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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1. INTRODUCTION

This guideline summarises the testing requirements for the certification of analytical reference standards used in 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

The guideline presents the minimum requirements for a Certificate of Analysis for chemical reference standards used for identification or content determination to support analytical and bioanalytical work. It aims at offering a benchmark about the essential tests required to have well characterised standards and a strong foundation for the 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 quantitated in the analytical method.

CoA: Certificate of Analysis:

Document that reports the tests done and results obtained on a product 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 considered unpractical.)

IS: Internal Standard:

Reference compound used in the analytical method for normalisation and to improve robustness. It is not generally quantitated in the analytical method, however the ratio to the analyte is essential for quantification.

Isobaric:

Compounds that possess the same molecular mass.

LC-MS/MS:

Liquid chromatography coupled with tandem mass spectrometry detection.

NMR: Nuclear Magnetic Resonance:

Technique used to prove the identity of a compound. It is generally, 1H-NMR or 13C-NMR, but other nuclei observation can be done. It must be noted that it 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:

Value by which the amount of product present in a reference standard is measured. (It must 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 data generated by independent workers. Relevant in work done and evaluated by regulatory bodies, or just quality conscious companies.

4. PURPOSE

The objective of this guideline is to describe the content of a CoA for a reference standard used by analytical and bioanalytical laboratories for GLP or GLP-like analysis (MHRA, ANVISA, Swiss Medic, EMEA, MHLW, NGCMA, etc.). The guideline is not intended for use in GCP or GMP activities. The primary goal is to safeguard the foundations on which the analytical data is generated and will not be compromised by standards that are not characterised properly. This guideline will describe 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 condition, expiry date
- Raw data, authentication and QA for regulated work

5. TESTS REQUIREMENTS FOR ANALYTES

All analytes should be fully characterised 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 % (So, 1 mg of reference standard equals 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 characterisation of the reference standard.

6. TESTS REQUIREMENTS FOR INTERNAL STANDARDS

An IS is often used in the analytical method. These ISs are not quantitated in the method and do not require the same level of characterisation. 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 (D, ^{13}C, ^{15}N) and will be required to be free of significant level of unlabeled analyte or isobaric contaminants. This isotopic contamination can be easily surveyed by mass spectrometry testing. For example, the CoA of an IS like "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 in %.
- 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 is to be noted that this level of testing could be suitable for any molecules not quantitated in the method, like a qualitative marker for example.

7. ADDITIONAL INFORMATION

The Certificate of Analysis should be completed by adding 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

8. RECOMMENDATIONS

It is highly recommended that the standards used in the analytical methods have a complete and adequate Certificate of Analysis.

9. CONTRIBUTORS

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