determining the suitability of methodology for providing useful analytical data. This is a value judgment in which the performance parameters of the method are compared with the requirements for the analytical data ..." (5). In the USP method adoption procedure, a method or an array of methods (monograph) is submitted for consideration to the USP Staff, usually by the (NDA) innovator firm. The submitted method should have been extensively validated in accordance with USP requirements (6). Although the testing of the ruggedness including collaborative testing of the method is also suggested ("reproducibility" assessment), that level of testing is seldom obtained. The submission is reviewed by the USP Staff and then by the Committee of Revision (7) for consideration. Only the Committee Members can perform the final act which is to adopt the method as "official." The Committee Members may choose to only review the methods or they may test them before: (a) rejecting the proposed methods or changes; (b) returning them to the submitter for additional information; or (c) recommending that they be published in the Pharmacopeial Forum (PF) for consideration by the industry as a whole. When methods or monographs are published in the PF it is expected that all interested parties will not only review them but also assure themselves that the methods as proposed are applicable to their products. Problems encountered with a particular method or monograph are reported along with the supporting data to the USP Staff, who collates the materials and submits them to the Committee Members for their consideration. The Committee Members review the information and may ask the method submitter to: (a) furnish additional data; (b) perform additional testing; or (c) modify the proposal and resubmit it for publication in the PF; or the Committee Members may adjudicate that the encountered problems are not significant, thus allowing the method adoption.

As noted above, in the USP procedure the submitter of the method is expected to have performed suitable linearity, sensitivity, accuracy, precision, and ruggedness testing before submitting the method to the USP for consideration. The method should demonstrate suitable "repeatability" throughout this process. When the method is tested by the other interested parties, they also informally assess the method's "repeatability" in their own laboratories on their own products and possibly other products. The "reproducibility" of the methods is not determined. The problems with this limited testing have been discussed by Karpinski, who notes the deficiencies in not determining "reproducibility" (8). The deficiencies in this informal validation process (for elevation of a method to Legal Reference Method status) are overcome by the: (a) required concurrent use of USP Reference Standards; (b) relatively wide acceptance limits when compared to the "repeatability" or "reproducibility" Coefficients Of Variation (COV); (c) small dynamic range of the analyte; (d) well-defined non-interfering matrices; and (e) independent multiple "repeatability" assessments by the interested parties. These points apply as well to Limit

Tests for low-concentration impurities where only one point in the dynamic range need be of acceptable variation.

An extensive review of collaborated pharmaceutical methods of analysis for products containing analyte concentrations between 0.1 and 100% of the dosage form has shown that, for well-validated methods, the "repeatability" COV ranged from 0.5 to 1.25% while the "reproducibility" COV ranged from 1.0 to 2.5% (9). Since the CGMP regulations require that all products be formulated with the intent to provide 100% of the active ingredients (10), one would expect that 95% of individual analyses would lie between 95 and 105% of the stated value (i.e., +twice the largest "reproducibility" COV range) while the pharmacopeial acceptance limits for individual unit analysis (which include the production variance) usually range from 85 to 115% (11). Potential problems with the USP monograph approach are significantly further reduced since " ... substances are regarded as unsuitable and prohibited unless ... they do not interfere with the assays and tests prescribed for determining compliance with the Pharmacopeial standards (12)." This places the onus on the individual manufacturers to demonstrate or validate that each individual component and the aggregation of components as they are formulated do not interfere or bias the analyses. The USP process develops these public standards, which are used as Legal Reference Methods, i.e., applicable to all parties in the marketplace, with the advice and tacit consent of the involved industries.

The FDA's NDA/ANDA review process is vulnerable to the same method validation deficiencies as the USP process. Methods of analysis that have been validated for the specific product in accordance with USP guidance or FDA Guidelines (13) are submitted by the applicant to the FDA. The methods of analysis, and samples consisting of finished dosage forms, bulk drugs, and excipients, are submitted to one or two FDA laboratories for validation (14). The applicant is expected to assess the usual analytical parameters cited earlier and, as mentioned, the approving officials compare the "repeatability" of the methods in the three (i.e., applicant and two FDA) laboratories. As in the USP case, there is no determination of "reproducibility" or formal assessment of "repeatability." These private standards, i.e., applicable to the new-drug manufacturer only, were for many years considered to be proprietary and therefore not subject to release. This ruling meant that only the manufacturer and the FDA could determine if an approved product was what the manufacturer purported the product to be. This problem was addressed in the mid-1970s when it was determined that the method of analysis would be made available through the Freedom of Information Act after a product was approved for marketing. These private standards are Legal Reference Methods which the applicant and the FDA agree are suitable to regulate the product.

If the methods of analysis for a product are not included in the USP or an FDA approved drug application, the manufacturer's control and release methods of analysis must be validated outside of the compendial and new-drug contexts, but still in accordance with FDA CGMP Regulations (15). The manufacturer must obtain the validation data for the product and have it available for FDA scrutiny. If the FDA must determine whether the product complies with its specifications, the FDA must analyze samples using these methods unless it can show that the methods are not adequate to assess the quality of the product. Among the validation data that should be available for FDA scrutiny are all of the analytical dimensions discussed in the USP and FDA validation guidelines.

The next testing level in the hierarchy consists of the methods of analysis of the AOAC, as published in their "Official Methods of Analysis." These methods of analysis have been developed according to the extensive AOAC collaborative study protocols (16). The AOAC adopts a method as "Official" only after careful review and a determination that acceptance criteria are fully met. These protocols and adoption procedures were developed primarily under the tutelage of FDA scientists and statisticians concerned with food analysis during the AOAC's long tenure in the FDA (17). The "repeatability" and "reproducibility" of the AOAC methods are assessed and published before they are adopted.

If the products are not covered by any of the above testing procedures, FDA analysts must develop and validate methods that are suitable to assess the quality of the products in question. In summary, the Legal Reference Methods for the active drug substance in pharmaceutical products as developed through the USP and FDA processes are suitable to regulate the products because the concentration of the analyte in the matrix is generally adequately high. As has been empirically shown by Horwitz et al. for well-behaved methods of analysis, the "reproducibility" of a method (among-laboratory COV in percent) is approximately related exponentially to the analyte concentration by the equation:

$$\text{COV} = 2 \exp(1 - 0.5 \log c),$$

where c is the concentration of the analyte given in powers of ten, i.e., $1 \text{ ppm} = 10 \exp -6$ (18). For the overwhelming majority of pharmaceutical products the concentration of the active drug substance ranges from ca. $10 \exp -2$ to $10 \exp 0$ (from 1 mg of active drug substance in a 100 mg tablet weight, up to a direct compressible product); such products would be expected to have analytical COV "reproducibility" values ranging from approximately 4 to 2% respectively. Actual pharmaceutical analysis collaborative studies generally show lower COVs than these (9). These lower COVs may be due to the lesser complexity of pharmaceutical matrices compared to foods which, in turn, reduces the analytical complexity of the methods, thereby reducing the relative errors. This has been discussed by Hall and Selinger in their rationalization of the empirical relationship advanced by Horwitz (19).

Although FDA's validation procedures are currently adequate to address such products, it appears that more systematic and extensive validation procedures will become necessary as the concentration of the active drug substance in the formulated product decreases and/or the matrices become less tractable. In addition, it will be necessary to re-evaluate the acceptance ranges for low concentration products in order to accommodate the greater analytical variances and the increased manufacturing variances that will occur. Although it will remain desirable to keep the concentration of the active substance in the matrix as high as possible in order to keep the analytical variances at a minimum, the technical manufacturing limits, and clinical efficacy and bioavailability performance will ultimately determine the matrix components and their levels.

It should be noted that impurity tests which have either pass/fail or defined limits have correspondingly wider acceptance limits to accommodate the larger variances associated with these lower concentrations.

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