European Pharmacopoeia Reference Standards

European Pharmacopoeia Training for Homoeopathic Products

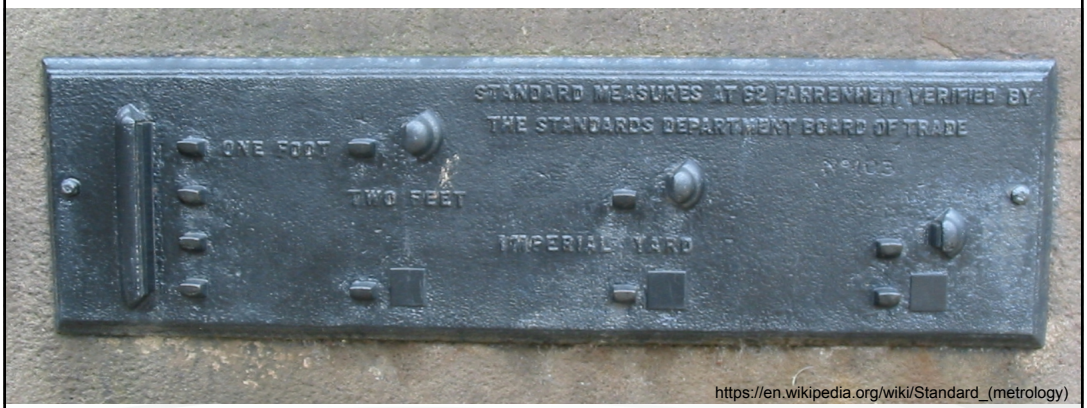
6th December 2016

Strasbourg, France

Dr Matthias Weber

EDQM, Council of Europe

Motivation



https://en.wikipedia.org/wiki/Standard_(metrology)

Reference Standards

Avogadro constant:
1 mol =
$6.022140857(74)\times10^{23}$ mol^{-1}

https://en.wikipedia.org/wiki/Avogadro_constant

European Pharmacopoeia Reference Standards

- References and Definitions
- Establishment
- Reference standards used in herbal monographs
- Labeling and use of Ph. Eur. RS
- Secondary Standards

Ph. Eur.
Reference Standards

References and Definitions

Ph. Eur. General Notices

The European Pharmacopoeia Commission establishes the official reference standards, which are alone authoritative in case of arbitration.

These reference standards (RS) are available from EDQM.

Ph. Eur. Chapter 5.12. Reference Standards

Content:

- Terminology
- Use of Ph. Eur. Reference Standards
- Establishment of Reference Standards
- Manufacturing, Labelling, Storage and Distribution
- Re-Test Programme for Ph. Eur. Reference Standards

Ph. Eur. Chapter 5.12. Reference Standards

- Reference standard" (RS) is a general term covering reference substances, preparations and spectra.
- European Pharmacopoeia reference standard
  A reference standard established under the aegis of and adopted by the European Pharmacopoeia Commission.

Ph. Eur. Chapter 5.12. Reference Standards

European Pharmacopoeia **chemical reference substance (CRS)**

A substance or mixture of substances intended for use as stated in a monograph or general chapter of the European Pharmacopoeia. CRSs 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.

European Pharmacopoeia **herbal reference standard (HRS)**

A herbal drug preparation (usually an extract) or a herbal drug intended for use as stated in a monograph or general chapter of the European Pharmacopoeia. Unless otherwise specified, HRS are designated as primary reference standards for their intended use.

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EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL

Health systems and products
Medicinal products – quality, safety and efficacy

Brussels, 28 March 2014

EudraLex

The Rules Governing Medicinal Products in the European Union

Volume 4
EU Guidelines for
Good Manufacturing Practice for
Medicinal Products for Human and Veterinary Use

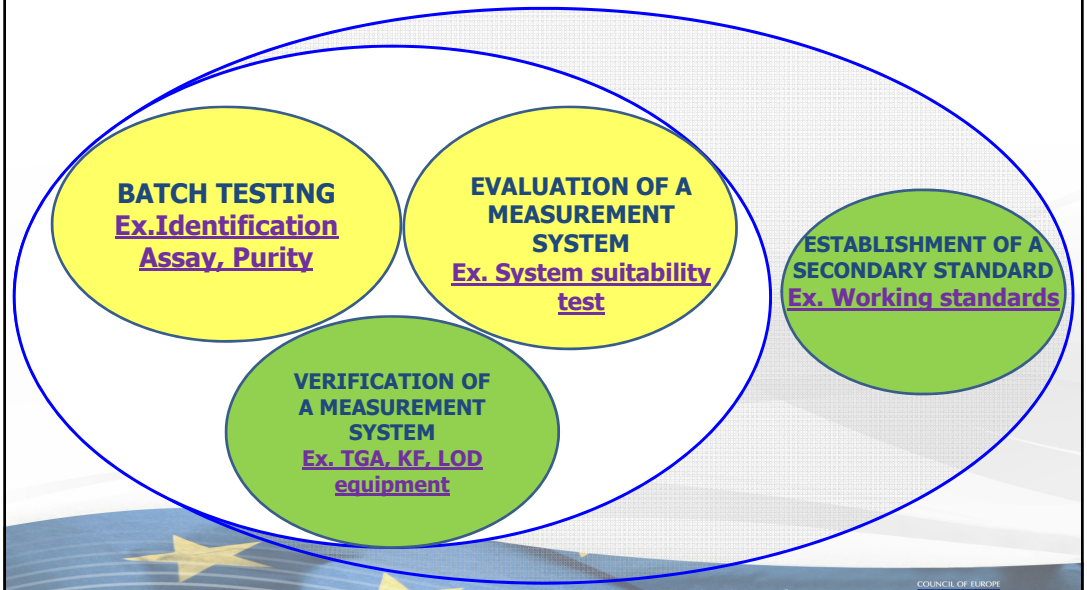
Part 1
Chapter 6: Quality Control

6.20
Reference standards should be established as suitable for their intended use. Their qualification and certification as such should be clearly stated and documented.

Whenever compendial reference standards from an official source exist, these should preferably be used as primary reference standards unless fully justified (the use of secondary standards is permitted once their traceability to primary standards has been demonstrated and is documented).

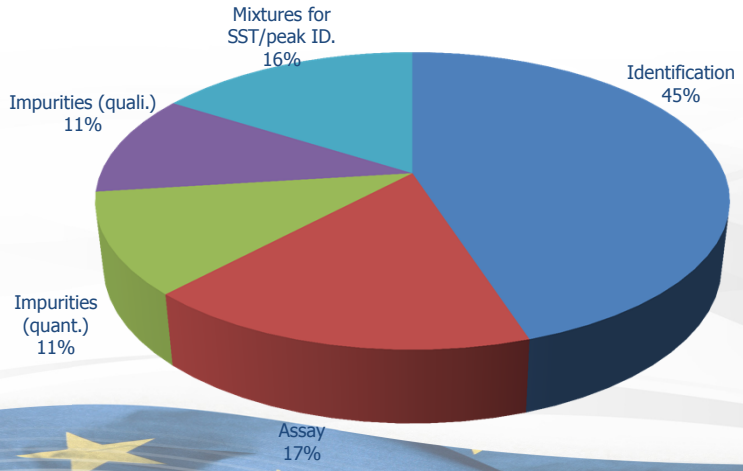
These compendial materials should be used for the purpose described in the appropriate monograph unless otherwise authorised by the National Competent Authority.

Use of Ph. Eur. Reference Standard



Types of Ph. Eur. Reference Standards

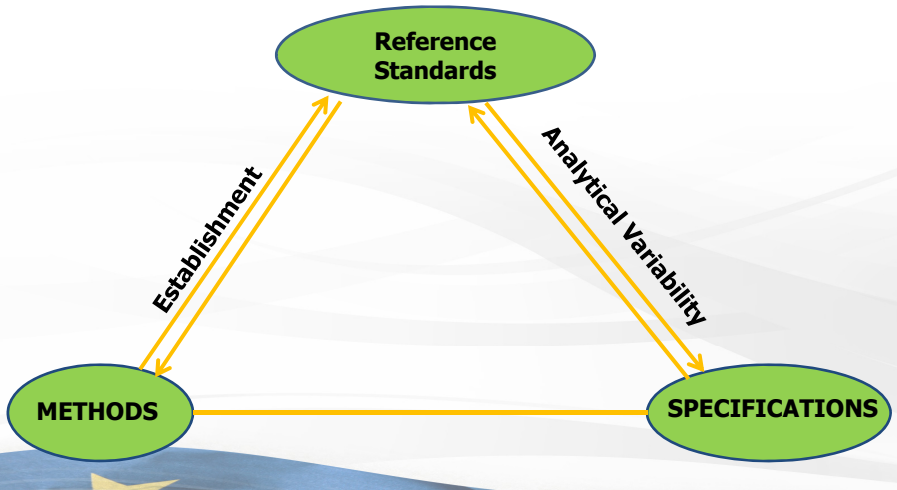
Total number of Ph.Eur. RS 15/06/16: 2713



Ph.Eur. Reference Standards

Establishment

European Pharmacopoeia Monographs and Reference Standards



Step 1: Characterisation of bulk material

- Verification of identity, structure and compliance with the monograph (when applicable)
- Determination of purity
- Confirmation of identity and purity by alternative methods (NMR, qNMR, MS, elemental analysis, titration, DSC, etc.)

(Ph. Eur. general text 5.12 Chapter 4. ESTABLISHMENT OF REFERENCE STANDARDS)

Step 2: Characterisation after subdivision in vials

- Determination of homogeneity
- Content assigned by mass-balance (if required)
- Assigned value checked by qNMR (when applicable)

- ✓ Technical approval by Group of Experts
- ✓ Adoption by Ph. Eur. Commission

(Ph. Eur. general text 5.12 Chapter 4. ESTABLISHMENT OF REFERENCE STANDARDS)

Assigned content

For a European Pharmacopoeia chemical reference substance established for assay purposes, the assigned content is usually calculated from the values obtained from the analyses performed for the determination of impurities (organic, inorganic, water and solvents) by applying the principle of mass balance; other suitable methods may also be used. When possible, the assigned content is confirmed by comparing with the result obtained by an independent method.

$$X(\%) = (100\% - water - residual\ solvents - inorganics) * \frac{(100\% - total\ related\ substances)}{100\%}$$

(Ph. Eur. generaltext 5.12 Chapter 4. ESTABLISHMENT OF REFERENCE STANDARDS)

Ph.Eur.
Reference Standards

Herbal Monographs

Expert Groups

Monographs of HOM (Homoeopathic raw materials and stocks of herbal material) working party
[about 16 herbal monographs]

Monographs of expert group 13A (Phytochemistry A)
[about 123 monographs]

Monographs of expert group 13B (Phytochemistry B)
[about 136 monographs]

Monographs of TCM (Traditional Chinese Medicines) working party
[about 44 monographs]

(updated 01/2016; Ph. Eur. 8.8)

Chemical Reference Substance (CRS) in herbal monographs

Qualitative CRS

Chemical reference substance used for peak identification or system suitability in the (HP)TLC test, LC test or LC assay
(only if the substance is not available)

Quantitative CRS

Chemical reference substance used as external standard for an LC test or an LC assay with an assigned content

Herbal Reference Standards (HRS)

Qualitative HRS

Herbal reference standard used for identification or adulteration in the (HP)TLC test or for peak identification or system suitability in the LC test or LC assay

Quantitative HRS

Herbal reference standard used as external standard for an LC test or for the LC assay with an assigned content of one or more components

Homoeopathic Example 1

Ph.Eur. monograph 2486 for *Anamirta cocculus ad praeparationes homoeopathicas*

Content: minimum 0.80 per cent of picrotoxinin ($C_{15}H_{16}O_6$; M_r 292.3) (dried drug).

ASSAY

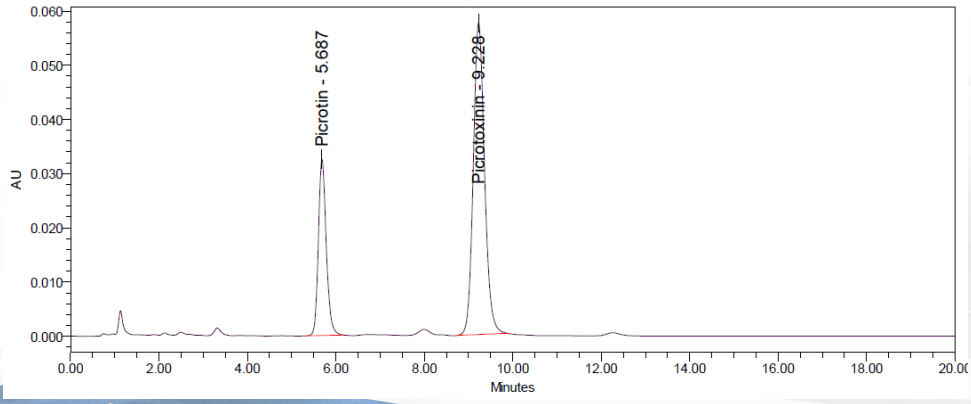
Liquid chromatography (2.2.29).

Reference solution. Dissolve 5.0 mg of picrotin CRS and 5.0 mg of picrotoxinin CRS in 10.0 mL of acetonitrile R. Dilute 2.0 mL of the solution to 20.0 mL with the mobile phase.

Picrotoxinin CRS is used for system suitability and as external standard in the LC assay for the content of picrotoxinin

"as is" content of picrotoxinin CRS 1: **98.3% $C_{15}H_{16}O_6$**

Chromatogram ref. solution



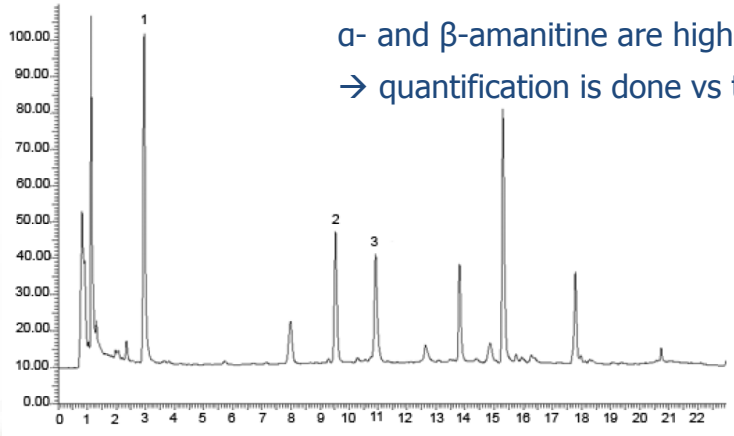
Homoeopathic Example 2

Ph.Eur. monograph 2290 for *Amanita phalloides ad praeparationes homoeopathicas*

Content: 0.001 per cent *m/m* to 0.010 per cent *m/m* for the sum of α-amanitine and β-amanitine ($C_{39}H_{54}N_{10}O_{14}S$; M_r 919).

Reference solution. Dissolve 10.0 mg of tryptophan CRS in mobile phase A and dilute to 20.0 mL with mobile phase A.

Tryptophan CRS used for as external standard in the LC test for α-amanitine and β-amanitine.



1. tryptophan 2. β-amanitine 3. α-amanitine

α- and β-amanitine are highly toxic and unstable

→ quantification is done vs tryptophan

Ph.Eur.
Reference Standards

Labelling and use

Labelling

Includes: name of the reference standard, name of the supplier, batch number and any other information necessary to the proper use of the reference standard.

Labelling

If used as an assay standard the following information is also given:

- the assigned percentage content;
- or, the content in mg or mL of the chemical entity in the container;
- or, the assigned potency (for biological assays or microbiological assays) in units either per mg or per vial.

An explanatory leaflet is considered as part of the labelling.

Reference Standard Leaflets

European Directorate for the Quality of Medicines & HealthCare
European Pharmacopoeia (Ph. Eur.)
7, Allée Kastner CS 30026, F-67081 Strasbourg (France)
Tel. +33 (0)3 88 41 20 35 Fax. + 33 (0)3 88 41 27 71
For any questions: www.edqm.eu (HelpDesk)

INFORMATION LEAFLET Ph. Eur. Reference Standard

Tryptophan CRS batch 2

1. **Identification**

Catalogue code: T2610000 Unit Quantity: ca 100 mg

2. **Scientific Information**

2.1 Intended use

Reference Standard for laboratory tests as prescribed in the European Pharmacopoeia only.
Established for use with the monograph(s): 1272, 2290.

2.2 Analytical information related to intended use, when applicable

The "as is" content is : **100.0% C11H12N2O2 (for 2290 only)**

2.3 Uncertainty of the assigned value, when applicable

According to ISO Guide 34 and ISO Guide 35, for this Pharmacopoeial standard the uncertainty of the assigned value is not stated since it is considered to be negligible in relation to the defined limits of the method-specific assays for which the reference standard is used. Please also refer to Ph. Eur. chapter 5.12.

Reference Standard Leaflets

2.4 Validity

A statement on the validity of the batch (Batch Validity Statement) can be printed directly from the EDQM website (Reference Standards Database).

2.5 Instructions for use

Allow the closed container to equilibrate at ambient temperature before breaching to avoid uptake of moisture.Use "as is". Do not dry/desiccate before use. Once the container has been breached, stability of the contents cannot be guaranteed. It is for immediate use.

3. **Storage conditions**

Store the original container at +5°C ± 3°C, protected from light. The container should not be opened until required for use.

4. **Safety**

Hazard Classification

For laboratory use only. Handle in accordance with good occupational hygiene, safety and laboratory practices and take precautions to avoid exposure.

For substances subject to GHS/CLP classification, the corresponding safety data sheet can be accessed via the EDQM website (Reference Standards Database) or is available upon request from the EDQM (Helpdesk-FAQ section).

5. **Shipping conditions**

Please check shipping conditions on the EDQM website (Reference Standards Database).

M. Weber 24/1

Cat. Code: T2610000 Date of issue: 19/10/2015 Rev.2 1/2

Reference Standard Leaflets

6. **Warranties, Liability and disputes**

a) Warranties

The Council of Europe does not offer any warranty concerning the quality or safety of any item supplied, the absence of any defects, or its fitness for any particular purpose except that of use as a Ph. Eur. CRS, BRP or RS for use as reference standards in tests and assays carried out in accordance with the official methods of the European Pharmacopoeia (Ph. Eur.) by professionals with the necessary technical skills. In particular, the Council of Europe (EDQM) does not guarantee that the items will meet the customer's specific expectations. The Council of Europe also does not guarantee that the purchase or use of the items will not infringe any intellectual property rights, in particular patents.

The Council of Europe (EDQM) only guarantees that the items meet the above conditions in the moment they are handed over to the carrier being responsible for the delivery of the item to the purchaser and that the carrier and the purchaser have received clear and accurate instructions for the item's delivery and reception.

No other warranty, either express or implied, is given by the Council of Europe (EDQM).

b) Liability

The Council of Europe (EDQM) shall not be liable for the failure to meet the requirements of the legislation of the country where the items are delivered. It is the purchaser's responsibility to check with the local, regional or national authorities and to make sure that the goods or services that they intend to order may be imported or used in that country. The purchaser is solely responsible for the choice of items, their storage from the time of delivery and their use.

In no event shall the Council of Europe (EDQM) be liable for any damages due to the use of items, including, but not limited to, lost profits, loss of use, costs of procurement of substitute goods, services or systems, or for any indirect, special, incidental, punitive, or consequential damages, however caused and, whether in contract, tort or under any other theory of liability, whether or not the Purchaser has been advised of the possibility of such damage.

c) Disputes

In accordance with the provisions of article 21 of the General Agreement on the Privileges and Immunities of the Council of Europe, all disputes between the Council of Europe (EDQM) and the customer as regards the application of this contract shall be submitted, if a mutual agreement cannot be reached between the parties, to arbitration as laid down in Order No. 481 of the Secretary General, approved by the Committee of Ministers.

7. **Citation**

Users shall ensure that any reference made to an EDQM Reference Standard in any publication, presentation or public document (ex. scientific articles, data sheets for kits) bears the exact name, and catalogue code of the Reference Standard and the exact name and address of EDQM as given on the first page of this information leaflet.

8. **Adoption**

The present reference standard has been officially adopted by the European Pharmacopoeia Commission.

9. **Signature**

This document is electronically signed by:

Dr Pierre Leveau
Head of the Quality, Safety and Environment Division

M. Weber 24/11/2016 ©2016 EDQ

Instructions for Use

Powder-filled CRS

Content (m/m) assigned on an « as is » basis.

To be weighed – No need to dry

Freeze-dried CRS

The value assigned corresponds to the amount per vial, for example 2.05 mg/vial.

To be reconstituted – Not weighed

Ph. Eur. Reference Standards

→ are not necessarily suitable for other purposes.

If a 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.

Any value assigned to a reference standard is valid for the intended use and not necessarily for other uses.
[Ph.Eur. Chapter 5.12.]

What is expected from the User

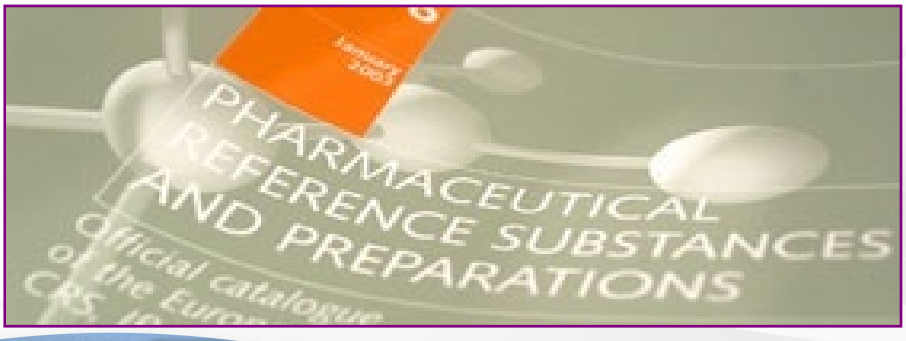
Immediately before using a Ph. Eur. Reference standard, the following shall be checked:

- that the reference standard batch number be current at the time of use. A real-time batch validity statement is available on line;
- that the container/closure system integrity be kept, i.e. absence of visible defects originating from shipping;
- that the reference standard after receipt has been stored at the conditions prescribed in the Ph. Eur. CRS catalogue;
- allow the RS to equilibrate to lab temperature. before opening

Reference Standards Catalogue

Available online. Updated daily. English only.

store.edqm.eu



Online Ph. Eur. RS Database

crs.edqm.eu

Search European Pharmacopoeia Reference Standards

The database provides specific information including:

Availability
Origin
Storage and dispatching conditions
Presentation
Assigned Content
Batch Validity Statement
Complementary information (for ex. chromatogram)
Safety Data Sheet

Batch Validity Statement (BVS)

Catalogue Code	Y0001528
Name	Picrotoxinin
Current batch number	1
Unit quantity	40 mg
Sale unit	1
Used in monograph(s)	2486
Assigned content	See leaflet
Additional information	
Leaflet	click to download the leaflet
SDS	Click to download SDS
CAS Registry Number	17617-45-7
Presentation	
Origin	click to download Origin Of Goods.pdf
Proposed Import HS code	293220
EDQM long term storage conditions	+5°C ± 3°C
Dispatching conditions	Ambient temp.
UN Code	2811
Shipping group	A1A
Price	79 EUR
Availability	Available
Sales restriction	No

Batches

batch 1 is valid at this date

Print BVS

Batch Validity Statement (BVS)

BATCH VALIDITY STATEMENT
EUROPEAN PHARMACOPOEIA REFERENCE STANDARDS (CRS) & (BRP)
This Batch Validity Statement has to be used in conjunction with Ph. Eur. general chapter 04/2015:51200 Reference Standards.

European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe
Postal address: 7 Allée Kastner CS 30026 F - 67081 STRASBOURG (France)
Phone: +33 (0)3 88 41 30 30
Fax: +33 (0)3 88 41 27 71
Internet : http://www.edqm.eu

Name	Picrotoxinin
Catalogue code	Y0001528
Batch number*	1
Assigned value	See leaflet
Validity	Batch 1 is valid at the printing date: 2016-11-24
Additional information	
Storage conditions	The standard is intended for immediate use. Recommended EDQM storage conditions for unopened containers : +5°C ± 3°C
Safety data	Safety Data Sheet is available from the detailed view or upon request.
Leaflet	Click on the hyperlink to download the leaflet containing the instructions for use, **if available** (Adobe Acrobat Reader version 5 or higher, or the corresponding browser plug-in is needed to open the file) click to download the leaflet

Ph.Eur.
Reference Standards

Secondary Standards

Ph. Eur. Chapter 5.12. Reference Standards

Secondary standard. *A standard whose property value is assigned by comparison with a primary standard of the same property or quantity.*

4-5. SECONDARY STANDARDS

A secondary standard should exhibit the **same property** or properties **as the primary standard**, relevant for the test(s) for which it is established. The extent of testing may not be as comprehensive as is required for the establishment of a primary standard. The secondary standard is established by comparison with the primary standard to which it is traceable. **An official primary standard is used wherever possible for establishment of secondary standards.**

Commercial standard

Certificate of Analysis

Secondary pharmaceutical reference standard

HWI ANALYTIK GMBH pharma solutions

Silybum marianum extract

Article no: 0513-50-01
Batch: HWI01368
Storage: airtight container, protected from light at < -15°C under inert gas
Expiry date[1]: 05/2017

Parameter	Method [2]	Requirement	Result
Characters			
Appearance	organoleptic	yellowish brown powder	complies
Identification	Ph. Eur. monograph "Milk Thistle Dry Extract"	Silicristin	complies
		Silidianin	complies
		Isosilibinin A	complies
		Isosilibinin B	complies
		Silybin A	complies
		Silybin B	complies
Assay	Ph. Eur. monograph "Milk Thistle Dry Extract"; calibration standard: primary reference standard Silybin batch HWI00090	Sum of Silybin A + B	257.96 mg/g

Suitability for Ph. Eur. compliance testing not demonstrated

« traceable « to the corresponding HWI primary pharmaceutical standard (whose content is determined by quantitative NMR)

The eight Ph. Eur. CRS take home messages

- Reference standards are analytical benchmarks that serve two major purposes: qualitative and quantitative
- Ph. Eur. reference standards are official, legally binding standards, constituting an essential part of Ph. Eur. monographs
- Ph. Eur. policy on reference standard is reflected in general chapter 5.12.
- Ph. Eur. ref. standards are described in the EU GMP Regulations
- Ph. Eur. ref. standards are established for the intended purpose
- Ref. standards for herbals/homeopathics bear some specificities
- Questions → helpdesk www.edqm.eu/register
- Information → website www.edqm.eu

Thank you very much for your attention.