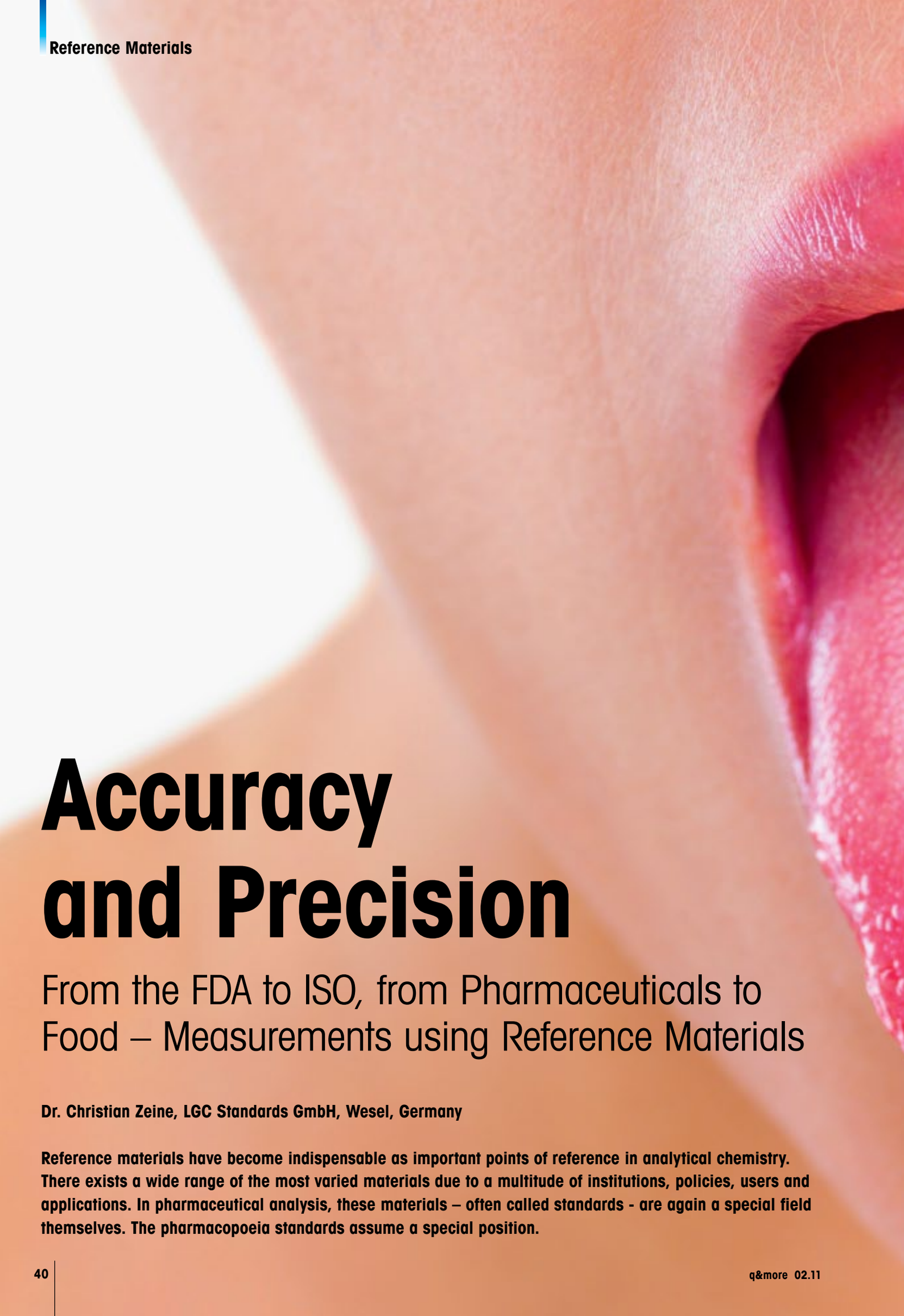

Accuracy and Precision

From the FDA to ISO, from Pharmaceuticals to Food – Measurements using Reference Materials

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Reference materials have become indispensable as important points of reference in analytical chemistry. There exists a wide range of the most varied materials due to a multitude of institutions, policies, users and applications. In pharmaceutical analysis, these materials – often called standards - are again a special field themselves. The pharmacopoeia standards assume a special position.

The general principle

There are two major groups of reference materials: reference standards and matrix reference materials.

Reference standards are reference materials that are as pure as possible and are used to calibrate an analytical method. The content of the standard is known and with it the resulting measurement signal can be related to it. The unknown sample to be measured also provides a measurement signal and, using a proportional calculation, conclusions can be drawn as to the content of the unknown sample.

Matrix reference materials are materials with which a particular analyte has been accurately determined within a specific matrix, such as the pesticide hexachlorobenzene (analyte) in cod liver oil (matrix).
Matrix reference materials are used in the method validation. In this connection, correctness and precision determine the accuracy of a method [1]. In addition, matrix reference materials are also used for quality control in daily routine analysis.

Depending on which documentation and which "analytic family tree" a reference standard or a matrix material has, this standard and this material can then have the status of a Certified Reference Material (CRM).

Reference material (RM) and certified reference material (CRM)

The VIM (Vocabulaire International de Métrologie, International Vocabulary of Metrology) defines RM and CRM as follows [2]:

Reference Materials

Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

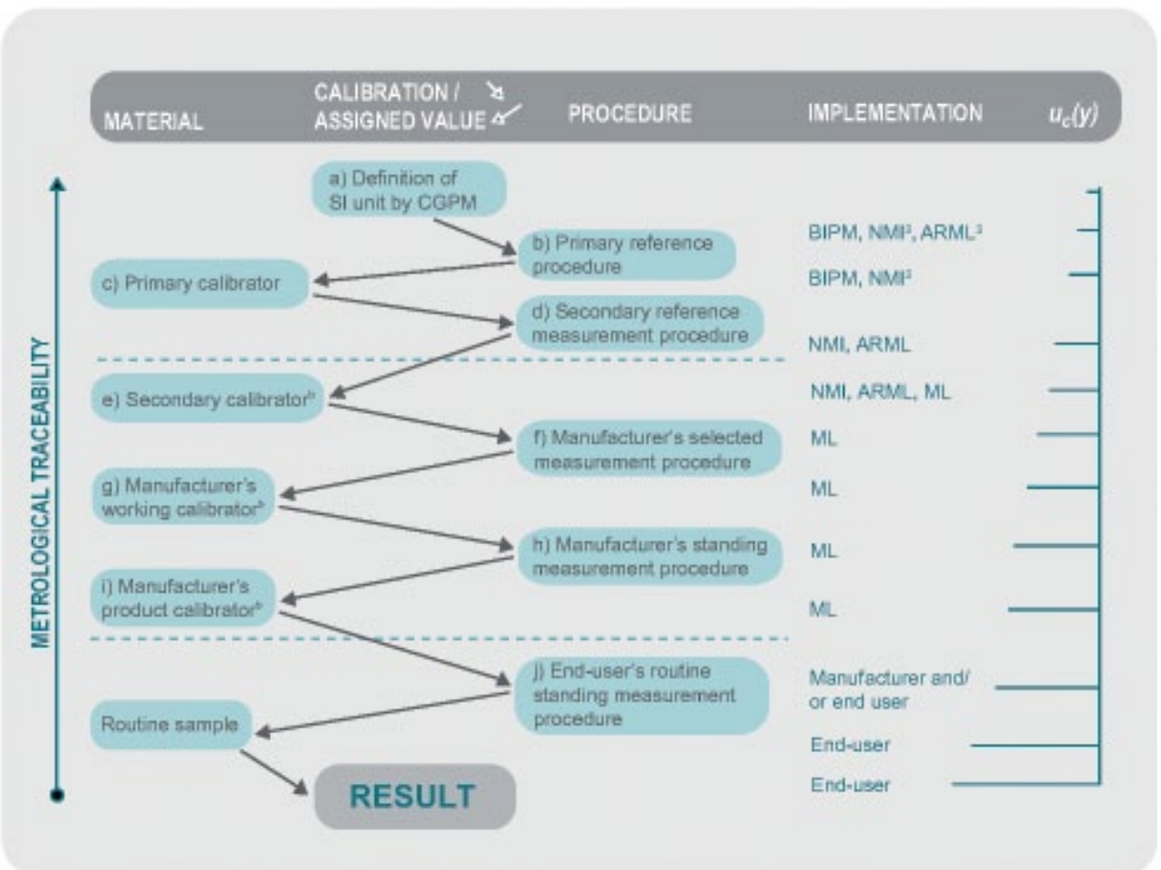

Fig. Traceability chain in accordance with ISO Guide 17511 (ML: Manufacturer Laboratory; ARML: Accredited Reference Measurement Laboratory; NMI: National Metrology Institute, BIPM: Bureau International des Poids et Mesures).

Certified Reference Material

A reference material characterised by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that states the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

An RM thus differs from a CRM in three fundamental points:

- a validated measurement method,
- an indication of the associated uncertainty,
- a statement regarding the metrological traceability.

Both the CRM features, uncertainty and metrological traceability, are considered in detail below.

Uncertainty

No matter how accurate a method is, no method is able to deliver the true value of a particular characteristic. During a measurement there will always exist factors which cause the measurement result to differ from the true value. Since the extent of random errors can also vary from measurement to measurement, it is equally impossible to obtain precise details about such deviations. However, it is possible, by a thorough examination of the individually measured components, to estimate the uncertainty (standard uncertainty) of each measurement component. The components with the largest contributions to uncertainty are then combined, using the rules of error propagation, to give a total uncertainty (combined standard uncertainty). Finally, this total uncertainty is subjected to statistical analysis. It is now important to determine a so-called confidence interval from the usually limited number of samples, from which one can assume with a certain probability that the actual ("true") value lies in this interval. The statistical considerations boil down to the fact that the overall uncertainty - depending on the sample size - is multiplied by a greater or lesser "coverage factor" [3]. These procedures, here considerably simplified, are then finally found for example in the following measuring data (also from [3]):

*Total nitrogen: (3.52 ± 0.14) %w/w **
**The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95 %.*

Traceability

According to the definition of traceability [2] it must be possible to trace the result of measurement to a reference through an unbroken chain of calibrations. The individual calibrations each carry a measurement uncertainty.

The so-called traceability chain again illustrates this situation (Fig., based upon [4]).

Regarding traceability, a CRM should also contain information as to how a particular value was safeguarded by comparison with other materials. Some certificates of analysis and reports of the IRMM (Institute for Reference Materials and Measurements) are exemplary and still unequalled in this respect. The following quoted text [5] gives an example:

The measurements of A2M, AAG, AAT, ALB, C3c, C4, HPT, IgA, IgG, IgM, TRF, and TTR were calibrated with ERM-DA470 …
… The certified values for AAG, AAT, TRF, and TTR in ERM-DA470 were obtained by calibration with pure proteins … Consequently, the certified mass concentrations, for AAG, AAT, TRF and TTR in ERM-DA470k/IFCC are traceable to the International System of Units (SI) …

Tab. ISO Guides, Series 30-35

ISO-Guide 30, Second Edition 1992	Terms and definitions used in connection with reference materials
ISO-Guide 31, Second Edition 2000	Reference materials – contents of certificates and labels
ISO-Guide 32, Second Edition 1997	Calibration in analytical chemistry and use of certified reference materials
ISO-Guide 33, Second Edition 2000	Uses of certified reference materials
ISO-Guide 34, Third Edition 2009	General requirements for the competence of reference material producers
ISO-Guide 35, Third Edition 2006	Reference materials – general and statistical principles for certification

For the protein AAG in the certified reference material ERM-DA470k/IFCC, the calibration of the measuring method was carried out with the older CRM ERM-DA470. For the determination of AAG in ERM-DA470, in turn, the measurement method was calibrated with the pure protein, which for AAG ultimately permits – via conversion using the molecular weight – traceability to the SI units in moles or kilograms.

The ISO Guides 30–35

The ISO Guides 30–35 provide guidance regarding where special attention must be paid with RM and CRM (Tab.). Here, Guide 34 is the central document. An accreditation according to this guide is possible and is now sought by many producers of reference materials.

Guides 30-35 are used in all areas of analytical chemistry. Since the accreditation of the USP, Guides 30–35 are becoming more accepted in the pharmaceutical industry as well, although a parallel world has been formed with respect to reference standards there.

Pharmaceutical analysis

Pharmacopoeia standards

Pharmacopoeia standards have an official status and must no longer be recognised separately by the regulatory authorities when they are used according to the Pharmacopoeia. However, improper use of methods outside of the Pharmacopoeia invalidates the official status.
Strictly speaking, one has then only a fine chemical, whose suitability for the unofficial purpose must be completely checked again.

Pharmacopoeia standards are delivered without a certificate of analysis. All important information on how to use the standard in accordance with Pharmacopoeia, is given only on the label, and sometimes on a separate sheet (information sheet, leaflet).

Primary reference standards

If no Pharmacopoeia standard is available, the user is urged to provide other standards for comparison. In general, this is achieved by the user applying a primary standard for each analytical purpose, which

- is either directly used in the analysis (e.g. if this analysis is not performed frequently)
- or when he can - with relatively little additional effort – calibrate it with a secondary standard, which he has available in larger quantities and – instead of the expensive primary standards – he can use for the frequent routine analysis.

The FDA describes this process quite well in one of its Guidances for Industry [7].

The following list (adapted from [8]) gives guidance points as to what may be required for the establishment of a primary standard. It applies to the standards for active ingredients:

- Purity above 95 %
- Complete verification of identity
- Purity (test for organic impurities)
- Residual solvents
- Water
- Loss on drying
- Sulphate ash
- Content calculation (for example from the purity, residual solvent and water analyses), for the plausibility test an additional independent method is often also called for (e.g. titration or DSC (differential scanning calorimetry))

The analysis results are documented in the form of an analysis certificate.

Secondary reference standards

They are derived from analytical comparisons with primary standards and are used - particularly for frequently performed routine analysis – instead of primary standards, which are characterised as being laborious and costly [7].

The qualifications required by the authorities often are:

- No detailed proof of identity required, often a comparison with a primary standard method is sufficient (e.g. by IR)
- Determination of the content by comparison of peak area with a primary standard

Often, the establishment of a reference standard is outsourced to external service providers. Here, the

assignment with a primary standard is naturally associated with higher costs, because the analytical and documentary work involved is disproportionately high.

Reference standards for impurities

Based on the ICH (International Conference on Harmonization [9, 10]), impurities (VUs) are all substances in drugs that are not the actual active ingredient or an excipient for the manufacture of the drug. Both the FDA [11] and the EMA (European Medicines Agency [12]) have made it clear in their guidances or guidelines that the monographs of the USP and Ph.Eur. are often not sufficient to guarantee the quality of a generic drug in terms of VUs, and stipulate possible additional tests to be performed based on the ICH. The correct identification and quantification of a VU in a pharmaceutical product is of immense importance. The best way to ensure the correct identification and quantification is by use of precisely defined reference standards for VUs. These are often not provided by the pharmacopoeias, but by correspondingly established commercial suppliers [13]. Instead, many pharmaceutical laboratories are working only with fine chemicals, in which merely the chromatographic purity is known. From the regulatory side this approach is often accepted as long as only the identity is protected. The reason for this is the almost certain overestimation of the VU in the actual product, with this approach, so that at no time is there a risk for the patient. But one must be fully aware of the fact that this approach has significant disadvantages for the pharmaceutical manufacturers:

- Overestimation of the level of the VU in the product and therefore probably more frequent exceeding of the ICH threshold for qualification (with the consequence of possibly unnecessary, expensive and time-consuming Tox studies)
- Unnecessarily short shelf-life of the product
- Possible freezing of batches in the quality control or at the incoming and out-going controls, which may well be still acceptable.

Conclusion

A reference standard is not simply a reference standard. It is important, not only in pharmaceutical analysis, to know exactly what application the reference standard can be used for. Above all, pharmacopoeia standards should, as far as possible, only be used as prescribed by the currently valid version of the Pharmacopoeia, as otherwise this invalidates the official status of the standard.

The FDA and EMEA have also decided that it is often necessary to test the VUs in addition to the Pharmacopoeia. Special reference standards for impurities help immensely in the correct identification and quantification of these substances in active ingredients and medicines.

Literature from the author:

Christian Zeine studied chemistry at the Westphalian Wilhelms-University in Münster, where he received his doctorate in 1998. After project work at the University, from 1999 on, there followed activities at manufacturers of medical and *in-vitro* diagnostics products, including at B. Braun Melsungen. Since 2002 he has worked in the product management for pharmaceutical reference materials at LGC Standards in Wesel. LGC Standards is a leader in the field of reference materials for various analytical applications and part of the international LGC Group.

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