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Good Laboratory Practice in Doping Control

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Introduction

Good Laboratory Practice (GLP) deals with the organization, process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported.[1] GLP practices are intended to promote the quality and validity of test data. GLP compliant studies and measurements are different from those performed independently from GLP rules. They regulate responsibilities of the study director, quality assurance unit and of other personnel. They require control and test articles to be identified and well characterized equipment must be designed and tested to meet the documented analytical requirements. While the basics of GLP are described elsewhere,[2] this paper will discuss requirements and recommendations for analytical measurements. All GLP regulations usually include just a few non specific paragraphs on equipment, for example the United States FDA GLP regulations, coded as part 58 of chapter 21 of the Code of the Federal Regulations in 1978: 'Equipment used in generation, measurement, or assessment of data shall be of appropriate design and adequate capacity to function according to the protocol." This means equipment and analytical processes should be validated before and during their routine use. Therefore this paper will mainly discuss various validation activities recommended for analytical laboratories.

Validation processes in the laboratory

Validation has been defined by many organizations and authors. Although the wording is different, the sense is always the same: (a) specify the intended use, (b) test if the specifications are met and (c) document. One of today's commonly accepted definitions of validation can be found in the guideline *General Principles of Validation* from 1987:[3] 'Establishing documented evidence which provides a high degree of assurance that a specific

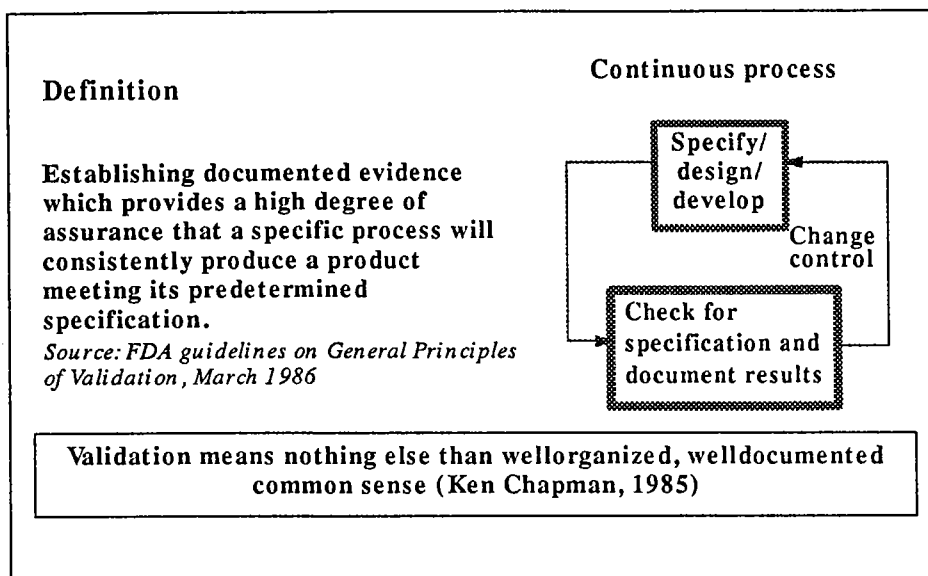process will consistently produce a product meeting its predetermined specifications and quality attributes."

Definition

Continuous process

Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification.
Source: FDA guidelines on General Principles of Validation, March 1986

Specify/ design/ develop

Change control

Check for specification and document results

Validation means nothing else than wellorganized, welldocumented common sense (Ken Chapman, 1985)

Figure 1. Commonly used definition of validation[3]

This definition is very well thought out and each word has a special significance. Most important in this definition are the words documented, high degree of assurance, specific process, consistently, and predetermined specifications.

The *EURACHEM/WELAC Guidance on Interpretation of the EN 45000 Series of Standards and ISO/IEC Guide 25*[5] defined validation of data and equipment in appendix C1.11 as 'The checking of data for correctness, or compliance with applicable (of data processing) standards, rules and conventions. In the context of equipment rather than data, validation involves checking for correct performance etc." The same guide also describes the objective of method validation: "Validation of a method establishes, by systematic laboratory studies, that the performance characteristics of the method meet the specifications related to the intended use of the analytical results."

Validation efforts in an analytical laboratory can be broken down into separate components addressing the equipment, the analytical method run on that equipment, the analytical system and finally the analytical data. The various validation activities in an analytical laboratory are illustrated in Figure 2.
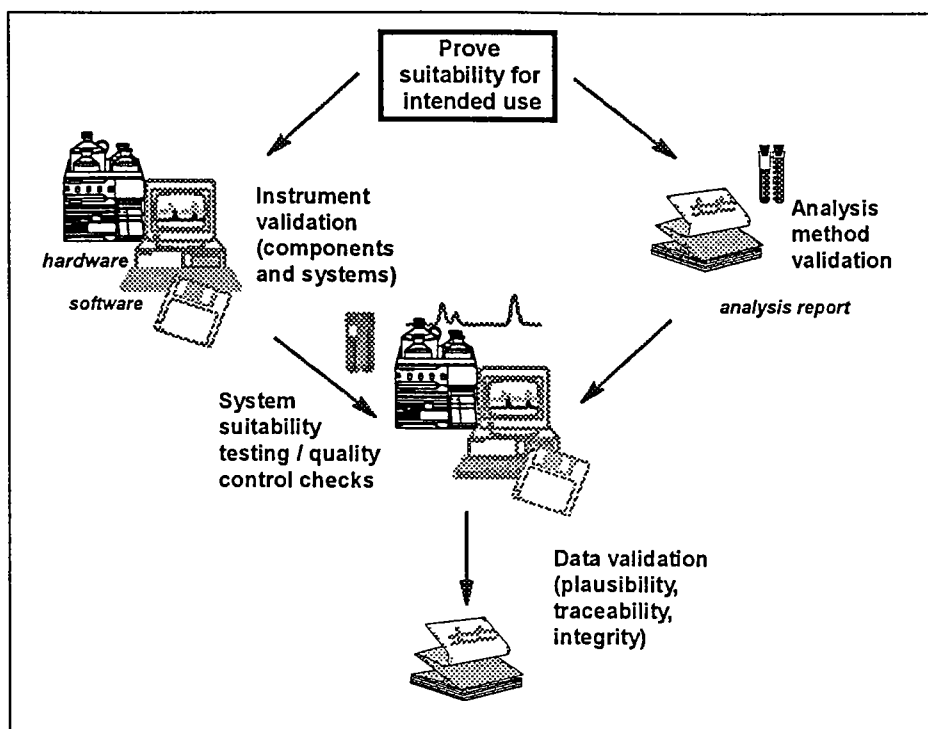
Figure 2. Validation in the analytical laboratory[4]

Analytical equipment hardware should be validated prior to routine use and, if necessary, after repair and at regular intervals. Computer systems should be validated during and at the end of the development process and, if necessary, after software updates. Computer system validation includes the validation process during software development at the development site and the qualifications of the individual products at the user's site.

Method validation covers testing of significant method characteristics, for example, limit of detection, limit of quantitation, selectivity, linearity and ruggedness. If the scope of the method is that it should run on different instruments, the method should also be validated on different instruments. Only when it is clearly specified that the method will always run on the same instrument, can validation efforts be limited to that instrument. Methods should be validated at the end of method development prior to routine use and whenever any method parameter has been changed.

A system combines instrument, computer and method. In chromatography it also includes a column and reference material for calibration. This validation, usually referred to as system suitability testing, tests a system against documented performance specifications, for the

specific analytical method. Analytical systems should be tested for system suitability prior to and during routine use, practically on a day to day basis. The analysis of well characterized quality control samples and a comparison of the actual values with expected results in form of quality control charts is also a valuable technique to prove a system's suitability for a specific task.

When analyzing samples the data should be validated. The validation process includes documentation and checks for data plausibility, data integrity and traceability. A complete audit trail that allows the final result to be traced back to the raw data should be in place.

Other tasks are equally important in ensuring reliable and accurate data: all laboratory work staff should be adequately qualified, and their qualifications should be documented. Standards for instrument calibration and quality control checks should be checked following documented plans.

While details on how to validate analytical hardware, software and complete computerized systems are described in references 4, 7 and 8, this paper will discuss validation associated analytical methods.

Validation of Methods for Biological Samples

Method validation is the process used to establish that the performance characteristics of an analytical method meet specifications that relate to the intended use of the analytical results. Methods need to be validated before their introduction into routine use and whenever the method is changed. To obtain the most accurate results, all of the method variables should be considered, including sampling procedure, sample preparation, chromatographic or electrophoretic separation, detection, data evaluation and the matrix of the intended samples.

Type of methods and their validation requirements

A laboratory applying a specific method should have documentary evidence that the method has been appropriately validated. 'The responsibility remains firmly with the user to ensure that the validation documented in the method is sufficiently complete to meet his or her needs."[6] This holds for standard methods, for example, from ASTM, ISO or USP, as well as for methods developed in-house.

The WELAC/EURACHEM interpretation guide[5] differentiates between three categories of methods: standard methods, methods developed in-house, either based on literature information or developed from scratch and generic methods.

When standard methods are used, full validation may not be required if the there is evidence that the standard method has been validated within the scope the laboratory is applying the method. In any case laboratories should verify their own ability to achieve satisfactory performance against documented performance characteristics. For an HPLC method this means that the key criteria such as detection limits, and precision of retention times and peak areas as well as the specified resolution between analytes should be verified on the system as used for sample analysis.

Methods developed in-house should be fully validated before use. Method accuracy should be validated by comparison with other techniques or by using certified reference material. All methods should be fully documented including validation data, limitations of applicability, procedure for quality control, and calibration.

Parameters for Method Validation

The parameters of what constitutes a validated chromatographic method have received considerable attention in the literature and from regulatory agencies.[9-18] The *Guidance on the Interpretation of the EN 45000 Series of Standards and ISO/IEC Guide 25* includes a chapter on the validation of methods[5] with a list of nine validation parameters. The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use[12] has developed a draft consensus text on the validation of

analytical procedures. The document includes definitions for eight validation characteristics. The United States Pharmacopoeia has published specific guidelines for method validation for compound evaluation.[10]
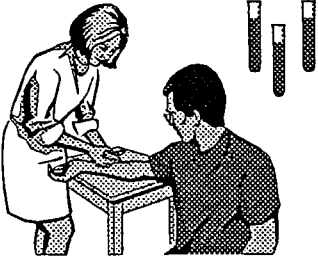
Parameters frequently used for method validation are

- Selectivity

- Range

- Linearity

- Accuracy

- Limit of detection

- Limit of quantitation

- Ruggedness (robustness)

- Stability

- Precision (repeatability, reproducibility)

There are no official guidelines referring to biological fluids. Clinical and pharmaceutical laboratories use methodology published in the literature.[16-18] The most comprehensive document was published as the 'Conference Report of the Washington Conference on *Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies* held in 1990 (sponsored by the American Association of Pharmaceutical Scientists, the Association of Official Analytical Chemists or AOAC and the US FDA, among others).[17] The report presents guiding principles for validation of studies in both human and animal subjects that may be referred to in developing future formal guidelines. Figure 3 summarizes the recommendations.

- Single sample analysis can be sufficient
- Multilevel calibration (5 to 7 points)
- 15% standard deviation, 20% close to detection limit
- Extensive use of quality control samples for each run
  - Duplicate measurements
  - 3 concentrations (at LOQ, medium, high concentration)
  - at least 4 of the six measurements must be within 20% of the nominal value, 2 of the six amy be outside, but not at the same concentration

Reference: Shah et al., Analytical methods validation: Bioavailability, bioequivalence and pharmacokinetic studies, Eur. J. Drug Metabolism and Pharmakokinetics, 1991, 16(4). 249/255

Figure 3. Method validation recommended for biological samples[17]

Internal and External Quality Control

The characteristics of equipment alter over time due to contamination and normal wear and tear. HPLC UV detector flow cells become contaminated, pump piston seals abrade and UV detector lamps lose intensity. These changes will have a direct impact on the performance of analytical hardware. A plan should be set up that ensures the system is under control. Ongoing activities may include preventive instrument maintenance, calibration, performance verification and calibration, system suitability testing, analysis of blanks and quality control samples and any combination thereof.

It is recommended to do critical tests automatically prior or/and and in between a series of routine samples. The types and frequency of tests depend on the criticality of data and on the stability of the system. This may be a daily system suitability test using a standard and/or single or duplicate quality control sample analysis with control charts plotting the average and or difference. If the linearity is critical control samples should cover different concentration ranges.
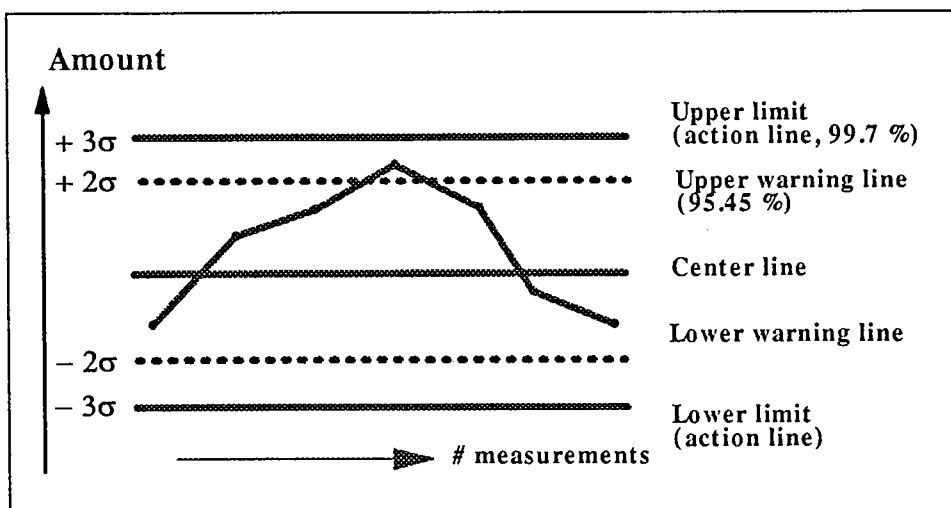

Figure 4. Analysis of well characterized quality control samples and the construction and interpretation of quality control charts provide evidence of on-going performance qualification. The HP ChemStations' data base generates quality control plots with warning and action limits.[4]

Proficiency testing by interlaboratory comparisons

Proficiency testing by interlaboratory comparisons is important to assess the regular technical competence of participating laboratories to generate comparable analytical data. ISO/IEC Guide 43[18] as developed by ISO/CERTICO in response to a request arising from the international laboratory accreditation conference (ILAC82) covers guidance on development and operation proficiency testing. At ILAC94 a revision of the guide was presented in draft form with the title 'Proficiency Testing by Interlaboratory Comparisons" that includes a statistical guidance on treatment of data from proficiency testing.[20]

In a typical proficiency testing scheme, portions of a well characterized test material are distributed on a regular basis to participating laboratories for analysis. The laboratories analyze the samples using methods and standards usually applied for that sample and send the results back to the organization that distributed the test material. Depending on the degree of agreement to the true value the laboratories are scored and receive a report which enable them to review how well they have performed in the test. The results are confidential to the laboratory and the organizer, but clients of the laboratory and the accreditation body may request the test results.

Typically neither calibration standards are send with the sample nor are the analytical methods mandated. However, laboratories are advised to report the method because this may be used to get information if the method itself may be a source of the deviation to the true results, if there is any.

Advantages for laboratories:

- External and independent assessment of data quality for specific tests
- A means of demonstrating the data quality to customers, accreditation bodies and regulatory agencies
- A motivation to improve analytical quality
- Information on the performance characteristics of analytical methods and the quality of reference material

The proficiency testing process should follow a protocol which has recently been developed by a collaboration of scientist from many countries under the joint organization of ISO, IUPAC and AOAC International.[21]

One of the goals of the procedure was to find a way to convert the data of the laboratories into scores that are easy to understand and of universal applicability. The method recommended in the protocol, therefore, is based on simple statistics with no scaling. Each result (x) is converted into a 'z' score according to the equation:

$$z = (x-y)/\sigma$$

where y is the 'assigned' value, the best estimate of the 'true' concentration of the analyte. Sigma (σ) is the 'target' value for the standard deviation of values of x. It describes the previously specified acceptable variability between the laboratories and is related to the ruggedness of the analysis method. Z-scores of between ± 2 σ will occur in 95% of all cases and are regarded as satisfactory. Z-scores between 2 and 3 are considered to be 'questionable' and will occur in 5% of all cases but those outside the range ± 3 σ are considered as unsatisfactory. Results are plotted to visualize them as easy as possible and are sent to each laboratory.

Documentation and Archiving

The raw data and final results of all tests and sample results should be recorded, documented and archived. Raw data should be defined as either being the paper print-outs or the electronically archived records. In any case traceability of the final results to the raw data and data integrity should be ensured. If the electronic data are defined as raw data, it is recommended to store chromatographic conditions and integration parameters together with the raw data file.

For example, the HP's HPLC, CE and GC ChemStation's data handling preserves traceability and integrity by storing initial raw measurement data together with instrument conditions and the instrument's logbook in a single checksum protected, binary coded register file.

The ChemStation records the pre-column pressure and temperature of the column compartment before, during and after the HPLC runs and stores these profiles. In a CE run, the ChemStation stores actual current, voltage and power and capillary temperature and pressure. This facilitates traceability of data to analytical parameters. Checksum is a programming terminology for an arithmetic operation performed on the data immediately after generation, the product of which is stored with the data. Future access to the data is subject to the same arithmetic check. Numerical matches confirm that data has not been tampered with, while mismatches draw attention to possible data corruption.
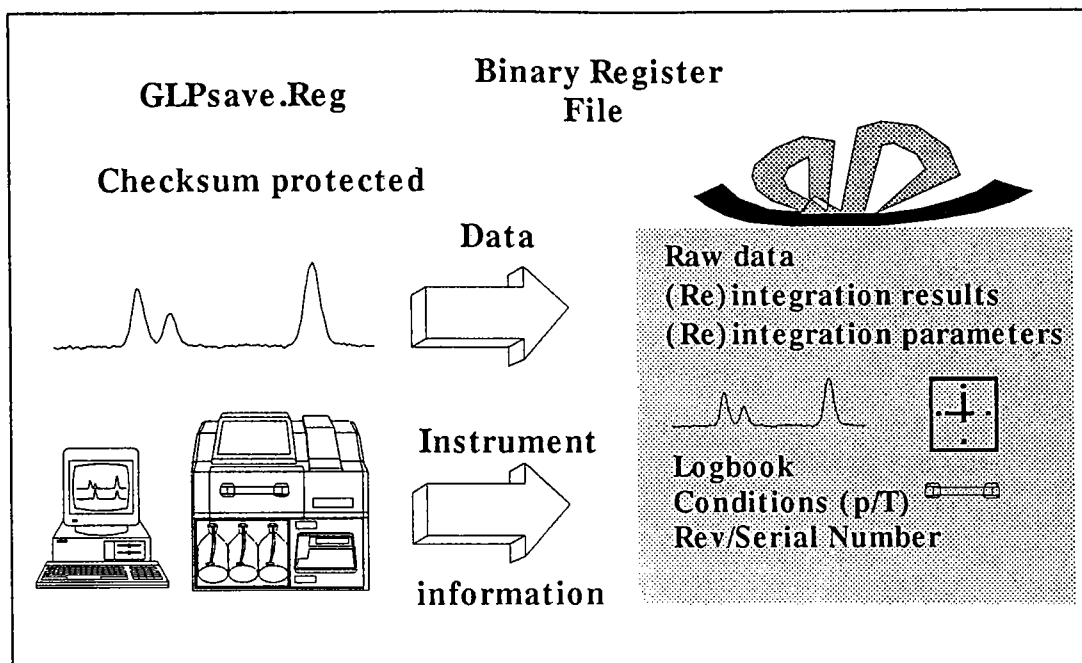
Figure 5. In the HP GC, HPLC and CE ChemStations, instrument parameters are stored together with raw data in checksum protected binary register files.[4]

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