

# PURPOSES AND USES OF EUROPEAN PHARMACOPOEIA REFERENCE STANDARDS

*Official European Pharmacopoeia Reference Standards (Ph. Eur. RSs) are essential for the application of the quality control tests described in the Ph. Eur. texts.*

## QUALITATIVE USE

### RS FOR IDENTIFICATION – MAIN SUBSTANCE

*Identification of a substance by spectroscopic techniques such as IR absorption spectrophotometry or NMR spectrometry, or by chromatographic separation techniques (LC, GC, TLC)*

Example:  
Goserelin for NMR identification CRS

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### RS FOR PEAK IDENTIFICATION OF IMPURITIES

*Identification of impurities in a test for related substances using a liquid chromatographic method (LC)*

Example:  
Buserelin synthetic peptide

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### RS FOR SYSTEM SUITABILITY

*Verification that a measurement system is operated within the boundaries of its validation scope, often in a test for related substances using a liquid chromatographic method (LC)*

Example:  
Risperidone for system suitability CRS

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## QUANTITATIVE USE

### RS FOR PHYSICO- CHEMICAL ASSAY

*Quantitative determination of an active pharmaceutical ingredient (API) in an assay by using mostly chromatographic separation techniques (LC, GC)*

Example:  
Raltegravir potassium CRS

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### RS FOR MICROBIOLOGICAL ASSAY OF ANTIBIOTICS

*Determination of the potency of APIs by comparing the inhibition of growth of sensitive micro-organisms and the corresponding CRS*

Example:  
Amphotericin B for  
microbiological assay CRS

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### RS USED AS EXTERNAL STANDARD

*Quantitative determination of an impurity in a test for related substances by using mostly chromatographic separation techniques (LC, GC)*

Example:  
Rifamycin B CRS

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### RS FOR EQUIPMENT QUALIFICATION

*Certified reference material used to qualify equipment according to established quality management system procedures*

Example:  
Sodium aminosalicylate  
dihydrate for equipment  
qualification CRS

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**WHO USES THEM?**

## RS FOR IDENTIFICATION – MAIN SUBSTANCE

Identification of a substance by spectroscopic techniques such as IR absorption spectrophotometry or NMR spectrometry, or by chromatographic separation techniques (LC, GC, TLC)

Example: **Goserelin for NMR identification CRS**

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### IDENTIFICATION

Carry out either tests A and B or tests B and C.

A. Nuclear magnetic resonance spectrometry (2.2.64).

Preparation: 13 mg/mL solution in 0.2 M deuterated sodium phosphate buffer solution pH 5.0 R containing 20 µg/mL of deuterated sodium trimethylsilylpropionate R.

Comparison: 13 mg/mL solution of goserelin for NMR identification CRS in 0.2 M deuterated sodium phosphate buffer solution pH 5.0 R containing 20 µg/mL of deuterated sodium trimethylsilylpropionate R (dissolve the contents of a vial of goserelin for NMR identification CRS in this solvent to obtain the desired concentration).

Operating conditions:

- field strength: minimum 300 MHz;

- temperature: 25 °C.

Results: examine the <sup>1</sup>H NMR spectrum from 0 ppm to 9 ppm; the <sup>1</sup>H NMR spectrum obtained is qualitatively similar to the <sup>1</sup>H NMR spectrum obtained with goserelin for NMR identification CRS.

*<sup>1</sup>H NMR spectrum of goserelin for NMR identification CRS*

## RS FOR PEAK IDENTIFICATION OF IMPURITIES

*Identification of impurities in a test for related substances using a liquid chromatographic method (LC)*

Example: **Buserelin synthetic peptide**

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### RELATED SUBSTANCES

Liquid chromatography (2.2.29).

Test solution. Dissolve 5.0 mg of the substance to be examined in 5.0 mL of the mobile phase.

Reference solution (a). Dissolve the contents of a vial of *D-His-buserelin* CRS (impurity A) in the mobile phase. Dilute an appropriate volume of this solution in the mobile phase to obtain a final concentration of 1 mg/mL. Add 1.0 mL of the test solution to 1.0 mL of this solution.

Reference solution (b). Dissolve the contents of a vial of *buserelin* CRS in the mobile phase. Dilute an appropriate volume of this solution in the mobile phase to obtain a final concentration of 1.0 mg/mL.

Reference solution (c). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

*Chromatogram obtained with reference solution (a)*

## RS FOR SYSTEM SUITABILITY

*Verification that a measurement system is operated within the boundaries of its validation scope, often in a test for related substances using a liquid chromatographic method (LC)*

Example: **Risperidone** for system suitability CRS

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### SYSTEM SUITABILITY

Reference solution (a):

- the chromatogram obtained is similar to the chromatogram supplied with *risperidone* for system suitability CRS;
  - *peak-to-valley ratio*: minimum 1.5, where  $H_p$  = height above the baseline of the peak due to impurity D and  $H_v$  = height above the baseline of the lowest point of the curve separating this peak from the peak due to risperidone.
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*In this example, the system suitability test contains both qualitative and quantitative aspects.*

## RS FOR PHYSICO-CHEMICAL ASSAY

*Quantitative determination of an API in an assay by using mostly chromatographic separation techniques (LC, GC)*

Example: **Raltegravir potassium CRS**

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### 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*.

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*The “as is” content of the reference standard is usually assigned by mass balance based on data generated in an inter-laboratory study.*

RS FOR MICROBIOLOGICAL ASSAY OF ANTIBIOTICS

Determination of the potency of APIs by comparing the inhibition of growth of sensitive micro-organisms and the corresponding CRS

Example: Amphotericin B for microbiological assay CRS

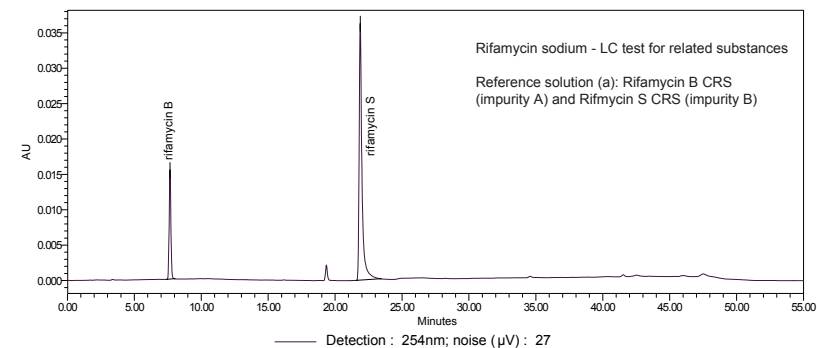
Table 2.7.2.-1 - Diffusion assay

| Antibiotic        | Reference substance                          | Solvent to be used in preparing the stock solution | Buffer solution (pH) | Micro-organism                                     | Medium and final pH (± 0.1 pH unit) | Incubation temperature |
|-------------------|----------------------------------------------|----------------------------------------------------|----------------------|----------------------------------------------------|-------------------------------------|------------------------|
| Amphotericin B    | Amphotericin B for microbiological assay CRS | Dimethyl sulfoxide R                               | pH 10.5 (0.2 M)      | Saccharomyces cerevisiae ATCC 9763 IP 1432-83      | F - pH 6.1                          | 35-37 °c               |
| Bacitracin zinc   | Bacitracin zinc CRS                          | 0.01 M hydrochloric acid                           | pH 7.0 (0.05 M)      | Micrococcus luteus NCTC 7743 CIP 53.160 ATCC 10240 | A - pH 7.0                          | 35-39 °c               |
| Bleomycin sulfate | Bleomycin sulfate CRS                        | Water R                                            | pH 6.8 (0.1 M)       | Mycobacterium smegmatis ATCC 607                   | G - pH 7.0                          | 35-37 °c               |

## RS USED AS EXTERNAL STANDARD

*Quantitative determination of an impurity in a test for related substances by using mostly chromatographic separation techniques (LC, GC)*

Example: Rifamycin B CRS



RS FOR EQUIPMENT QUALIFICATION

Certified reference material used to qualify equipment according to established quality management system procedures

Example: Sodium aminosalicylate dihydrate for equipment qualification CRS

SUGGESTED ACCEPTANCE CRITERIA

Taking into account inter-laboratory standard deviation as well as the mean intra-laboratory standard deviation obtained in the inter-laboratory study for the value assignment, the result of a measurement performed (following the above experimental conditions) is considered acceptable if the mean of 3 replicate determinations falls within the following limits:

Loss on drying (2.2.32.):

167.2 mg/g to 172.0 mg/g

Semi-micro determination of water (2.5.12.):

165.4 mg/g to 177.8 mg/g

Micro determination of water (2.5.32) –

liquid sample introduction:

167.3 mg/g to 173.7 mg/g

It is understood that a laboratory may apply a different approach to set acceptance criteria.

Equipment control chart (example)

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## EUROPEAN PHARMACOPOEIA REFERENCE STANDARDS – WHO USES THEM?

QUALITY CONTROL LABORATORIES  
OF PHARMACEUTICAL COMPANIES

QUALITY CONTROL LABORATORIES OF MANUFACTURERS  
OF SUBSTANCES FOR PHARMACEUTICAL USE  
(E.G. ACTIVE PHARMACEUTICAL INGREDIENTS, EXCIPIENTS,  
CONTAINERS, CLOSURES, HERBALS, BIOLOGICALS)

OFFICIAL MEDICINES CONTROL LABORATORIES (OMCLS)

COMMUNITY, ARMY OR HOSPITAL PHARMACIES

ACADEMIA