Cooperation Centre for Scientific Research Relative to Tobacco

CORESTA Guide N° 20

Biomarker Studies – Requirements for the Certification of Analytical Reference Standards

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Biomarkers Sub-Group

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Biomarker Studies - Requirements for the Certification of Analytical Reference Standards

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1. INTRODUCTION

The aim of this guideline is to summarize the testing requirements for the certification of analytical reference standards used by the CORESTA Biomarkers Sub-Group. This is viewed as essential to reinforce the validity of the analytical and bioanalytical results generated by the industry and independent testing laboratories.

2. FIELD OF APPLICATION

This guideline presents the minimum requirements for a certificate of analysis for chemical reference standards used for identification or content determination in support of analytical and bioanalytical investigations. It aims to offer a benchmark regarding the essential tests required to have well characterized standards and a strong foundation for chemical analysis.

This guideline is applicable for current and future work undertaken by the CORESTA Biomarkers Sub-Group.

3. ABBREVIATIONS AND TERMINOLOGY REFERENCES

Analyte:

Compound that is quantified in the analytical method.

CoA: Certificate of Analysis:

Document that reports the tests done and results obtained on a compound to be used in an analytical/bioanalytical method.

HPLC:

High pressure liquid chromatography. In this context, it is a method used to assess the purity of the reference standards (technically a non-destructive method but recovery of the injected product will be impractical).

IS: Internal Standard:

Reference compound used in the analytical method for normalization and to improve robustness. It is not generally quantified in the analytical method, however the ratio of the analyte to the internal standard is essential for quantification.

Isobaric:

Compounds that possess the same molecular mass.

LC-MS/MS:

Liquid chromatography coupled with tandem mass spectrometric detection.

NMR: Nuclear Magnetic Resonance:

Technique used to prove the identity of a compound. It is generally, 1H-NMR or 13C-NMR, but other nuclear observations can be carried out. It should be noted that this is a non-destructive method.

MS: Mass Spectrometry:

Technique used to prove the identity of a compound and a detector used in analytical methods. It provides molecular mass information for the compound. It is a destructive method.

Potency:

The value by which the amount of target compound present in a reference standard is measured. (It should take into account water content, residual solvents, counter ion, purity, residual metals if applicable, etc.)

Purity:

Value by which the integrity of a compound is measured. Different techniques can be used to assess the purity of a product. The most common and accepted method is HPLC-UV profile but others like GC-FID, GC-MS, quantitative TLC, HPLC-ELSD, etc. may also be used.

QA: Quality Assurance:

Reviews of the laboratory processes and data generated by independent workers. Relevant for work performed for, and evaluated by, regulatory bodies, or as a best practice within quality conscious companies.

4. PURPOSE

The objective of this guideline is to describe the required content of a CoA for a reference standard used by analytical and bioanalytical laboratories for biomarker analysis in preclinical and clinical studies (MHRA, ANVISA, Swiss Medic, EMEA, MHLW, NGCMA, etc.). The guideline is intended for use in bioanalytical campaigns in compliance with GLP, GCP and GCLP. The primary goal is to safeguard the foundations on which the analytical data is generated and to ensure that the integrity of the analytical data will not be compromised by standards that are not adequately characterized. This guideline describes the essential testing requirements for Analytes and Internal Standards.

Other considerations are:

- Physicochemical data about the compound (e.g. name, molecular formula, molecular weight, lot number, appearance)
- Storage conditions and expiry date
- Raw data, authentication and QA for regulated work

5. TESTS REQUIREMENTS FOR ANALYTES

All analytes should be fully characterized by the technology available in order to assess its potency. Generally, two different tests should be used to prove the identity of the analyte (e.g. NMR and Mass Spectrometry). Also, purity of the product should be determined (e.g. HPLC-UV). Potency of the analyte must then be calculated, taking into account counter ions, water content, residual solvents (basically, all contaminants that are not detectable by the analytical method used for assessment of purity). It is important to understand that an analyte could be 98 % pure by HPLC-UV and only have a potency of 41.5 %. For example, the CoA of an analyte like “nicotine tartrate salt” could contain the following test results:

- ^{1}H-NMR: Conforms to structure
- Mass spectrum: Conforms to structure (Protonated molecular mass of 163 m/z)
- HPLC-UV Purity: 98 %
- Water content: (by Karl-Fisher method) 8.2 %
- Residual solvents: Dichloromethane 0.2 % and Dioxane 1.2 %
- Counter ion content: Tartrate 48.1 %
- Potency: 41.5 % (i.e. 1 mg of reference standard is equivalent to 415 µg of Nicotine)

A wide variety of tests and techniques can be used to assess the different values shown above.

It is beyond the scope of this guideline to review all of them, but it must be understood that they all have advantages and limitations that could impact the characterization of the reference standard.

6. TESTING REQUIREMENTS FOR INTERNAL STANDARDS

An internal standard (IS) is often used in an analytical method. These ISs are not quantified in the method and do not require the same level of characterization. Potency is not required to be determined. However, the IS must still be tested. Generally, two different tests should be used to prove the identity of the analyte (e.g. NMR and Mass Spectrometry). Also, purity of the product should be determined (e.g. HPLC-UV). More than likely, an IS used in LC-MS/MS methods will be labeled with stable isotopes (^{2}H, ^{13}C, ^{15}N) and will be required to be free from significant levels of unlabeled analyte or isobaric contaminants. This isotopic contamination can be easily surveyed by mass spectrometry testing. For example, the CoA of an IS such as "Nicotine-d_3" could contain the following test results:

- ^{1}H-NMR: Conforms to structure
- Mass spectrum: Conforms to structure (protonated molecular mass of 166 m/z with no significant level of 163 m/z present, the mass of unlabeled nicotine). If a ratio of unlabeled /labeled product can be measured, it should be reported as a percentage.
- HPLC-UV Purity: 95 %

In the case of an IS labeled with stable isotopes, mass spectrometry will be an essential test that can ensure the validity of the IS for its intended use.

It should be noted that this level of testing could be suitable for any molecules not quantified in the method, such as a qualitative marker for example.

7. GENERAL INFORMATION ON THE RE-CERTIFICATION OF STANDARDS, WHEN THE CERTIFICATE OF ANALYSIS HAS EXPIRED

When the proven period of stability for a reference standard approaches its end, the end user may pursue two options. The first is the purchase of fresh material that is within the certified expiry period. The second is to have the current material recertified. Recertification can be performed by the supplier/manufacturer or in-house by the end user.

Once a certificate of analysis has reached its retest/expiry date, an evaluation of the testing that has been performed should be completed. If stability data demonstrating a limit in the stability of the reference standard under specific storage conditions already exist, then the only acceptable approach is the purchase of fresh reference standard material. If, however, a limit in the stability has not been determined, it would be appropriate to perform a recertification if there is sufficient material available to do so. It is important to note that reference standards may decompose over time by various reactions such as oxidation, radical formation, hydrolysis, etc. If the recertification results confirm that a significant change in the reference standard material has occurred then a determination of the material's fitness for use should be evaluated.

The following cases where the expiry/retest date has been reached will be reviewed in more detail:

- - Reference standard retest/expiry date
- - Formulations of reference standards and retest/expiry date
- - Re-assessment of the storage conditions following retest/expiry date

8. REFERENCE STANDARD RETEST/EXPIRY DATE

To prove the integrity of the standard after a long-term storage, it should be re-analyzed for purity and an updated certificate of analysis should be issued. For analytes, it is strongly recommended that the residual solvents and water content are re-analysed. These volatile impurities can change over the course of storage and have an impact on the potency of the analyte. Alternatively, the standard can be re-analysed (quantified) against a new batch/lot of the respective compound with proven stability.

9. FORMULATIONS OF REFERENCE STANDARDS AND RETEST / EXPIRY DATE

For ease of use, reference standards can be sourced or prepared in formulations of known concentration.

The method of preparation by the supplier should be traceable and should be done using certified reference standard materials and/or be certified after formulation. As a minimum, a purity analysis should be made on the formulation to show the integrity of the standard (considering that one of the most common ways used to store such formulations is by using a glass vial that is sealed with an open flame).

Formulations in break-sealed ampoules will have the advantage of not being exposed to the ambient atmosphere and volatile content will not change over long-term storage. Once they have reached the retest/expiry date, only a purity check would then be required to re-certify the standards.

For stable labeled internal standards, an evaluation for the consistency of the isotopic labeling could be viewed as sufficient to establish the fit-for-purpose use of the material. Stability for the stable labeled internal standard may be implied from the unlabeled reference material, if they are stored at a similar concentration and under identical conditions (storage container, temperature, exposure to light).

10. RE-ASSESSMENT OF THE STORAGE CONDITIONS FOLLOWING RETEST / EXPIRY DATE

If significant degradation of the reference standard is observed after re-certification, it is recommended to modify the storage conditions (usually to colder temperature) and to shorten the time before the next retest date to evaluate the impact of change in storage *vs* degradation. Assuming the decomposition mechanisms are known, an additive could also be used as a stabilizer. Obviously, careful consideration must be made to ensure that the additive does not interfere with the method of analysis in which the reference standards will be used, and that the potency value is adjusted accordingly.

11. ADDITIONAL INFORMATION

The certificate of analysis should be complemented by the following information:

Physicochemical data:

- Compound name
- Molecular formula
- Molecular weight (MW)
- Lot number
- Appearance

Handling data:

- Storage conditions [temperature and special precautions (e.g. Protect from light, hygroscopic, etc.)]
- Test date
- Expiry date

Regulatory data:

- Supporting analytical data
- Raw data availability
- Standardized format
- Authentication of data
- Quality Assurance review

12. RECOMMENDATIONS

It is highly recommended that the standards used in the analytical methods have complete and adequate certificates of analysis.

13. CONTRIBUTORS

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