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Pharmaceutical quality control: the reference standards labyrinth

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Executive Summary

A pharmacopoeial RS is – in most cases – a primary standard specialized for one specific purpose. It should be used for the purpose(s) described in its corresponding monograph(s), and is not automatically suitable for other purposes. To service these, reference standards from other sources should be considered. This applies to both API and impurity RSs.

Secondary RSs are compared to primary standards prior to first use. The concern in the analytical community is that it is difficult to establish traceability for quantitative secondary standards to pharmacopoeial primary standards. The latter lack the uncertainty figures which would be necessary to realize a proper traceability of secondary to primary standard.

Research materials provide valuable support in early method development. However, their use as reference standards at a later stage in method validation and release testing of finished dosage forms bears risks for both patients and – from an economic standpoint – manufacturers.

Audience and content

This white paper has been prepared for pharmaceutical professionals who are new to the subject of reference standards (RSs) in pharmaceutical quality control (QC) and work in QC or stability testing teams in the pharmaceutical industry. It also addresses new starters working in the development, validation and transfer of analytical methods for QC and stability testing departments.

It could be helpful too for experienced staff and/or management in the above-mentioned teams, who want to refresh and update their knowledge on the topic.

This white paper explains the different types of RS used in the pharmaceutical departments mentioned above, and the intended uses of such standards. It also explores the limitations of pharmacopoeial RSs, and secondary standards based thereon. Additionally, it offers advice on alternative primary standards that are accompanied by a detailed certificate of analysis. Impurity RSs and research materials are also covered.

The different types of reference standards

In the pharmaceutical area, there are four major types of reference standards:

- **Primary RS**
- **Pharmacopoeial RS (for monograph use)**
- **Secondary RS**
- **Impurity RS**

Apart from the pharmacopoeial standards, all of the above come with certificates of analysis (CoAs), or – in the case of secondary RSs – with a comparison statement. The information provided on the CoA should be suitable with regard to the specific use planned for the corresponding RS.

There is a fifth type – research materials[1] – which is often used at the very beginning of analytical research and development, but not normally for method development, validation, transfer or quality control. Research materials come with minimal characterization data, which makes them useful for basic development and identification work, but they cannot be called reference standards, as defined by any regulatory or standardization body.

Primary reference standards

In the pharmaceutical context, a primary reference standard is a standard for which the properties (usually identity, very often also purity/assay values) have been characterized by certain analytical techniques, without, however, being compared to any other standard of the same kind. The definition for a primary standard in the GMP guideline Q7 of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) states[2]:

Reference Standard, Primary
A substance that has been shown by an extensive set of analytical tests to be authentic material that should be of high purity. This standard can be: (1) obtained from an officially recognized source, or (2) prepared by independent synthesis, or (3) obtained from existing production material of high purity, or (4) prepared by further purification of existing production material.

The ICH description does not refer to the unique position of the primary RS, in that it is not compared to any other RS. But this is clarified by the WHO definition,[3] which mentions that *"a designated primary chemical reference substance is one that… [has] the appropriate qualities within a specified context, and whose assigned content … is accepted without requiring comparison with another chemical substance."*

Possible *"set[s] of analytical tests"* (ICH) and respective *"appropriate qualities"* (WHO) to characterize primary standards are described later in this white paper.

[1] Also referred to as analytical standards.
[2] ICH guideline Q7, accessed on Jan 22, 2019 under https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q7/Step4/Q7_Guideline.pdf
[3] WHO Technical Report Series 943 (2007) accessed on Jan 22, 2019 under http://apps.who.int/medicinedocs/documents/s14139e/s14139e.pdf

When to use a primary standard?

In pharmaceutical quality control, the use of reference standards to calibrate the analytical procedure is mandatory when measurements are performed with relative methods such as HPLC in combination with a UV or MS detector. These measurements need to be traceable to a primary standard. This requirement is realized either by using the primary standard directly for the calibration purposes, or by using a secondary standard (see later) which is compared to the primary one.

ICH guideline Q7 states:
11.17 Primary reference standards should be obtained as appropriate for the manufacture of APIs. The source of each primary reference standard should be documented. Records should be maintained of each primary reference standard's storage and use in accordance with the supplier's recommendations[4]. Primary reference standards obtained from an officially recognized source are normally used without testing if stored under conditions consistent with the supplier's recommendations.

Officially recognized sources, however, are not specified in Q7. The FDA does mention sources in their Guidance for Industry on Analytical Procedures and Methods Validation for Drugs and Biologics[5] but, interestingly, does not refer to these institutions as an official or definitive list:

Reference standards can often be obtained from the USP and may also be available through the European Pharmacopoeia, Japanese Pharmacopoeia, World Health Organization, or National Institute of Standards and Technology.

Instead, the FDA states that *"reference materials from other sources should be characterized by procedures including routine and beyond routine release testing"* and that producers *"should consider orthogonal methods for reference material characterization"*.

This approach reads differently, but is exactly in line with the definition for primary RSs in ICH Q7, discussed earlier. For primary RSs, both the ICH guideline and FDA guidance allow other sources than the "officially recognized sources". Independent manufacturers can provide such primary standards, ideally characterized by processes like those outlined in the general text 5.12. of the European Pharmacopoeia (Ph.Eur)[6].

Acetylation of 2,6-Dimethylaniline during the synthesis of Lidocaine. (Impurity N-(2,6-Dimethylphenyl) acetamide, MM0102.08)

[4] The passage *"use in accordance with the supplier's recommendations"* in ICH guideline Q7 is of considerable relevance when working with pharmacopoeial RSs (see next chapter). The *"supplier's recommendations"* for those standards are to use them only in combination with the pharmacopoeial methods described in the monographs. Other uses are not automatically authorised, and are the sole responsibility of the user.

[5] *FDA Guidance for Industry Analytical Procedures and Methods Validation for Drugs and Biologics*, July 2015, accessed on Jan 22, 2019 under https://www.fda.gov/downloads/drugs/guidances/ucm386366.pdf

[6] Ph.Eur. 9th edition, chapter 5.12. This chapter also states that reference standards by the European Pharmacopoeia *"are in general primary standards, except for those (notably antibiotics) that are calibrated in International Units. The latter are secondary standards traceable to the international standard"*. As a first approximation, this is also valid for reference standards from other compendial sources.

Correct use of primary reference standards: what to keep in mind?

In essence, a primary RS needs to be fit for its intended purpose. A pharmacopoeial RS has been shown to be fit for its compendial purpose, but has not been demonstrated to be fit for any other purpose: this needs to be proven by the user. Consequently challenges of compendial standard use for non-compendial purposes have been reported in regulatory inspections. Other primary standards with fully documented CoAs can be used for most applications, providing they have been characterized appropriately.

If a primary RS is used to establish a secondary standard then the secondary RS can only be used for the same purpose as the primary one.

Pharmacopoeial reference standards

Reference standards from the pharmacopoeias – also often referred to as compendial RSs – are, in principle, primary standards that have a special status for use in connection with the monograph methods for which they have been designed.

This position is specified in the USP (United States Pharmacopeia) general chapter <11>[7]:

When approved as suitable for use as comparison standards ... in the United States Pharmacopeia (USP) or National Formulary (NF), USP RS also assume official status and legal recognition in the United States. Assessment of the suitability for use in other applications rests with the user.

It is also described in the European Pharmacopoeia (Ph.Eur.) in general text 5.12.[6]:

European Pharmacopoeia reference standards are shown to be suitable for their intended purpose; they are not necessarily suitable for other purposes. Any value assigned to a reference standard is valid for the intended use and not necessarily for other uses.

So, strictly speaking, the special status of a pharmacopoeial RS can only be assumed when the standards are used as required by the monographs. For anything other than these monograph purposes, compendial RSs stand on the same level as RSs from other sources. This means that you need to prove suitability of a compendial RS for the desired non-compendial purpose in the same way you would need to for any other RSs. This is clearly mentioned in the Ph.Eur. chapter 5.12., and can be seen as a blueprint for other compendial RS use as well:

If a European Pharmacopoeia reference standard is to be used for any purpose other than that for which it has been established, its suitability for the new use has to be fully demonstrated and when applicable, to be described in the marketing authorization application.

The use of compendial RSs for non-monograph applications is also made difficult by the fact that certificates of analysis, which might otherwise provide a rich data set of characterization testing results, are normally not provided by the compendial institutions.

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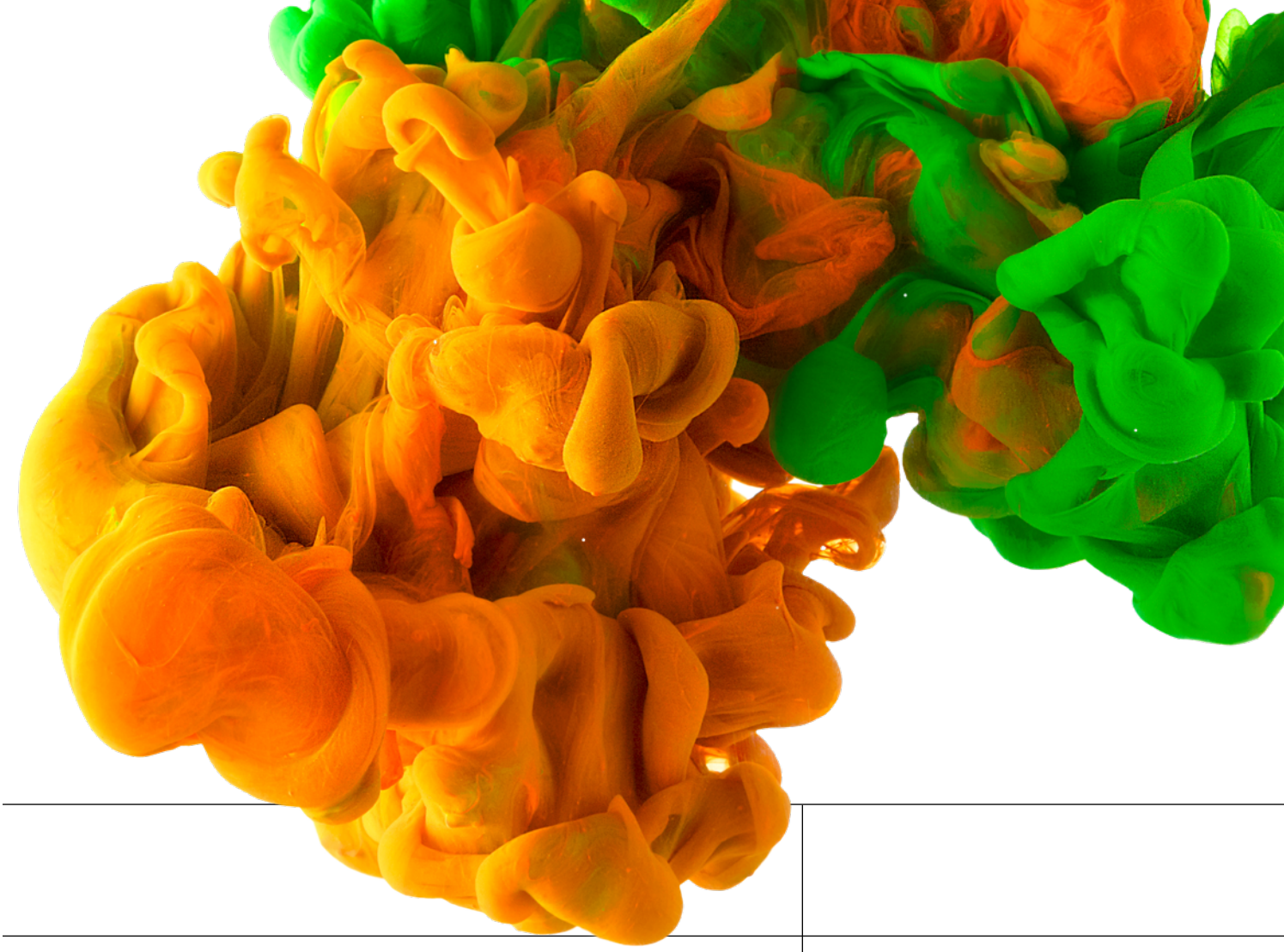
[7] United States Pharmacopeia, General Chapter <11>: Reference Standards.



When can I calculate with 100% assay value?

One of the most frequently asked questions for compendial RSs

In the analytical community, people often ask what assay can be assumed when a compendial reference standard (CRS) is not provided with a specific value. The USP writes about the assay*: *"Unless a reference standard label bears a specific potency or content, assume the reference standard is 100.0% pure in the official application."*

The crucial word in this instruction is "official", here meaning "compendial". If the compendial purpose in the accompanying monograph(s) is only a qualitative one (e.g. identification by IR, peak identification or system suitability test), then the CRS cannot be used for quantitative purposes assuming 100% assay.

The European Pharmacopoeia has a clearer statement on this**:

If a CRS has no assigned content, this should in no case be interpreted as if the content were 100 per cent. For assay purposes, the content explicitly assigned must be used and in no case should the user interpret this as if the content were 100 per cent.

The European Pharmacopoeia publishes a slightly different position, meanwhile, on quantitatively used impurity RSs: these are assumed to have an assay of 100%, unless they are labeled with a specific value. A value will be specified once the assay is below 95.0%.***

* USP 41, general chapter ‹11›

** See Ph. Eur. FAQ under https://www.edqm.eu/en/Helpdesk-1683.html?rubrique=318, accessed on Jan 22, 2019

*** Ph.Eur. 9th edition, chapter 5.12.

Impurity reference standards

Everything discussed so far can also be applied to another special group of reference materials: impurity reference standards (IRSs). Although the term 'primary impurity standard' does not really exist, any impurity standard that is not compared to another material of the same chemical structure and which is fit for its designated purpose can be considered such a primary material.

IRSs are designed to detect, identify, quantify and qualify impurities in a drug substance in accordance with the ICH guidelines (Q3A to Q3D and Q7). ICH Guidelines 3A to 3D regulate the approach to impurities in the pharmaceutical industry. ICH does not prioritize at any point any given range of standards over another, whether officially recognized or not, for use within the pharmaceutical industry.

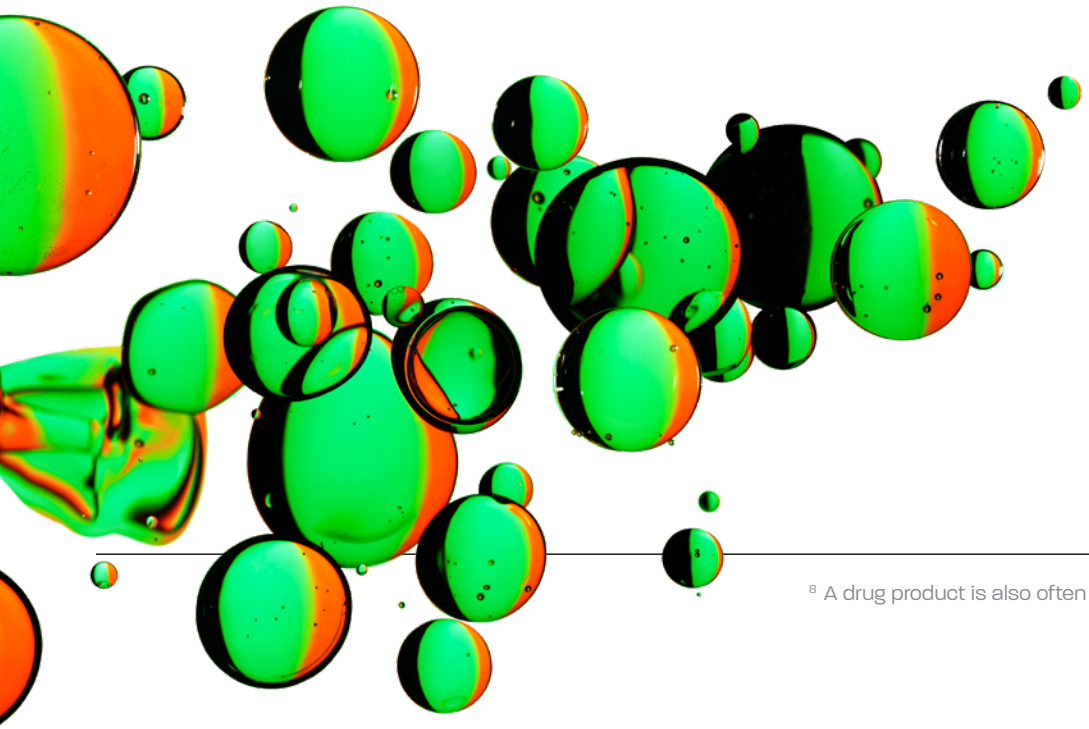
According to these guidelines, impurities can be primarily classified into either drug substance or drug product impurities, referring in the first case to any component in the drug substance which is not the chemical entity itself. A drug product impurity, meanwhile, is any chemical entity present that is not the drug substance or an excipient used to manufacture the drug product[8]. Based on the cause triggering their appearance, impurities can be classified as starting materials, byproducts, intermediates, degradation products or reagents/ligands and catalysts. Finally, by their chemical nature, impurities can be classified as organic, inorganic or solvents.

Pharmacopoeial IRSs, like all compendial materials, are designed for the specific monograph purposes. For other purposes, IRSs from other sources suitable for the intended purpose should be preferred, for two major reasons:

– The use of a pharmacopoeial IRS outside the monograph can be challenged by regulatory authorities. Customers have reported to us that authorities have insisted on a CoA for pharmacopoeial IRSs used for non-compendial purposes. A CoA is not provided by pharmacopoeial sources, rendering the material unsuitable for use without separate detailed internal characterization.

– Furthermore, supply of pharmacopoeial IRSs can be insecure. For example, there have been occasions where a change in a monograph has resulted in a neat impurity standard being replaced by a system suitability or peak identification mixture, usually consisting of a mix of the API and traces of one or more impurities. Such a replacement is not a problem if you work according to the monograph methods. But if you work with a pharmacopoeial material for non-compendial purposes, you might face a challenge replacing the neat IRS if it is not available anymore. If you have stated the use of that specific material in your dossier, you might even need to report the change to the authorities. Cessation of supply is also a risk with any catalogue reference standard provided by a third party, but switching between suppliers is significantly easier when the materials are "primary" and accompanied by CoAs.

In addition, the current regulation situation – especially for the finished dosage forms dealt with in ICH guideline Q3B – often requires additional impurity testing, on top of the requirements of the pharmacopoeial monographs. It is also often the case then that there are no suitable impurity standards available from compendial sources, and the support of experienced commercial IRS manufacturers thus becomes necessary.



[8] A drug product is also often called a finished dosage form (FDF).

Essentials of secondary standards

Secondary reference standards are, as the name suggests, second-line materials. They consist in each instance of a material that is compared against the primary material, and used instead of that[9].

It does not matter whether the secondary standard is compared against a pharmacopoeial primary standard, or against a primary standard obtained in-house or from a third source[10]. Of particular note, a secondary standard can only be used for the same purposes as the primary standard. Thus if a primary standard was designed solely for a qualitative purpose (i.e. identification via IR, system suitability test or peak identification), then to use the corresponding secondary standard for quantitative purposes is not valid. For example, a large number of Ph.Eur. reference standards for APIs have been set up for IR comparisons only, and should not be used as a basis for quantitative secondary standards.

In addition, the EDQM (European Directorate for the Quality of Medicines) does not recommend measuring secondary standards against even their quantitative materials[11]. This is because:

- While pharmacopoeial primary RSs have a determined measurement uncertainty (MU) in line with the requirements of the relevant pharmacopoeial monograph, the value of this uncertainty is not currently specified.

- The comparison of a candidate material for a secondary RS to the pharmacopoeial RS will result – by the principle of uncertainty propagation – in a larger but non quantifiable MU for the secondary standard, by virtue of the fact that the MU for the pharmacopoeial material is not published

- This unknown but enlarged MU for the secondary RS might no longer be appropriate for the acceptance interval specified in the relevant monograph.

For the same reason, the use of a pharmacopoeial RS with a non-compendial method can be difficult to justify on the basis that the uncertainty of its value assignment is unknown.

Research materials

Research materials are often used at the very beginning of analytical research and development, but should not be employed when the methods are fully developed. During validation and implementation in the QC or stability testing lab, they are usually replaced with better-characterized materials, especially when any quantitative purpose is connected with their usages.

Research materials come with only basic characterization data (e.g. identity and chromatographic purity) that makes them useful for basic development and identification work. They cannot be considered reference standards. Research materials can be distinguished from reference standards by their relatively simple CoA.

The use of research materials in implemented methods is not often observed, and if so then it is mainly restricted to identification purposes. As their purity is often overstated, use for quantitative applications risks an overestimation of the amount of an analyte. For quantitative applications, a thoroughly characterized reference standard is preferred over a research material that is accompanied by inadequate levels of supporting measurement data.

[9] ICH Q7 also states in the definitions section: *"Reference Standard, Secondary: "A substance of established quality and purity, as shown by comparison to a primary reference standard, used as a reference standard for routine laboratory analysis."* The FDA GFI referenced under footnote 4 directs users to the Q7 as well.

[10] Some sources (e.g. Ph.Eur. chapter 5.12., see footnote 6) state that secondary RSs should be set up against the compendial primary RS whenever possible. This can be upheld of course only when the compendial primary RS has been used within the compendial purpose, or has been shown suitable by other measures.

[11] A. Lodi, presentation at EDQM Training "European Pharmacopoeia Reference Standards", 19 April 2016, Strasbourg

Conclusions

Primary reference standards (RSs) can come from pharmacopoeias or from other sources. They need to be suitable for their intended purpose.

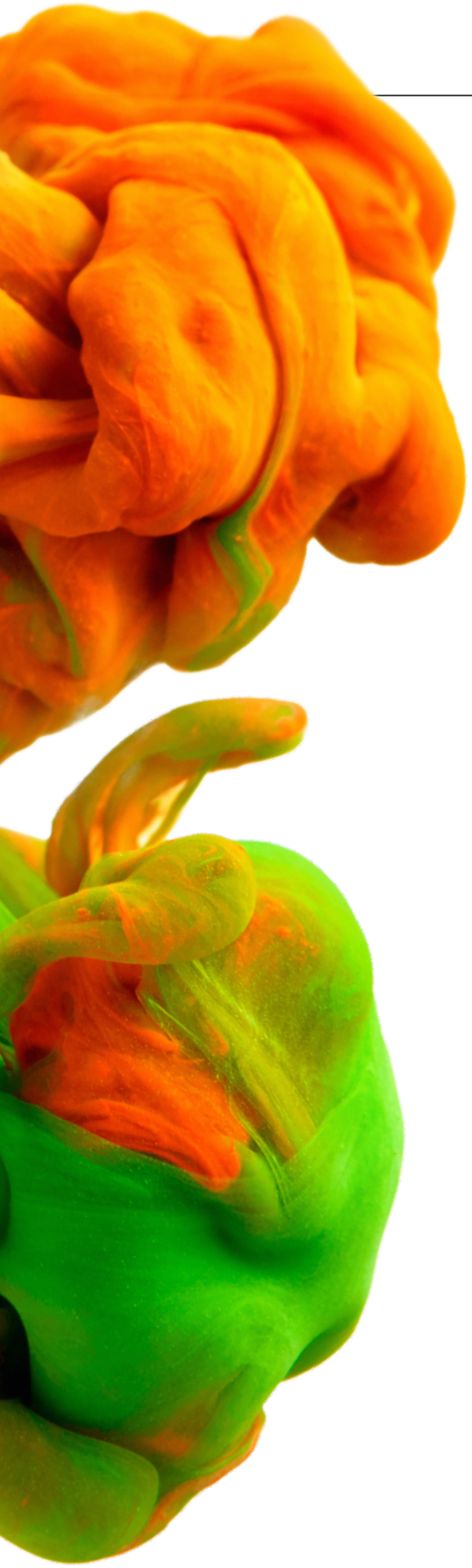
A pharmacopoeial RS is – in most cases – a primary standard specialized for one specific compendial purpose. It is not automatically suitable for other purposes.

Secondary RSs are compared to primary standards prior to first use.

Research materials provide valuable support in early method development. However, their use as reference standards at a later stage in method validation and release testing of finished dosage forms bears risks for both patients and – from an economic standpoint – manufacturers as well.

About the author

Martina Christiane Kotthaus received her PhD in organic chemistry from the Westphalian Wilhelms-University in Münster, Germany, and performed her postdoctoral research in the laboratories of Prof. M. Schlosser at the University of Lausanne, Switzerland. She has more than 20 years' experience in the pharmaceutical industry, starting as a research chemist in drug discovery at Hoechst Roussel Vet/Intervet, then moving into customer manufacturing of APIs, before various positions in R&D at DSM and Patheon (Thermo Fisher Scientific). Since January 2019 Martina has worked as Site Director at LGC GmbH in Luckenwalde, Germany. She has published many articles in international journals and is the inventor of several patents.



About Mikromol
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At Mikromol we combine an incomparable depth of pharmaceutical knowledge with 25 years of manufacturing experience and scientific acumen to ensure our portfolio of over 5,000 Impurity, Active Pharmaceutical Ingredient (API) and Excipient reference standards is of the highest quality. We go beyond the standard, supporting you with the highest accreditation for reference standard purity and a comprehensive Certificate of Analysis, to help you achieve greater analytical certainty.

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Phase separation during the liquid-liquid extraction of Enalapril Maleate. (Impurity Imidazole, MM0015.02)