THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

Ph. Eur. reference standards Establishment

2019 Training Session
"The European Pharmacopoeia"
Dr Jochen Pauwels
EDQM Laboratory Department

10 – 11 September 2019, Iselin, New Jersey, USA

OUTLINE

- Terms and definitions
- Establishment of reference standards: general principles
- Qualitative reference standards
- Quantitative reference standards

PH.EUR. REFERENCE STANDARDS

Terms and definitions

TERMS AND DEFINITIONS

5.12. REFERENCE STANDARDS

This chapter is published for information.

07/2018:51200
corrected 10.0

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

TERMS AND DEFINITIONS

ISO GUIDE 30 / Ph.Eur. 5.12.

Reference Material

Material, sufficiently **homogeneous** and stable with respect to one or more **specified properties***, which has been established to be **fit for its intended use** in a measurement process.

* quantitative or qualitative

TERMS AND DEFINITIONS

ISO GUIDE 30 / Ph.Eur. 5.12.

Certified Reference Material (CRM)

Reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a **certificate** that provides the value of the **specified property**, its associated **uncertainty**, and a statement of metrological **traceability**.

TERMS AND DEFINITIONS

ISO GUIDE 30 / Ph.Eur. 5.12.

Primary measurement standard

A standard designated or widely acknowledged as having the highest metrological qualities and whose **property value is accepted without reference** to other standards of the same property or quantity, within a specific context.

Secondary measurement standard

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

TERMS AND DEFINITIONS

Ph.Eur. 5.12.

Reference Standard (RS)

General term covering reference substances, preparations and spectra.

European Pharmacopoeia reference standard (Ph.Eur. RS)

A reference standard **established** under the aegis of and **adopted** by the European Pharmacopoeia Commission.

TERMS AND DEFINITIONS

Ph.Eur. 5.12.

European Pharmacopoeia chemical reference substance (CRS)

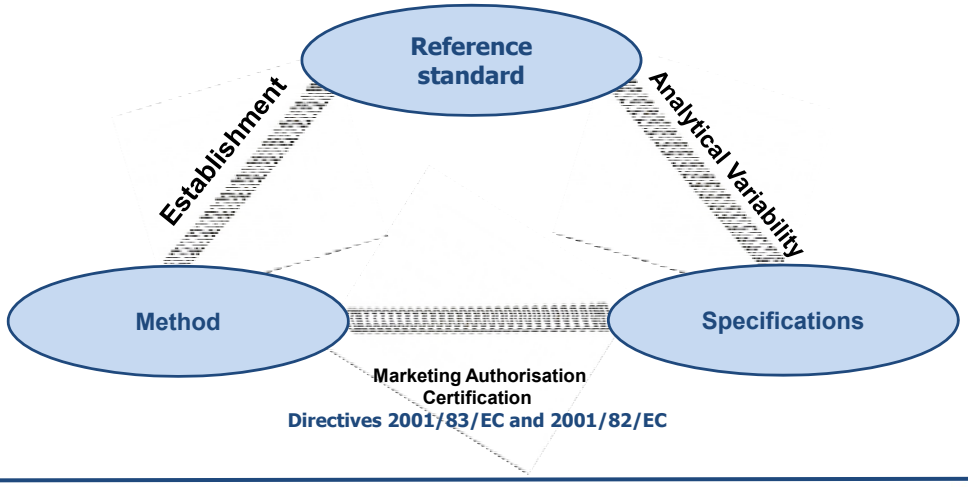
Substance or mixture of substances intended for **use as stated in** a monograph or general chapter of the **European Pharmacopoeia**.

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.

Note: HRS and BRP are other types of RS.

TERMS AND DEFINITIONS

EUROPEAN PHARMACOPOEIA
COMPENDIAL STANDARD = MONOGRAPH + REFERENCE STANDARD



TERMS AND DEFINITIONS

REFERENCE STANDARDS
LEGAL ASPECTS

Ph.Eur. General Notices

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

These reference standards are available from EDQM.

TERMS AND DEFINITIONS

REFERENCE STANDARDS
LEGAL ASPECTS

EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL

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

EU guideline for GMP * Part 1 – 6.20

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.

* Eudralex Volume 4, EU guidelines for good manufacturing practice for medicinal products for human and veterinary use

PH.EUR. REFERENCE STANDARDS

Establishment of reference standards: general principles

ESTABLISHMENT: GENERALS

5.12. REFERENCE STANDARDS

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

Intended purpose

ESTABLISHMENT: GENERALS

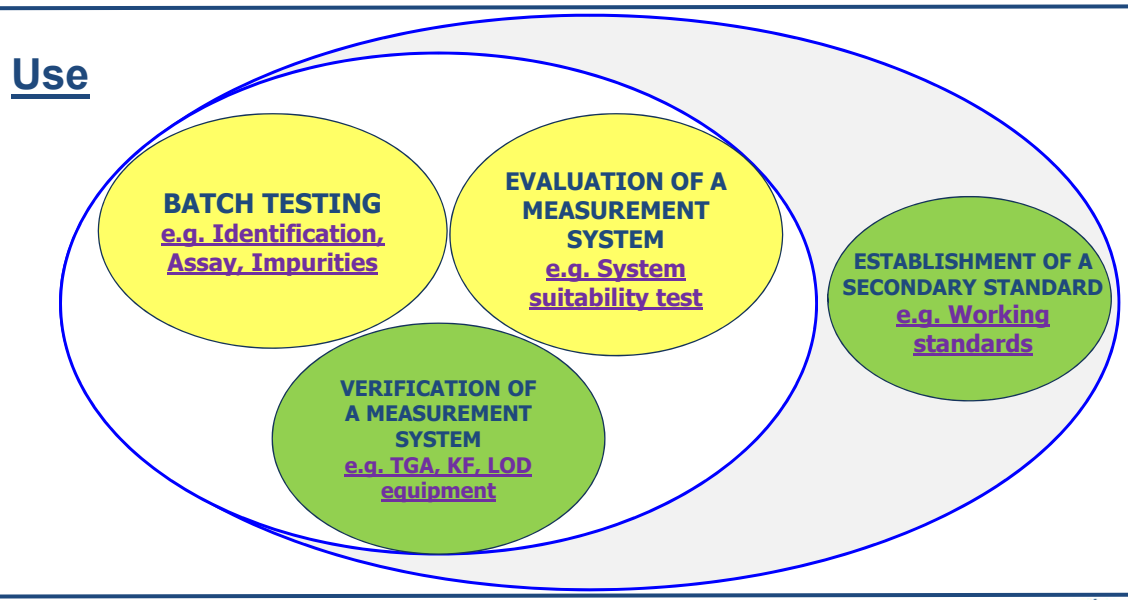
EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL
Health systems and products
Medicinal products – quality, safety and efficacy

EU guideline for GMP * Part 1 – 6.20

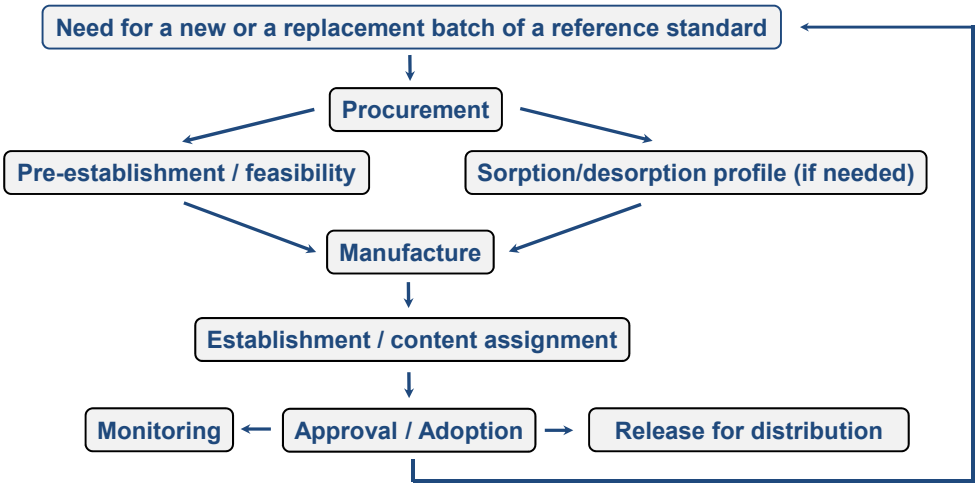
Reference standards should be **established as suitable for their intended use**. Their qualification and certification as such should be clearly stated and documented.

* Eudralex Volume 4, EU guidelines for good manufacturing practice for medicinal products for human and veterinary use

ESTABLISHMENT: GENERALS



ESTABLISHMENT: GENERALS



PH.EUR. REFERENCE STANDARDS

Establishment of qualitative RS

ESTABLISHMENT OF QUALITATIVE RS

- **Single substance RS subject of a Ph. Eur. monograph**

  → Key quality attribute = **identity**.

  → Verification of: * identity (full structural elucidation: NMR, QTOF-MS)

  * compliance with relevant requirements of monograph

  * intended use

  → Overall, characterisation is less elaborated than for RS used quantitatively.

ESTABLISHMENT OF QUALITATIVE RS

- **Single substance RS not subject of a Ph. Eur. monograph (e.g. impurity)**

  → Key quality attribute = **identity**.

  → Verification of: * identity (full structural elucidation: NMR, QTOF-MS)

  * intended use

  → Overall, characterisation is less elaborated than for RS used quantitatively.

ESTABLISHMENT OF QUALITATIVE RS

- **Mixture RS**

  → Key quality attributes: identity of impurities, homogeneity, fitness for purpose

  → **Identity of impurity peaks**

  Spiking with authentic impurity samples

  → **Homogeneity**

  In particular for compounded mixtures (evaporation, lyophilisation)

ESTABLISHMENT OF QUALITATIVE RS

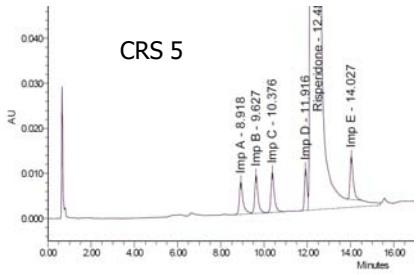
- **Mixture RS**

  → **Fitness for purpose**

  * Using method of intended use
  * Amount of each impurity (detectable and/or suitable for system suitability)
  * System suitability assessment, if applicable
  * Chromatogram for the RS leaflet

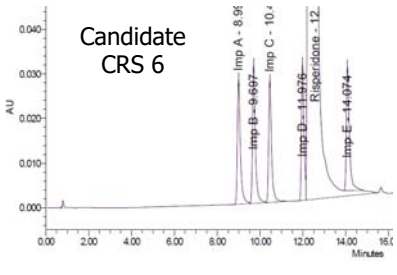
ESTABLISHMENT OF QUALITATIVE RS

- **Example fitness for purpose: Risperidone for system suitability CRS**



Peak Results

	Name	RT	RT Ratio	RR	Area	%Area	Height	Amount (%)	Rs	Hp/Hv
4	Imp D	11.916	0.95		76281	0.15	9250	0.142	6.37	3.26



Peak Results

	Name	RT	RT Ratio	RR	Area	%Area	Height	Amount (%)	Rs	Hp/Hv
	Imp D	11.976	0.95		248692	0.49	30638	0.467	6.94	7.02

Monograph 01/2011:1559 corrected 7.4 for risperidone: peak-to-valley ratio impurity D ≥ 1.5

PH.EUR. REFERENCE STANDARDS

Establishment of quantitative RS

ESTABLISHMENT OF QUANTITATIVE RS

- **RS used as external standard in related substances test (impurities)**

A candidate RS is characterised for:

Parameter	Method
Identity	Structural elucidation (NMR, qTOF-MS)
Identity of counter-ion	Various methods; specific or screening
Related substances	Method of intended use (LC/GC)
Volatile impurities	Loss on drying, thermogravimetry or water (+ residual solvents)
Inorganic impurities	Sulfated ash (if amount allows) or screening
Homogeneity	Method of relevant parameter

Content by mass balance or quantitative NMR, **assigned only if below 95.0 %.**

Confirmation of assigned content by orthogonal methods e.g. elemental analysis.

ESTABLISHMENT OF QUANTITATIVE RS

Example orthogonal techniques: Phenobarbital impurity A CRS 1

Analytical results:

- Identity: **confirmed**
- Loss on drying: **0.1 %**
- LC-purity: **99.7 %**
- Content by quantitative NMR (expressed 'as is', as free base): **79 %**
- Elemental analysis: **does not match** the theoretical composition
- **Identification of nitrate as the counter ion** (not on CoA)
- Quantification of nitrate by ion-exchange chromatography: **20.6 %**

→ **Good match with quantitative NMR and elemental analysis**

ESTABLISHMENT OF QUANTITATIVE RS

- **RS used as external standard in related substances test (impurities)**

Stoichiometric conversion factor

- Applied for new batches of impurity RS to be used as external standards, if the RS is supplied in a **different salt form** than the substance to be examined.
- (If required) provided in the **leaflet** accompanying the RS, together with instructions for its use.
- Given separately from the assigned content, if any.
- Can vary from batch to batch of the RS.

Note: a stoichiometric conversion factor can also be provided if the substance to be examined is a hydrate (even if the salt form is the same).

ESTABLISHMENT OF QUANTITATIVE RS

Example stoichiometric conversion factor: Rivastigmine impurity D CRS 2

- Rivastigmine impurity D CRS is used as external standard in the Ph.Eur. monographs for **rivastigmine** and **rivastigmine hydrogen tartrate**
- Rivastigmine impurity D CRS 2 is supplied as hydrogen tartrate salt
- The hydrogen **tartrate salt** of impurity D has a molecular mass of 400.4
- Impurity D as a **free base** has a molecular mass of 250.3
- The calculated **stoichiometric conversion factor** for use of rivastigmine impurity D CRS 2 in the Ph.Eur. monograph for rivastigmine is: 400.4 / 250.3 = **1.3** (rounded to one decimal)
- For use in the Ph.Eur. monograph for rivastigmine hydrogen tartrate no stoichiometric conversion factor is needed

ESTABLISHMENT OF QUANTITATIVE RS

Example stoichiometric conversion factor: Rivastigmine impurity D CRS 2

INFORMATION LEAFLET Ph. Eur. Reference Standard

Rivastigmine impurity D CRS batch 2

Identification

Catalogue code: Y0001515 Unit Quantity: ca 10 mg

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): 2629, 2630.

2.2 Analytical information related to intended use, when applicable

Rivastigmine impurity D CRS 2 is supplied as the hydrogen tartrate salt.

For the calculation of the amount of impurity D in monograph 2629 for rivastigmine, multiply the peak area of impurity D obtained with reference solution (a) by a stoichiometric conversion factor of Mr A / Mr B = 1.6.

For the calculation of the amount of impurity D in monograph 2630 for rivastigmine hydrogen tartrate, no stoichiometric correction is required.

Note: Molecular masses used for the calculation of the stoichiometric conversion factor in this leaflet:
Mr A: Rivastigmine impurity D as hydrogen tartrate salt: C14H22N2O2 * C4H6O6 --- 400.4 g/mol
Mr B: Rivastigmine impurity D as free base: C14H22N2O2 --- 250.3 g/mol

ESTABLISHMENT OF QUANTITATIVE RS

- **RS used for assay**

A candidate RS is characterised for:

Parameter	Method
Identity	Structural elucidation (NMR, qTOF-MS)
Compliance with monograph	As in monograph, relevant requirements only
Volatile impurities	Residual solvents (GC)
Inorganic impurities	Sulfated ash
Homogeneity	Usually loss on drying or water (and/or residual solvents)

If required, **inter-laboratory study** for parameters significantly contributing to assigned content: related substances (LC/GC), water / loss on drying, residual solvents.

Content is assigned by mass balance.

Confirmation of assigned content by orthogonal methods e.g. quantitative NMR, elemental analysis.

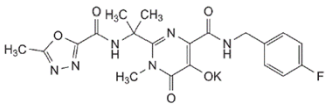
ESTABLISHMENT OF QUANTITATIVE RS

Example inter-laboratory study: Raltegravir potassium CRS 1

04/2018:2887

RALTEGRAVIR POTASSIUM

Raltegravirum kalicum



$C_{20}H_{20}FKN_6O_5$ M_r 482.5
[871038-72-1]

DEFINITION

Potassium 4-[[(4-fluorophenyl)methyl]carbamoyl]-1-methyl-2-[2-[(5-methyl-1,3,4-oxadiazol-2-yl)formamido]propan-2-yl]-6-oxo-1,6-dihydropyrimidin-5-olate.

Content: 98.0 per cent to 102.0 per cent (anhydrous substance).

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{20}H_{20}FKN_6O_5$ taking into account the assigned content of *raltegravir potassium CRS*.

ESTABLISHMENT OF QUANTITATIVE RS

Example inter-laboratory study: Raltegravir potassium CRS 1

Characterisation EDQM Lab

Test	Result	RSD	n
Appearance	Almost white powder	n/a	1
Mass spectrometry (in-house method) 2.2.43.	m/z found in accordance with sum formula	n/a	1
Nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	NMR spectra in accordance with structure	n/a	1
Identification reactions of ions and functional groups 2.3.1.	Positive reaction b) of potassium	n/a	1
Infrared absorption spectrophotometry 2.2.24.	KBr disc and ATR spectra recorded	n/a	1
Related substances by liquid chromatography 2.2.29. / 2.2.46.	See inter-laboratory study	-	-
Semi-micro determination of water 2.5.12.	See inter-laboratory study	-	-

Test	Result	RSD	n
Micro determination of water 2.5.32.	0.16 %	sd: 0.02	8
	Conditions: Direct introduction of about 50 mg	-	-
Residual solvents by headspace gas chromatography 2.2.28. / 2.4.24.	Acetonitrile and ethanol: see inter-laboratory study Sum of other residual solvents: below 0.10 % (Traces of toluene detected)	-	-
Differential scanning calorimetry (in-house method) 2.2.34.	Melting point above 205 °C → molar purity could not be determined	n/a	1
Quantitative nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	About 99.4 %	n/a	3
	Internal standard: maleic acid	-	-
Elemental analysis (contracted out to Solvias AG Switzerland)	C: 49.8 % (theory 49.8 %) H: 4.2 % (theory 4.2 %) N: 17.4 % (theory 17.4 %)	n/a	3

ESTABLISHMENT OF QUANTITATIVE RS

Example inter-laboratory study: Raltegravir potassium CRS 1

LC suitability

Liquid chromatography (2.2.29.)

System suitability	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Acceptance criterion
Resolution imp. E / raltegravir [ref. sol. (c), n = 1]	3.1	3.6	3.9	3.9	3.2	≥ 1.5
Symmetry factor raltegravir [ref. sol. (b), n = 1]	1.3	1.1	1.1	1.1	1.2	0.8 to 1.5
Signal-to-noise ratio raltegravir [ref. sol. (b), n = 1]	45	151	38	68	136	≥ 35
RSD peak area raltegravir [ref. sol. (b), n = 3]	1.9 % 1.3 %	0.7 % 3.4 %	3.4 % 2.9 %	1.5 % 0.9 %	2.9 % 1.4 %	≤ 5.0 %
All system suitability requirements fulfilled?	yes	yes	yes	yes	yes	

ESTABLISHMENT OF QUANTITATIVE RS

Example inter-laboratory study: Raltegravir potassium CRS 1

LC results

Impurity	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Mean
Imp. C (RR[$] about 0.7)	0.159 % (RR 0.77)	0.143 % (RR 0.76)	0.143 % (RR 0.76)	0.184 % (RR 0.76)	0.144 % (RR 0.77)	
Imp. E (RR about 0.95)	0.046 % (RR 0.95)	0.042 % (RR 0.95)	0.044 % (RR 0.95)	0.048 % (RR 0.95)	0.043 % (RR 0.95)	
Imp. F (RR about 1.15)	0.040 % (RR 1.18)	0.036 % (RR 1.18)	0.039 % (RR 1.19)	< rep. threshold	0.038 % (RR 1.17)	
Imp. G (RR about 1.1)	0.059 % (RR 1.12)	0.055 % (RR 1.12)	0.053 % (RR 1.12)	< rep. threshold	0.054 % (RR 1.12)	
Unspec. imp. 1 (RR about 1.9)	< rep. threshold	< rep. threshold	< rep. threshold	< rep. threshold	0.096 % (RR 1.86)	
Sum of impurities	0.303 % n = 2	0.276 % n = 2	0.279 % n = 2	0.232 % n = 2	0.375 % n = 2	**0.29 % n = 5 sd: 0.05**

[$] Relative retention.

ESTABLISHMENT OF QUANTITATIVE RS

Example inter-laboratory study: Raltegravir potassium CRS 1

Content assignment

(100 % - water % - residual solvents %) x [(100 % - sum of impurities by LC %) / 100 %] =

99.1 % of $C_{20}H_{20}FKN_6O_5$

The estimated uncertainty is 0.10 %, i.e. negligible in relation to the content limits given in the monograph.

ESTABLISHMENT OF QUANTITATIVE RS

Example inter-laboratory study: Raltegravir potassium CRS 1

Uncertainty estimation

$$u_{IS} = \sqrt{\frac{\sigma_{LC}^2 + \sigma_w^2 + \sigma_s^2}{n}}$$

u_{IS} = standard uncertainty of inter-laboratory study
σ_{LC} = standard deviation total impurities by LC
σ_w = standard deviation water
σ_s = standard deviation residual solvents
n = number of participants

$$U_{exp.} = \sqrt{u_{IS}^2 + u_{hom}^2} \times k$$

$U_{exp.}$ = expanded uncertainty
u_{IS} = standard uncertainty of inter-laboratory study
u_{hom} = standard uncertainty of homogeneity
k = coverage factor (normally 2)

Note: The stability component of the uncertainty is not included as considered negligible based on existing data.

ESTABLISHMENT OF QUANTITATIVE RS

Example inter-laboratory study: Raltegravir potassium CRS 1

Leaflet

INFORMATION LEAFLET Ph. Eur. Reference Standard

Raltegravir potassium CRS batch 1

1. **Identification**

Catalogue code: Y0001943 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): 2887, 2938, 2939.

2.2 Analytical information related to intended use, when applicable

The "as is" content is : **99.1 % of C20H20FKN6O5**

No uncertainty

ESTABLISHMENT OF QUANTITATIVE RS

- **RS used as external standard in assay – assigned value**

  - RS = material filled as such in a suitable container

    Content (m/m), assigned on an 'as is' basis.

    TO BE WEIGHED – NO NEED TO DRY

  - RS = freeze-dried material

    Amount per vial, e.g. 2.05 mg/vial.

    TO BE RECONSTITUTED, NOT WEIGHED

ESTABLISHMENT OF QUANTITATIVE RS

Ph.Eur. 5.12.

A European Pharmacopoeia reference standard with an **assigned content / potency** for use in the assay of a substance for pharmaceutical use (…) **may be suitable to determine the content/potency of that substance in a pharmaceutical preparation** provided all of the following conditions are fulfilled:

– the chromatographic assay method described in the active substance monograph is employed;
– the applicability of the method to the particular pharmaceutical preparation (absence of interference) is verified by the user;
– any pre-treatment of the sample (e.g. extraction, filtration) is validated for the particular pharmaceutical preparation.

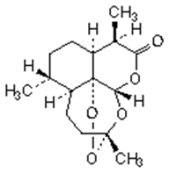
ESTABLISHMENT OF QUANTITATIVE RS

Example risks off-label use: Artemisinin RS (not Ph.Eur.)

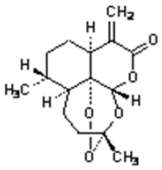
LC-UV assay method: RP C18 column – Isocratic elution
Detection wavelength: 210 nm
Limits: 97.0 % to 102.0 %

Content of the reference standard for LC-UV assay:
mass balance: 99.9 % (contains 0.1 % impurity A)
by quantitative NMR: 99.9 %

Artemisinin



Impurity A



Can the standard be used in a direct UV assay method at 210 nm?

ESTABLISHMENT OF QUANTITATIVE RS

Example risks off-label use: Artemisinin RS (not Ph.Eur.)

Difference in response at 210 nm
Impurity A needs a correction factor of 0.027 corresponding to a response factor of **37!**

Impurity A is **separated** in the LC-UV assay → no impact.

However, impurity A is **not separated** in the direct UV assay → the presence of 0.1 % of impurity A results in a UV signal at 210 nm which is equivalent to 3.7 % of artemisinin.

Conclusion:

The standard with an assigned content of 99.9 % is not suitable for use in a direct UV assay method.

ESTABLISHMENT OF QUANTITATIVE RS

Any **value** assigned to a reference standard is valid for the **intended use**.

If a reference standard (be it qualitative or quantitative) 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 by the user**.

ACKNOWLEDGEMENT

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Thank you for your attention

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