External reference standards or relative response factors:

Considerations for quantitation of impurities in pharmaceuticals

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1. Introduction

Accurate and reproducible quantitation of impurities in pharmaceuticals is an important part of drug development. Impurities must be controlled to levels that will ensure the quality of the product is as good or better than the quality of materials used in preclinical safety studies and clinical trials. International Council for Harmonisation (ICH) Q3A and Q3B guidelines for drug substances and drug products, respectively, provide regulatory expectations for investigation and control of impurities including process-related substances and degradation products. Thresholds for identification and safety qualification of impurities can be based on relative percentages or directly in milligrams of exposure, depending on the nature of drug substances and products[1]. Therefore, an accurate assessment of impurity levels is needed both to guide development efforts and during the entire lifecycle of the drug.

Determination of impurities for most pharmaceuticals is accomplished by HPLC with UV detection. Quantitation can be performed versus an external standard of the impurity itself or by comparison to the response of the active pharmaceutical ingredient (API). If the response (peak area/concentration) of the impurity is different than the response of the API, a relative response factor (RRF) may be applied to report a more accurate weight percentage result for the impurity. The RRF is defined in equation 1. Relative response factors can be applied whether an area normalisation procedure (% of total peak area) or a dilute solution of an API standard is used.

Equation 1.

$$RRF = \frac{\text{(Impurity response/Impurity concentration)}}{\text{(API response/API concentration)}}$$

Several factors influence the choice of impurity quantitation using impurity reference standards or the API with RRFs. This paper reviews these factors and discusses considerations for making an informed choice.

This article does not deal in great detail with impurity quantitation in pharmacopoeial monographs. However, it should be noted that different conventions for RRFs are used by the United States Pharmacopoeia (USP) and European Pharmacopoeia (Ph.Eur.) in impurity calculations. Awareness of these differences is important to avoid miscalculation of results, i.e. in the case that pharmacopoeia information is used for non-pharmacopoeial purposes. In Ph.Eur. monographs, a correction factor (CF) is applied when the responses of the impurity and API are different. The CF is defined as 1/RRF and appears in the numerator of impurity calculations as a multiplier. In updated USP monographs, RRFs are denoted as F and appear in the denominator of calculations. Some older monographs retain a different convention, so it is critical to understand how relative response factors or correction factors used in a specific situation have been defined and are being applied.

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

[1] ICH Q3A(R2), Q3B(R2).

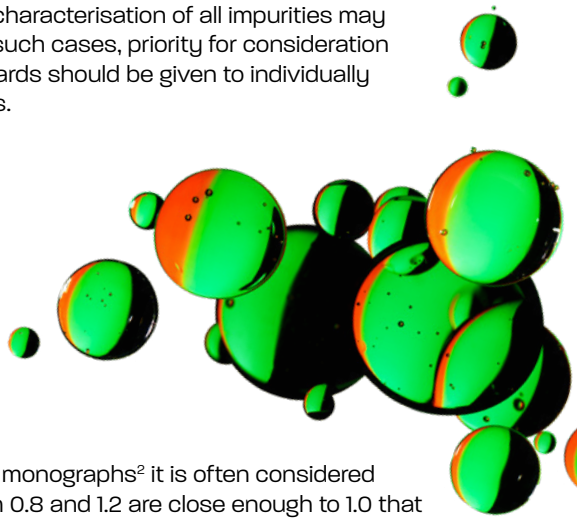
2. Motivation for quantitation vs the API

It is generally acknowledged that use of a standard of a given impurity is the most accurate and robust means of impurity quantitation. However, there are considerations that motivate quantitation vs. the API.

Impurity standards may be difficult to isolate or synthesise in adequate quantities needed for routine use. The standards must be adequately characterised to confirm identity and assess purity and this characterisation needs to be robustly monitored to account for potential changes over time. The availability and costs of standards have improved in recent years due to the emergence of commercial sources in dedicated impurity standard manufacturers, some of which are operating under and accredited to ISO 17034:2016 (General requirements for the competence of reference material producers), to provide the highest quality level possible in order to help improve accuracy.

Preparation of external impurity standard solutions may be more complicated or time-consuming for lab operations. This may be mitigated by freezing or refrigerating prepared standards, having confirmed whether this provides stability for extended periods.

Some drugs have complex impurity profiles, with many components, and characterisation of all impurities may not be feasible. In such cases, priority for consideration of reference standards should be given to individually specified impurities.

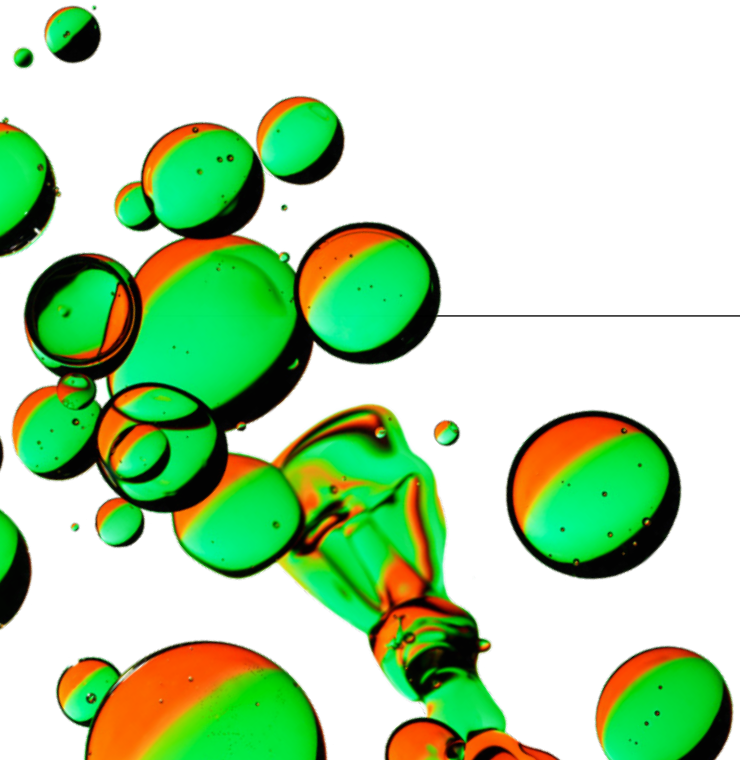


3. Assignment of RRFs

The use of API response and RRFs for accurate impurity quantitation requires an accurate assignment of the RRF. Determination of RRFs is accomplished by comparing the response of the impurity to that of the API for known concentrations of the compounds under the conditions of the HPLC analytical method. The reference standard of the API is often used for comparison since it has been thoroughly characterised and an assay value has been assigned to it. For preparation of impurity solutions of known concentration, a sample of the impurity is needed. Confirmation of the impurity identity is necessary, along with knowledge of the overall purity of the sample with which to assign an assay value. The RRF of the impurity is often calculated as the ratio of slopes of linearity plots for the impurity and API.

In pharmacopoeial monographs[2] it is often considered that RRFs between 0.8 and 1.2 are close enough to 1.0 that RRFs do not need to be applied, i.e., the RRF is taken as 1.0. This is not followed consistently, however. Moreover, for working with non-pharmacopoeial methods, this approach is only accepted by the authorities if the impurity is in fact overestimated, i.e. the true RRF is above 1.0, but calculation is done using an RRF of 1.0.

As discussed below, RRFs are subject to variability depending on the spectra of the compounds involved and experimental conditions of the analysis, such as instrument-specific factors. When methods are transferred into a laboratory or a pharmacopoeial method is implemented, it is advisable to verify the stated RRFs to ensure instrumental differences have not caused a significant discrepancy.



[2] USP general chapter 621, Ph.Eur. chapter 2.2.46.

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

4. Accuracy of RRF assignment

Correct impurity quantitation using an RRF is impacted by the accuracy of the RRF itself. A proper assessment of the assay value of an impurity sample is needed to assign the RRF accurately. As with API reference standards, the assay assignment for impurities by HPLC area normalisation alone is not adequate. Salt counterions, moisture, inorganic impurities, residual solvents, etc. need to be considered in assigning the assay. For example, isolation of an impurity by lyophilisation can leave high levels of water. Trifluoroacetate salts from preparative HPLC isolation or solvates from a synthesis crystallisation solvent may also be present. A white paper on standard characterisation describes approaches to assay assignment.[3]

An over-assessment of an impurity's assay leads to under-assessing the RRF, which causes the amount of an impurity quantified in samples to be over-reported. The potential impact could be that ICH thresholds are incorrectly exceeded, triggering unnecessary investigations or development efforts, as explained in more detail in the next paragraph.

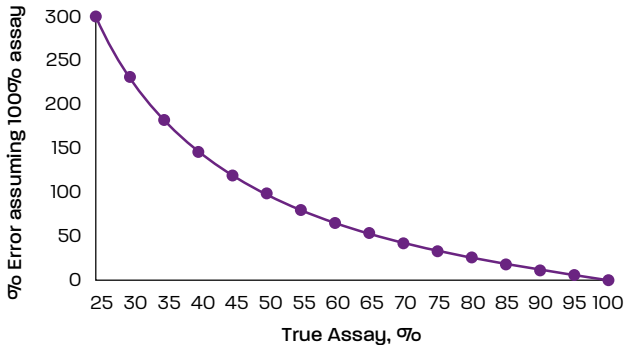
The percentage error in reporting an impurity level using an RRF increases as the assay value of an impurity standard decreases but was taken as 100% when calculating the RRF. If an assay value for an impurity standard is less than 100% but was assumed to be 100% instead, the RRF would be assigned a value lower than the "true" RRF. Then, when performing an impurity analysis, results would be divided by a lower RRF than the actual one, resulting in a higher calculated result for the impurity.

For example, if an RRF was assigned assuming an impurity standard had 100% assay that actually had only 70% assay, an overestimation error of 43% in impurity results would be obtained. In this case, if the impurity level was at 0.12%, it would be reported as 0.17% because of the incorrect RRF. The incorrect result exceeds the ICH qualification threshold of 0.15% in this example, where the actual result determined with an accurate RRF (or an accurately assigned external impurity standard) would be below the threshold. The impact of over-assessing the assay of an impurity sample when assigning RRFs is shown in Figure 1.

In such a case, an overestimation would result in hidden costs connected to qualification studies then required by ICH guidelines Q3A/Q3B. By the same mechanism one can see that overestimation can also lead to unnecessary out of specification (OOS) results. Following up these OOS results is considered an expensive undertaking. Investigation of OOS results by laboratory and quality staff can often take many hours. One cost estimate is $3000 for a straightforward investigation with a readily assignable cause[4]. Clearly, much greater costs may be incurred if manufacturing or product release are delayed.

The need for an accurate characterisation of the assay of an impurity sample to assign RRFs suggests that an impurity standard could be established and used instead of establishing and applying an RRF. The amount of impurity available compared to the amount needed for routine use may then become the deciding factor for use of the standard.

Figure 1.
Error in impurity results with inaccurately defined RRF due to over-assessment of impurity sample assay



[3] Look out for our next Mikromol white paper "Characterisation of non-compendial impurity reference standards: How good is good enough?"; https://www.lgcstandards.com/GB/en//Resources/Mikromol-White-Papers

[4] How painful are OOS investigations?, http://complectors.com/?p=64, accessed March 9, 2018.

Oxidation of Ufiprazole with Hydrogen Peroxide during the synthesis of Omeprazole. (Impurity 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl) methyl]sulphonyl]-1H-benzimidazole (Omeprazole Sulphone), MM0095.05).

5. Assay assessment for non-compendial impurity standards[5]

Before the assay of an impurity sample is assessed, data should be obtained to confirm the structure of the material. This is usually accomplished with spectroscopic techniques such as MS, NMR, and IR. Other data such as elemental composition may also support the identification of the sample.

An assay value is often calculated by mass balance where impurities are determined and subtracted from 100%. The key consideration for an accurate mass balance assignment is that all significant impurities have been considered. Other components in the sample, such as counterions or solvated solvents, including water, must also be accounted for.

Quantitative ^{1}H-NMR (qNMR) is useful in many cases for the determination of sample assay[6]. The area of a resonance peak or group of peaks specific to the compound can be compared to the area of a peak from an internal standard of a known compound. Knowing the number of protons that give rise to the respective peaks then allows calculation of the assay of the impurity sample.

qNMR can be especially helpful when an impurity cannot be obtained through external purchase or in-house synthesis. When an impurity needs to be extracted from the API or finished dosage forms at sufficient levels by prep-HPLC for identification and quantification, qNMR can be of enormous help to assign purity or assay values, given the small amount of material available.

The accuracy needed for the impurity assay assignment should consider the intended use of the impurity standard. For example, if a sample is used as a qualitative peak marker, it may be sufficient to confirm identity and establish that no other components that could produce significant peaks are present. The impurity identification and assay characterisation processes are described in more detail in a companion white paper.[7]

[5] Compendial impurity standards are out of scope here as they are intended for use only in combination with the pharmacopoeial methods. Their use for other purposes is not covered by the pharmacopoeias (e.g. USP general chapter 11, Ph.Eur. general text 5.12.).

[6] a) J Malmstrøm, L Hansen, A Ryager, H Olsen; J Pharm Sci, 94 (2005) 2549-2567. https://www.ncbi.nlm.nih.gov/pubmed/16200561
b) S Liu, C Hu; Anal Chim Acta, 602 (2007) 114–121. https://www.ncbi.nlm.nih.gov/pubmed/17936115

[7] Look out for Mikromol white paper “Characterisation of non-compendial impurity reference standards: How good is good enough?”. Impurity reference standards are produced ideally under a dedicated quality system such as ISO 17034:2016, which distinguishes them from research chemicals. The risk of using research chemicals interchangeably with reference standards is illustrated by a recent Broad Institute drug repurposing project, which found that 29% of the examined 8,500+ compound samples failed quality control, defined as a purity of less than 85%, even on products that were announced with much higher values (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5568558/).

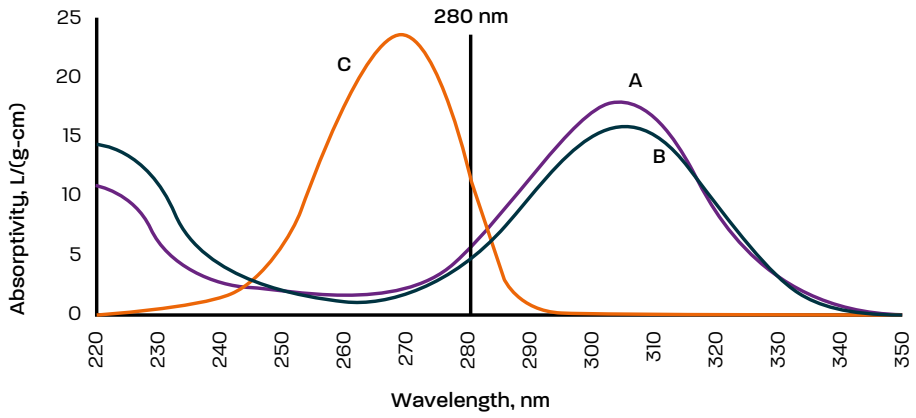
6. Other RRF considerations

Ruggedness

The consistency of RRFs from day to day, on different instruments, and in different laboratories is important for long-term use of a method. One major factor that affects RRF consistency is the UV spectra of the impurity and the API[8]. If small changes in wavelength cause relatively large changes in impurity and API responses, the RRF value is subject to high variability. This is especially problematic when the spectra of the impurity and API are sloping in opposite directions over a small range around the nominal detection wavelength. This is illustrated in Figure 2, where spectra for the API (A) and impurities B and C are shown. The detection wavelength was 280 nm to provide detection of both impurities. Examination of the spectra shows that a small wavelength decrease from 280 nm will give a higher response for impurity C and a lower response for the API. The opposite situation occurs for a small increase in wavelength.

The RRF for impurity C changes by about 30% with a ±2 nm change in wavelength from 280 nm. Small wavelength changes produce an insignificant change in RRF for impurity B since its spectrum changes in an almost identical way to that of the API. Unfortunately, RRF ruggedness with respect to small wavelength changes is not always evaluated in method development and validation. Other detector factors such as type of detector (variable-wavelength or photodiode array) and bandwidth or other settings may influence RRF ruggedness, but little information is published on these factors.

Figure 2.
UV spectra of drug substance A and impurities B and C. Detection wavelength of method is 280 nm



Range of RRF application

Is there a limit to the range for application of RRFs? For example, should RRFs be applied outside a range of about 0.1-10? One guideline suggests that the minimum RRF be limited to 0.2[9], but the choice is left to method development scientists. The purpose of the method will be a factor for this decision, but very low RRFs can introduce large errors or variability in quantitative results for impurities, since integration and chromatographic variability become magnified. Also, when trace analysis is being conducted for compounds such as mutagenic impurities, an external standard is recommended since acceptable thresholds are given by mass-based exposure to the compound.[10]

Method comparability

It is difficult to compare results between different methods if inaccurate or non-robust RRFs are used in one or both methods. This causes difficulties with comparability, especially if impurity specification limits are based on results with inaccurate RRFs and a method change is desired where an accurate result is obtained.

[8] BA Olsen, MD Argentine; J. Chromatogr. A, 762 (1997) 227-233. https://www.ncbi.nlm.nih.gov/pubmed/9098981
[9] EDQM, Technical Guide for the Elaboration of Monographs, 7th Ed., 2015
[10] ICH M7 Guideline on Mutagenic Impurities.

7. External standard considerations

As mentioned previously, availability, characterisation, and ease of preparation are factors to consider in the use of impurity reference standards. Standard solutions in single or multi-use vials that can be stored under conditions providing adequate impurity stability can reduce the quantities of impurity needed. Preparation of standard solutions for injection might also be more efficient with solutions that can be stored for extended time periods.

An external standard also serves as positive impurity peak identification rather than relying on relative retention times, which are subject to variability. Positive peak identification is especially helpful if several possible impurities are close in retention and their presence fluctuates from sample to sample.

Conclusions

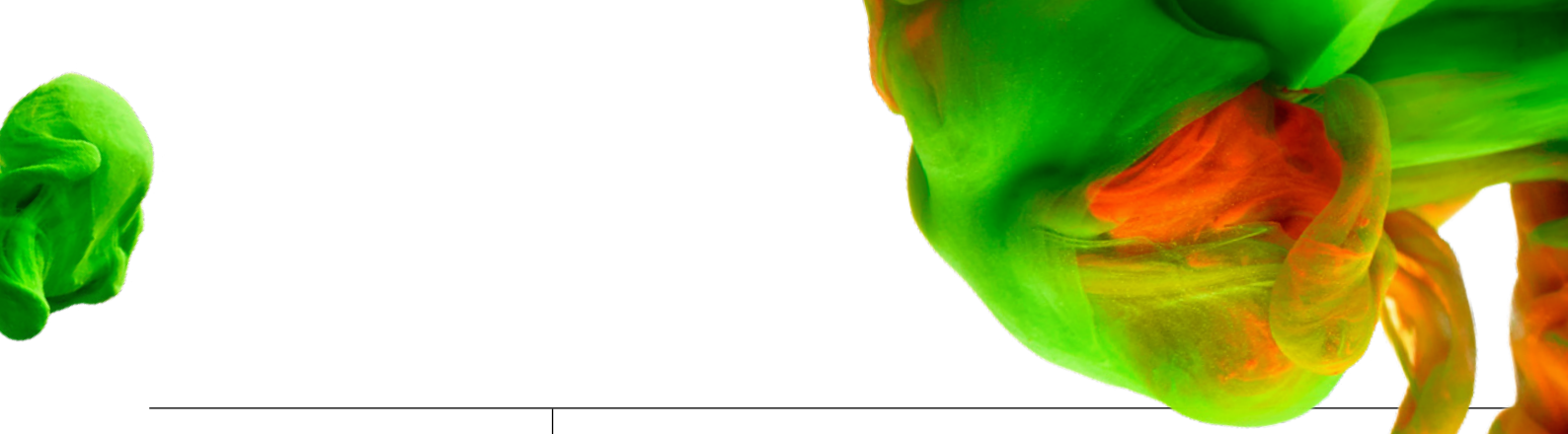
Quantitation of impurities using external standards provides accurate weight percentage results that prevent incorrect conclusions when comparing impurity levels to ICH impurity thresholds. External standards of impurities also provide accurate results that can provide the basis for comparability across the life cycle of the analytical method(s) for the impurity, i.e. to verify product is within specifications during routine quality control. Ideally, such reference standards are produced under a dedicated quality system such as ISO 17034:2016 (General requirements for the competence of reference material producers), representing the highest quality level possible. Quantitation versus the API response modified with relative response factors can also be used successfully under appropriate circumstances if the RRFs are assigned accurately. Reliable assignment of RRFs requires an impurity sample whose assay is accurately determined. Potential ruggedness issues with RRFs should be considered when incorporating RRFs in methods for routine long-term use.

About the authors

Bernard A. Olsen, Ph.D., received his undergraduate degree from Nebraska Wesleyan University in chemistry and his doctorate in analytical chemistry from the University of Wisconsin-Madison. He is an independent consultant providing expertise in chemistry, manufacturing, and control to the pharmaceutical industry. He has over 37 years of experience in drug development including 29 years at Eli Lilly, where he was a Senior Research Fellow and contributed to the development and support of over 25 commercial drugs and numerous developmental drugs. He has published and given invited lectures on a wide array of drug development and analytical topics including high performance liquid chromatography, method development and validation, impurity determination and control, genotoxic impurities, physical property characterisation, drug counterfeiting, regulatory aspects of drug development, and quality control. He is also a Fellow of the American Association of Pharmaceutical Scientists.

Dr. Christian Zeine studied chemistry at the Westphalian Wilhelms-University in Münster, Germany, where he received his doctorate in 1998 in organosilicon chemistry. After project work at the University, from 1999 on there followed activities at manufacturers of medical and in-vitro diagnostics products, including B. Braun Melsungen. Since 2002 he has worked at LGC Standards, serving as Global Senior Product Manager for pharmaceutical reference standards.

Christian is also the author of articles introducing the general topics of impurity testing and reference standards, and lectures on these subjects during seminars and symposia.



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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LGC is an international leader in the extended life sciences sector, providing a comprehensive range of reference materials, proficiency testing schemes, genomics reagents and instrumentation, as well as research and measurement services. We have cutting-edge expertise in measurement science, serving as the UK National Measurement Laboratory and Designated Institute for chemical and bio measurement. Operating out of 19 countries worldwide, our reference material manufacturing capability includes five facilities accredited to ISO 17034 or its predecessor ISO Guide 34, ensuring our products remain best in class.

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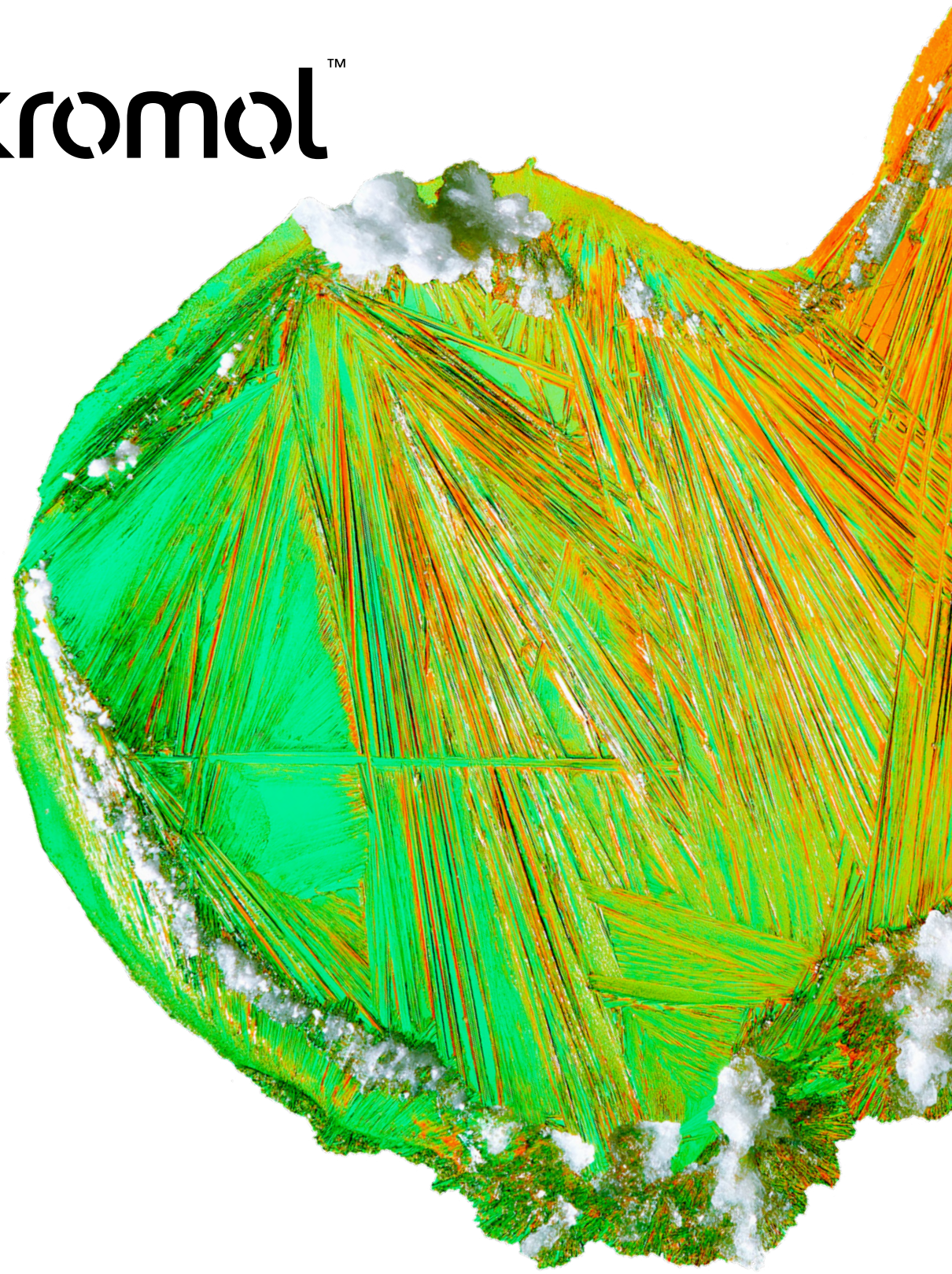

Crystallization of Metformin Hydrochloride after removal of Methanol from the reaction mixture. (Impurity Cyanoguanidine, MM0056.01).

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