UTILISATION OF LC-ASSAY STABILITY DATA TO CALCULATE RELIABLE REPEATABILITY AND INTERMEDIATE PRECISION RESULTS

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Keywords: precision, repeatability, stability

During studies to investigate the stability of a drug substance or a drug product, the same analytical procedure is applied to samples stored under defined conditions over a long time, often up to 5 years. These stability data provide an excellent source of reliable precision estimates, both with respect to time as well as to an increased number of data available.
In case of a significant decrease of the analyte, this systematic change in the data must be separated from the random part, i.e. the intermediate precision. This can be achieved by using the residual standard deviation of the regression line of content vs. storage time. If no significant decrease is observed, an analysis of variances can be directly applied to the data [1].
The results of a multi-company investigation are presented for LC assay procedures. 44 drug substances and drug products of various types subjected to 156 stability studies extending from 12 to 60 months, with 2915 assay values in total were included [2].
The results show impressively the large intervals where the individual precision parameters scatter. Distribution ranges and averages for repeatability, intermediate precision, and their ratio are mainly dependent on the type of drug product: Repeatabilities and intermediate precisions were found up to 0.8% and 2.2% for solutions, 1.6% and 1.1% for drug substances, 1.9% and 2.3% for tablets, 2.3% and 3.1% for creams, and 3.4% and 3.2% for a bath, respectively. These differences for the types of drug product may be explained by the influence of the sample and/or the sample preparation: The more complex, the higher the variability contribution. For the investigated examples, the impact of the analyte and of the concentration (dosage) seems to be of less importance.

[1] J. Ermer, H.-J. Ploss, J. Pharm. Biomed. Anal. 37/5 (2005) 859-870
[2] J. Ermer et al., J. Pharm. Biomed. Anal. 38/4 (2005) 653-663

UNCERTAINTY BUDGET IN PHARMACEUTICAL INDUSTRY

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Keywords: bias, conformity, uncertainty

Measurements in a pharmaceutical industry are usually carried out to ascertain the quality of a product or to control a process; in either case the measurement result serves to demonstrate that the value of the measurand is within specified limits. No method is without bias, the result of a measurement is therefore only an estimate of the value of the measurand.

Traditionally it is assumed that specifications duly reflect both bias and measurement uncertainty in such a way that conformity requires only that the measurement result falls within specified limits. In our age of globalisation this assumption can no longer be upheld, and external specifications have to be taken as real, rational expressions of limits not to be exceeded (ISO 10576-1, 2003). Demonstration of conformity therefore requires results to be corrected for bias and accompanied by a statement of their uncertainty, exactly as described in GUM (1995). Pharmaceutical industry has therefore over the last 5 years shown increasing interest in accreditation according to ISO 17025 (2004) and today uncertainty budgets are being developed for all so-called critical measurements.

The uncertainty of results obtained by methods under development or with limited practical experience has to be estimated by a bottom-up procedure, while results obtained by routine methods or standardized methods from the Pharmacopoeia can apply the top-down approach, using already existing statistical information. It is important to remember that the uncertainty of a particular result is independent of the method used for its estimation.

Several examples of uncertainty budgets for critical parameters based on the bottom-up procedure will be discussed, and it will be shown how the top-down method is used as a means of verifying uncertainty budgets, based on Type B experience [1].

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VALIDATION OF QUANTITATIVE NMR MEASUREMENTS

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Keywords: NMR, quantitation, validation

NMR is by definition a quantitative spectroscopic tool because the intensity of a resonance line is directly proportional to the number of resonant nuclei (spins). Quantitative NMR can be used primary method [1] according to the definition of the CCQM [2] enabling, in principle, a precise determination of the amount of substances. With the sensitivity enhancements due to stronger and stronger static magnetic fields including improved electronics the detection limits have been pushed down significantly. Quantitative NMR measurements for assessments of amount ratios and fractions and also for purities and contents by using internal standards have been described for different areas such as pharmacy, agriculture, material science, military purposes etc. However, the lack of a precise protocol that considers and controls the aspects of both measurement procedure as well as spectra processing and evaluation is responsible for the fact that quantitative investigations of identical samples performed in various laboratories may differ severely (deviations up to 90% relative to gravimetric reference values).

The validation of quantitative high resolution ^{1}H-NMR using single pulse excitation will be presented [3]. It considers all issues regarding linearity, robustness, specificity, selectivity and accuracy as well as influences of instrument specific parameters and data processing and evaluation routines, confirmed by national and international round robin tests. Furthermore, results of purity determinations will be discussed for selected representative pharmaceutical reference substances.

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[3] Malz, F., Jancke, H., J. Pharm. Biomed. In press.

PRECISION AND ITS OPTIMIZATION IN CAPILLARY ELECTROPHORESIS - MASS SPECTROMETRY

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Keywords: precision, capillary electrophoresis – mass spectrometry, electrospray ionisation

Liquid chromatography-mass spectrometry (LC-MS) is widely used for identification and quantification in e.g. pharmaceutical and biotechnological applications. For identification and characterization, capillary electrophoresis-mass spectrometry (CE-MS) has evolved into a strong alternative. The achievable precision for quantitative methods is still the main limitation for a general application of these instruments in analytical laboratories.
Similar to LC-MS, aspects of matrix influence on the electrospray process need to be carefully addressed when quantitative results are intended by CE-MS. Due to a more complicated coupling special emphasis needs to be put on the CE-MS interface, with the positioning of the capillary being the crucial parameter to ensure reproducible results.
Methodological aspects influencing the precision in CE-MS are summarized, evaluated and experimentally examined. Internal standards generally improve the precision independently on the instruments used. Experiments with different kinds of internal standards revealed that the precision can be doubled when changing from a structural related to an isotopically labeled internal standard. Thus a level of precision better than 5% percental relative standard deviation can be achieved.

UNCERTAINTY OF MEASUREMENT – EXPERIENCE OF A BUNDESWEHR CONTROL LABORATORY

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Keywords: testing laboratory, uncertainty

At present, estimating analytical uncertainty (i.e. uncertainty of measurement) in accordance with international standard ISO/IEC 17025 is a vital part of good analytical practice and the first step in determining acceptance criteria. Development and application of methods to estimate the measurement uncertainty is crucial for testing laboratories in order to achieve ISO 17025 accreditation.

Chapter 5.4.6.2 of ISO 17025 states: "In certain cases the nature of the test method may preclude rigorous metrologically and statistically valid calculation of uncertainty of measurement. In these cases the laboratory shall at least attempt to identify all the components of uncertainty and make a reasonable estimation, and shall ensure that the form of reporting of the result does not give a wrong impression of the uncertainty. Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data."

The following presentation elucidates the methods of reasonable estimation of measurement uncertainty. These methods are illustrated with examples from Bundeswehr microbiological and chemical food and drinking water laboratories.

[1] Allgemeine Anforderungen an die Kompetenz von Prüf- und Kalibrierlaboratorien DIN ENISO/IEC 17025 : 2000
[2] EURACHEM / CITAC Leitfaden „Ermittlung der Messunsicherheit bei analytischen Messungen"

EQUIVALENCE TEST OR CLASSICAL T-TEST? EVALUATION OF THE PERFORMANCE FOR METHOD TRANSFER

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Keywords: Equivalence test, method transfer, statistical test

The performance of the equivalence test principle in the context of analytical method transfers was investigated by means of a simulation study. An ISPE [1] design proposal and typical error contributions for pharmaceutical routine control have been used for the testing of accuracy. Acceptable results (probability of a correct decision) have been obtained here. For total variations above 0.4% RSD% the basic design was not sufficient. An overview for the number of additional series needed corresponding to higher variations has been developed based on further simulations.

[1] ISPE Good Practice Guide for Technology Transfer (2003)
www.ispe.org

TYPICAL VARIABILITY AND SOURCES OF VARIABILITY IN DRUG DISSOLUTION TESTING

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Keywords: variability, collaborative study, dissolution

Dissolution testing technique is a time-consuming and a labour-intensive test procedure. The manual technique is subject to analyst variability and there do exist several options to proceed as regards selecting apparatus and conditions, handling the drug product, sampling and analyzing.
On the basis of the data obtained from 29 international laboratories, the presentation provides an investigation into sources of variability in dissolution testing. The study design for an immediate release dosage form applying multiple point testing using the USP paddle apparatus is described. Apparatus set-up, calibration and experience of the operators are discussed, Total and Between Laboratory Variability and Between Analyst Variability were calculated.
In contrast to previous reports, the variability of the glibenclamide dissolution data was significantly lower. Total variances (s^2) were found from 19.37-43.52. Between Laboratory and Between Analyst variances ranged from 12.9-38.7 and the Within Analyst variances ranged from 4.95-5.60. The dissolution profiles and corresponding variances obtained by laboratories with little or no experience in glibenclamide dissolution testing were similar to those obtained by more experienced laboratories indicating sufficient robustness and capability. The smallest statistically detectable difference between two dissolutions runs was calculated (95%CI) to be 7% for one analyst, or 5 % if two analysts do testing.

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TARGET VALIDATION: NON-GELBASED PROTEOME RESEARCH FOR SYSTEMATIC AND QUANTITATIVE PEPTIDE ANALYSES

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Keywords: kinase inhibitor, LC-MS/MS, proteomics

Small molecular kinase inhibitors can interfere with cellular signal transduction pathways and therefore are of considerable interest for both pharmaceutical and academical research. Recent studies have demonstrated that inhibitor specificities are often not restricted to individual kinases [1] and some inhibitors target up to 64 different kinases activities. Kinase inhibitors can be applied in affinity chromatography and thereby give quantitative access on a significant part of the total human kinome. This strategy together with recent progress in quantitative non-gelbased proteomics [2] and phosphoproteomics [3] now favours comprehensive and systematic investigations of signal transduction pathways.
We selected the host-pathogen-interaction concerning *Listeria monocytogenes* to establish and validate technologies for (i) the specific enrichment of phosphorylated peptides and kinases and (ii) for their relative quantification based on iTRAQ-nanoHPLC-MS/MS analyses. *Listeria monocytogenes* is a Gram-positive bacterium and a model organism for facultative intracellular human pathogens. It induces its uptake even into non-professional phagocytotic cells by the presentation of virulence factors such as Internalin B (InlB) that interacts with the growth factor receptor tyrosine kinase c-Met (HGFR). We induced epithelial cells either with recombinant InlB or with the growth factor HGF that is the natural ligand of c-Met. Current results indicate to both common and individual signal specificities of InlB and HGF treated cells and furthermore helped to define statistical criteria for further quantitative LC-MS/MS analyses.

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[3] Ficarro S.B. et al., Nature Biotechnology **2002** Mar;20(3):301-5.

QUANTIFICATION BY PRE-DETERMINED CALIBRATION PARAMETERS VS. CLASSICAL APPROACH OF SIMULTANEOUS CALIBRATION IN LC

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Keywords: Precision; pre-determined calibration; control charts

One main root cause for variability in quantitative analysis is the preparation and analysis of an external standard. Depending on its stability the external standard solution is prepared for each analytical set separately or after certain intervals (e.g. weekly or monthly). The external standard solution is usually analysed on the analytical equipment (e.g. LC-system or spectrometer) daily for each analytical series, i.e. simultaneously with the samples. Based on the results of this determination the parameters for the calibration function are calculated according to the control test for each analytical series separately. These parameters are then used to calculate the content of the analyte in an unknown sample (**simultaneous calibration**).

While this calibration approach makes the analytical procedure robust for changes in the equipment, it finally leads to an additional contribution to the variance of the result and to an over-adjustment if the system is stable.

An alternative approach calculating the parameters of a calibration function for every analytical series is to take **pre-determined** parameters. This procedure requires verification, whether the pre-determined parameters are still valid for quantification, which is done by utilising a control chart.
In the presentation both approaches are compared to each other.

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