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Ph. Eur. Reference Standards: establishment and use

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Introduction

Outline

★ Terms and definitions,

★ Description, use and establishment of RS,

★ Reference Standards for general chapters and for equipment qualification,

★ Frequently Asked Questions (FAQ).

Terms and definitions

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Terms and definitions

EUROPEAN PHARMACOPOEIA
5.12. Reference standards

07/2018:51200
corrected 11.3

5.12. REFERENCE STANDARDS

This chapter is published for information.

EUROPEAN PHARMACOPOEIA
1. General notices

01/2023:10000

Terms and definitions – Ph. Eur. General chapter 5.12

Reference Material (RM)

Certified Reference Material (CRM)

Primary standard

Secondary standard

European Pharmacopoeia reference standard (Ph. Eur. RS)

European Pharmacopoeia chemical reference substance (CRS)

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 the measurement process.

* quantitative or qualitative

Terms and definitions – Ph. Eur. General chapter 5.12

Reference Material (RM)

Certified Reference Material (CRM)

Primary standard

Secondary standard

European Pharmacopoeia reference standard (Ph. Eur. RS)

European Pharmacopoeia chemical reference substance (CRS)

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

Terms and definitions – Ph. Eur. General chapter 5.12

Reference Material (RM)

Certified Reference Material (CRM)

Primary standard

Secondary standard

European Pharmacopoeia reference standard (Ph. Eur. RS)

European Pharmacopoeia chemical reference substance (CRS)

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.

Terms and definitions – Ph. Eur. General chapter 5.12

Reference Material (RM)

Certified Reference Material (CRM)

Primary standard

Secondary standard

European Pharmacopoeia reference standard (Ph. Eur. RS)

European Pharmacopoeia chemical reference substance (CRS)

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.

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

Terms and definitions – Ph. Eur. General chapter 5.12

Reference Material (RM)

Certified Reference Material (CRM)

Primary standard

Secondary standard

European Pharmacopoeia reference standard (Ph. Eur. RS)

European Pharmacopoeia chemical reference substance (CRS)

A reference standard **established** under the aegis of and **adopted** by the European Pharmacopoeia Commission (substances, preparations, spectra).

Terms and definitions – Ph. Eur. General chapter 5.12

Reference Material (RM)

Certified Reference Material (CRM)

Primary standard

Secondary standard

European Pharmacopoeia reference standard (Ph. Eur. RS)

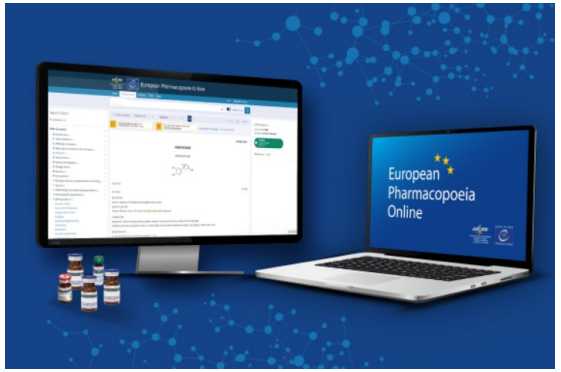
European Pharmacopoeia chemical reference substance (CRS)

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

Note: HRS and BRP are other types of RS.

Use of reference standards

Ph. Eur. General Notices



EUROPEAN PHARMACOPOEIA
1. General notices

01/2023:10000

- ★ *"Certain monographs require the use of reference standards, which can be chemical reference substances (CRSs), herbal reference standards (HRSs), biological reference preparations (BRPs) or reference spectra."*
- ★ *"Unless otherwise stated, the reference standards referred to in texts are alone authoritative in case of arbitration".*

These reference standards are available from EDQM.

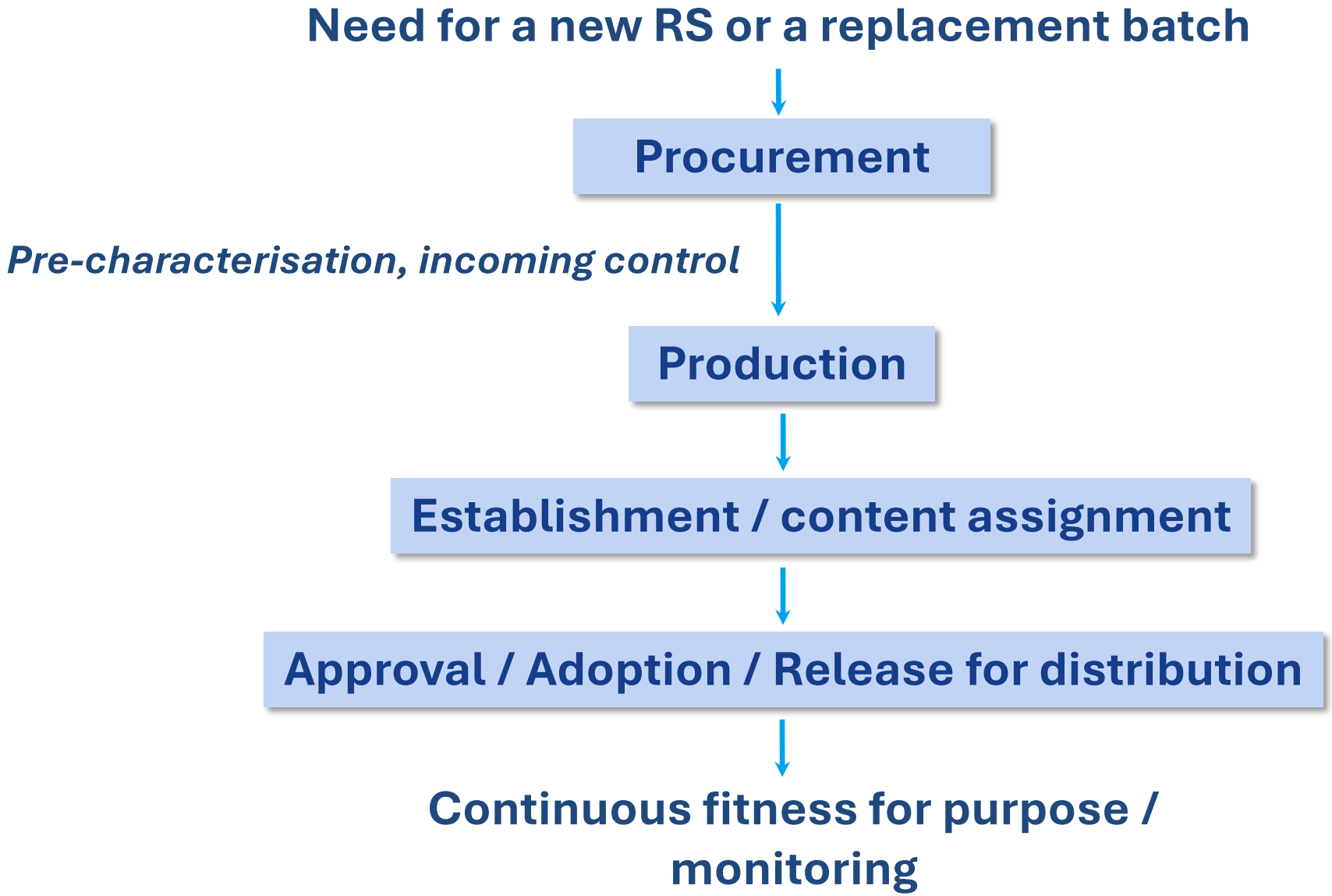
Description, use and establishment of RS

Use of Ph. Eur. RS

Description

- ★ Can be a substance subject of a monograph, an impurity, a mixture, etc…
- ★ Established for **their intended use in the Ph. Eur. only**.
- ★ If a reference standard (qualitative or quantitative) is to be used for any purpose other than for which it has been established, its **suitability for the other use** has to be **fully demonstrated by the user**.

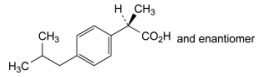
Ph. Eur. Reference Standard Process



Use of Ph. Eur. RS

IBUPROFEN

Ibuprofenum



$C_{13}H_{18}O_2$
[15687-27-1]

M_r 206.3

How to use?

- ★ As described in the Ph. Eur.
- ★ Information provided in the corresponding monograph:
  - ★ Substance to be weighed,
  - ★ Vial to be reconstituted (not weighed),
  - ★ Volume of solution or liquid to be taken.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 20 mg of the substance to be examined in 2 mL of *acetonitrile R* and dilute to 10.0 mL with mobile phase A.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

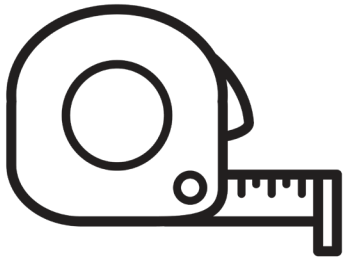
Reference solution (b). Dilute 1.0 mL of *ibuprofen impurity B CRS* to 10.0 mL with *acetonitrile R* (solution A). Dissolve 20 mg of *ibuprofen CRS* in 2 mL of *acetonitrile R*, add 1.0 mL of solution A and dilute to 10.0 mL with mobile phase A.

Reference solution (c). Dissolve the contents of a vial of *ibuprofen for peak identification CRS* (mixture of impurities A, J and N) in 1 mL of *acetonitrile R* and dilute to 5 mL with mobile phase A.

Use of Ph. Eur. RS

★ **Qualitative use:**

- ★ For identification of the main substance,
- ★ For peak identification of impurities,
- ★ For system suitability evaluation.



★ **Quantitative use:**

- ★ For physico-chemical assay,
- ★ For microbiological assay of antibiotics,
- ★ For quantification of impurities (purity test).

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Use of Ph. Eur. RS

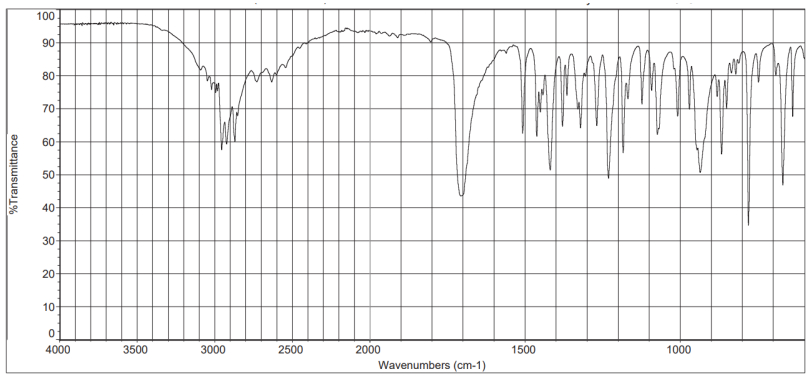
- **Qualitative use:**
  - For identification of the main substance,
  - For peak identification of impurities,
  - For system suitability evaluation.

- Quantitative use:
  - For physico-chemical assay,
  - For microbiological assay of antibiotics,
  - For quantification of impurities (purity test).

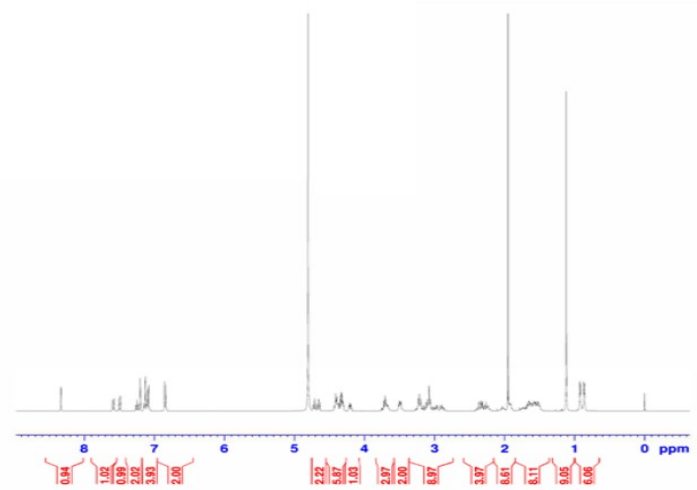
Ph. Eur. RS for identification

Identification of substances subject of a Ph. Eur. monograph by comparison with a CRS:

- **Spectroscopic techniques**
  - Example: Ph. Eur. monograph 01/2017:0721 corrected 9.6 for ibuprofen (identification C by IR)
  - Example: Ph. Eur. monograph 01/2023:1636 corrected 9.6 for goserelin (identification A by NMR)



IR spectrum of ibuprofen CRS

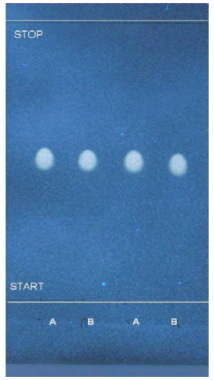


1H NMR spectrum of goserelin for NMR identification CRS

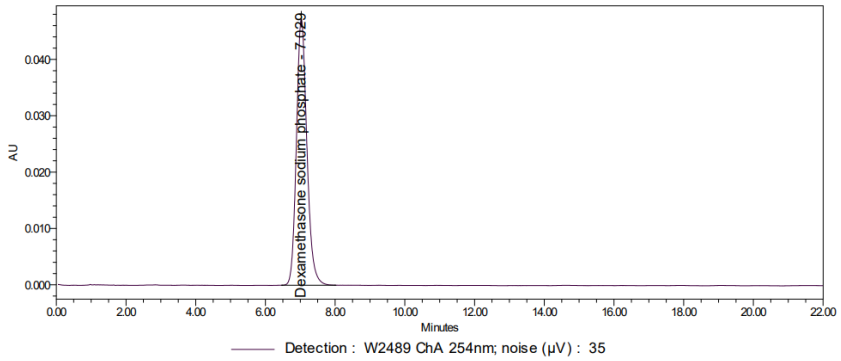
Ph. Eur. RS for identification

Identification of substances subject of a Ph. Eur. monograph by comparison with a CRS:

- **Chromatographic separation techniques**
  - Example: Ph. Eur. monograph 07/2021:0549 for dexamethasone sodium phosphate (identification D by LC)
  - Example: Ph. Eur. monograph 01/2017:0721 corrected 9.6 for ibuprofen (identification D by TLC)



TLC plate of ibuprofen CRS



LC chromatogram of dexamethasone sodium phosphate CRS

Ph. Eur. RS for identification

Establishment

★ Key quality attribute = **identity,**

★ Identity: **full structural elucidation** (NMR, MS, IR), whenever possible,

★ **Compliance** with relevant requirements of the monograph,

★ **Suitability** for its intended use.

Characterisation focused on the substance rather than impurities

Ph. Eur. RS for identification

Information provided on the leaflet

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

IBUPROFEN CRS batch 6

1. **Identification**

   Catalogue code: I0020000

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): 0721.

   2.2 Analytical information related to intended use, when applicable

   2.3 Uncertainty of the assigned value, when applicable

   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.

No assigned value indicated as not needed for its qualitative/intended use

Use of Ph. Eur. RS

★ **Qualitative use:**

- ★ For identification of the main substance,
- ★ For peak identification of impurities,
- ★ For system suitability evaluation.

★ Quantitative use:

- ★ For physico-chemical assay,
- ★ For microbiological assay of antibiotics,
- ★ For quantification of impurities (purity test).

RS for control of related substances (qualitative)

Qualitative RS for impurity control

- **Chromatographic** separation techniques (LC, GC, TLC),
- Batch testing: **identification of signals/peaks** (specified impurities or correction factor),
- **System suitability** testing: check the performance of a measurement system for application of the pharmacopoeial method.

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RS for control of related substances (qualitative)

RS strategy

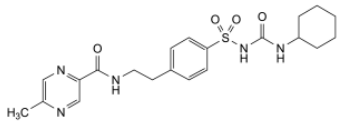
- ★ Single substance or mixture (with or without the main substance),
- ★ Single substance: can be a different salt from the main substance,
- ★ Alternative to RS: commercial reagent or in situ degradation,
- ★ If impurities are specified, batches containing the impurities normally available,
- ★ A chromatogram is supplied in the RS leaflet if referred to in the monograph.

RS for control of related substances (qualitative)

Use of single impurity: glipizide impurity A CRS 2

GLIPIZIDE

Glipizidum



$C_{21}H_{27}N_5O_4S$
[29094-61-9]

M_r 445.5

TESTS

Related substances. Liquid chromatography (*2.2.29*).

Reference solution (b). Dissolve 6.0 mg of *glipizide impurity A CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 1.0 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (c). Dissolve 2 mg of *glipizide impurity C CRS* in the solvent mixture and dilute to 100 mL with the solvent mixture. Dilute 1 mL of the solution to 100 mL with the solvent mixture.

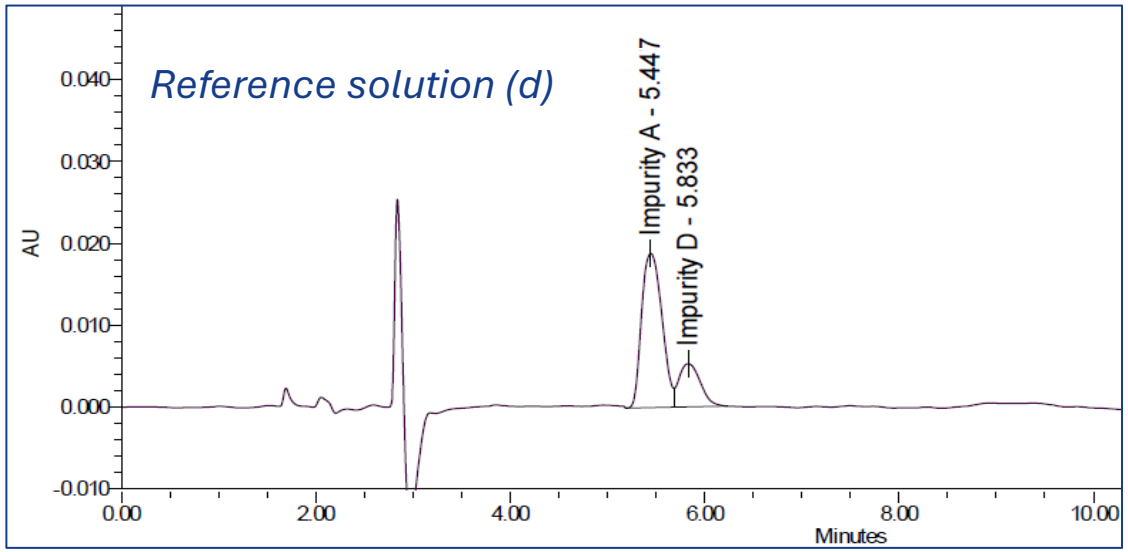
Reference solution (d). Dissolve 2 mg of *glipizide impurity D CRS* in the solvent mixture and dilute to 250 mL with the solvent mixture. Dilute 1 mL of the solution to 20 mL with reference solution (b).

Identification of impurities: use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention with reference to glipizide (retention time = about 22 min): impurity A = about 0.25; impurity D = about 0.27; impurity C = about 1.2.

System suitability: reference solution (d):

- *peak-to-valley ratio*: minimum 2.0, 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 impurity A.



No chromatogram provided with the leaflet

RS for control of related substances (qualitative)

Information provided on the leaflet

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

GLIPIZIDE IMPURITY A CRS batch 2

1. **Identification**

Catalogue code: G0342000 Unit Quantity: ca 15 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): 0906.

2.2 Analytical information related to intended use, when applicable

2.3 Uncertainty of the assigned value, when applicable

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.

No assigned value indicated as not needed for its qualitative use.

No information on the salt / form as not needed for its intended use

RS for control of related substances (qualitative)

Establishment: single substance RS not subject of a Ph. Eur. monograph (e.g. impurity)

- ★ Key quality attribute: **identity** (qualitative),
- ★ **Full structural elucidation**, when possible (e.g. MS, NMR, IR),
- ★ **Intended use**,
- ★ Characterisation is less elaborated than for RS used quantitatively,
- ★ Homogeneity if needed.

RS for control of related substances (qualitative)

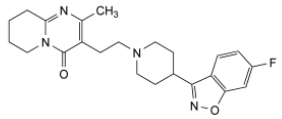
Use of RS mixture: risperidone for system suitability CRS 6

EUROPEAN PHARMACOPOEIA
Risperidone

01/2011:1559
corrected 7.4

RISPERIDONE

Risperidonum



$C_{23}H_{27}FN_4O_2$
[106266-06-2]

M_r 410.5

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 10 mg of *risperidone for system suitability CRS* (containing impurities A, B, C, D and E) in 1.0 mL of *methanol R*.

Identification of impurities: use the chromatogram supplied with *risperidone for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, D and E; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity K.

Relative retention with reference to risperidone (retention time = about 12 min): impurity A = about 0.7; impurity B = about 0.75; impurity C = about 0.8; impurity K = about 0.9; impurity D = about 0.94; impurity E = about 1.1.

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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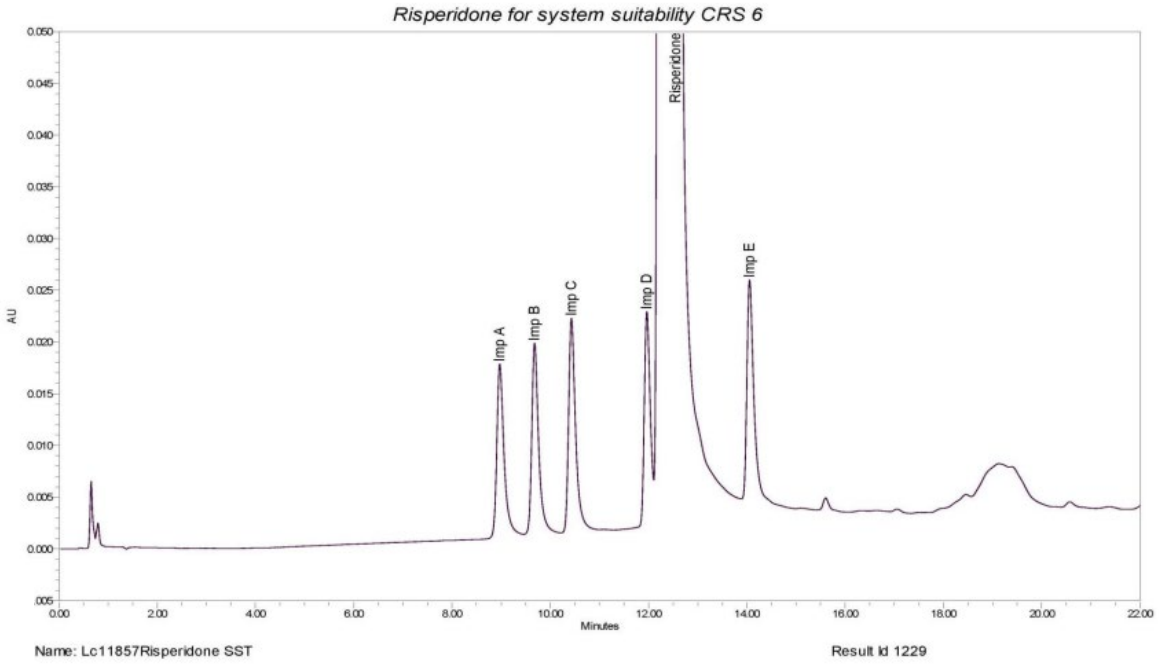
LIQUID CHROMATOGRAPHY REPORT



Chromatogram provided with the leaflet

RS for control of related substances (qualitative)

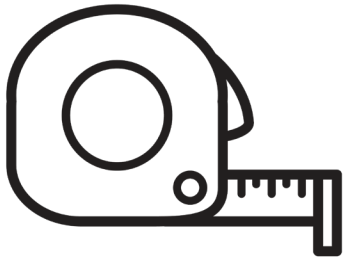
Establishment: mixture RS

- ★ Key quality attribute: **identity** (qualitative),
- ★ **Identity** of impurity peaks,
- ★ Spiking with **authentic impurity samples**, when possible,
- ★ **Intended use,**
- ★ Homogeneity if needed.

Use of Ph. Eur. RS

★ Qualitative use:

- ★ For identification of the main substance,
- ★ For peak identification of impurities,
- ★ For system suitability evaluation.



★ **Quantitative use:**

- ★ For physico-chemical assay,
- ★ For microbiological assay of antibiotics,
- ★ For quantification of impurities (purity test).

Assay RS

Description

- **Quantitative use** in assay procedures such as LC, GC, microbiology,
- In general, **substance compliant** with relevant requirements of corresponding Ph. Eur. monograph,
- Exceptional cases:
  - other salt form (e.g. **Montelukast dicyclohexylamine CRS** in the Ph. Eur. monograph for **montelukast sodium**),
  - other hydrate (e.g. **Rabeprazole sodium hydrate CRS** in the Ph. Eur. monograph for **rabeprazole sodium**),
  - lyophilised RS (e.g. **Tobramycin CRS** in the Ph. Eur. monograph for **tobramycin**).

Assay RS

Establishment

- ★ Key quality attributes: **Identity and content** (qualitative and quantitative),
- ★ Identity: **full structural elucidation** (NMR, MS, IR), whenever possible,
- ★ **Compliance** with relevant requirements of the monograph,
- ★ **Volatile impurities** (Loss on drying, residual solvents (HS-GC) and water),
- ★ **Inorganic impurities** (sulfated ash for screening, further testing may be required).

Characterisation focused on the substance and its impurities

Assay RS

Establishment (continued)

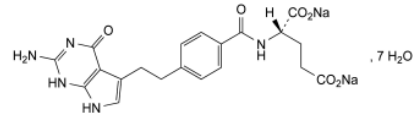
- **Content is assigned** based on mass balance approach (pharmacopoeial + complementary tests),
- **Uncertainty** of the assigned value is estimated and shall be negligible compared to content limits in the monograph,
- Homogeneity (LOD or water, residual solvents in specific cases),
- **Confirmation of assigned content** / purity by orthogonal methods (quantitative NMR, titration, ...), whenever possible,
- **Inter-laboratory study** for parameters with significant contribution to assigned content.

Assay RS

Example: Pemetrexed disodium heptahydrate CRS 3

PEMETREXED DISODIUM HEPTAHYDRATE

Pemetrexedum dinatricum heptahydricum



$C_{20}H_{19}N_5Na_2O_6,7H_2O$ M_r 597.5

[57166-29-1]

ASSAY

Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use or store them at 2-8 °C for not more than 24 h.*

Acetate buffer. Mix 1.7 mL of *glacial acetic acid R* and 900 mL of *water for chromatography R*, adjust to pH 5.3 with a 760 g/L solution of *sodium hydroxide R* in *water for chromatography R* and dilute to 1000 mL with *water for chromatography R*.

Test solution. Dissolve 30.0 mg of the substance to be examined in *water R* and dilute to 200.0 mL with the same solvent.

Reference solution. Dissolve 30.0 mg of *pemetrexed disodium heptahydrate CRS* in *water R* and dilute to 200.0 mL with the same solvent.

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (3.5 µm);
- *temperature*: 30 °C.

Mobile phase: *acetonitrile R*, acetate buffer (11:89 *V/V*).

Flow rate: 2.0 mL/min.

Detection: spectrophotometer at 285 nm.

Injection: 20 µL.

Run time: twice the retention time of pemetrexed (retention time = about 3 min).

Calculate the percentage content of $C_{20}H_{19}N_5Na_2O_6$ taking into account the assigned content of *pemetrexed disodium heptahydrate CRS*.

Assay RS

Example: Pemetrexed disodium heptahydrate CRS 3

Test	Result	%RSD	n
Appearance	White powder	n/a	1
Infrared absorption spectrophotometry 2.2.24.	Concordant with CRS 2	n/a	1
Mass spectrometry (in-house method) 2.2.43.	m/z found in accordance with sum formula	n/a	1
Identification reactions of ions and functional groups 2.3.1.	Positive identification reaction a) for Na	n/a	1
Nuclear magnetic resonance – other (in-house method) 2.2.33.	NMR spectra of CRS 2 and proposed CRS 3 are concordant	n/a	1

Identity check

Assay RS

Example: Pemetrexed disodium heptahydrate CRS 3

Test	Result	%RSD	n
Appearance	White powder	n/a	1
Infrared absorption spectrophotometry 2.2.24.	Concordant with CRS 2	n/a	1
Mass spectrometry (in-house method) 2.2.43.	m/z found in accordance with sum formula	n/a	1
Identification reactions of ions and functional groups 2.3.1.	Positive identification reaction a) for Na	n/a	1
Nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	NMR spectra of CRS 2 and proposed CRS 3 are concordant	n/a	1
Enantiomeric purity, Liquid chromatography 2.2.29. / 2.2.46.	Baseline separation between impurity E and pemetrexed	n/a	1
	Symmetry factor: 1.1	n/a	1
	Impurity E: 0.08%	n/a	2
Related substances by liquid chromatography 2.2.29. / 2.2.46.	Peak to valley ratio imp. B / imp. C: 7.8	n/a	1
	No impurity above reporting threshold	n/a	6
	Reporting threshold: 0.03%	-	-

Test	Result		%RSD	n
Semi-micro determination of water 2.5.12.	See inter-laboratory study		-	-
Residual solvents by headspace gas chromatography (in-house method) 2.2.28. / 2.4.24.	<0.10%		n/a	2
Assay by liquid chromatography 2.2.29. / 2.2.46.	78.7% (as is) External standard: CRS 2		0.41%	3
Quantitative nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	78.4% $C_{20}H_{19}N_5Na_2O_6$		0.37%	3
	Internal standard: dimethylmalonic acid		-	-
Elemental analysis (contracted out to SGS France)	Atom	Theoretical value[1] / Experimental value	-	-
	C	40.0 % / 40.2 %	-	3
	H	5.6 % / 5.5 %	-	3
	N	11.7 % / 11.6 %	-	3
	O	35.1 % / 34.6 %	-	3

[1] Theoretical values corrected for water content.

Assay RS

Example: Pemetrexed disodium heptahydrate CRS 3

Test	Result	%RSD	n
Appearance	White powder	n/a	1
Infrared absorption spectrophotometry 2.2.24.	Concordant with CRS 2	n/a	1
Mass spectrometry (in-house method) 2.2.43.	m/z found in accordance with sum formula	n/a	1
Identification reactions of ions and functional groups 2.3.1.	Positive identification reaction a) for Na	n/a	1
Nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	NMR spectra of CRS 2 and proposed CRS 3 are concordant	n/a	1
Enantiomeric purity, Liquid chromatography 2.2.29. / 2.2.46.	Baseline separation between impurity E and pemetrexed	n/a	1
	Symmetry factor: 1.1	n/a	1
	Impurity E: 0.08%	n/a	2
Related substances by liquid chromatography 2.2.29. / 2.2.46.	Peak to valley ratio imp. B / imp. C: 7.8	n/a	1
	No impurity above reporting threshold	n/a	6
	Reporting threshold: 0.03%	-	-

Test	Result	%RSD	n
Semi-micro determination of water 2.5.12.	See inter-laboratory study	-	-
Residual solvents by headspace gas chromatography (in-house method) 2.2.28. / 2.4.24.	<0.10%	n/a	2
Assay by liquid chromatography 2.2.29. / 2.2.46.	78.7% (as is) External standard: CRS 2	0.41%	3
Quantitative nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	78.4% $C_{20}H_{19}N_5Na_2O_6$	0.37%	3
	Internal standard: dimethylmalonic acid	-	-

Elemental analysis (contracted out to SGS France)	Atom	Theoretical value[1]	Experimental value	-	-
	C	40.0 %	40.2 %	-	3
	H	5.6 %	5.5 %	-	3
	N	11.7 %	11.6 %	-	3
	O	35.1 %	34.6 %	-	3

[1] Theoretical values corrected for water content.

Relevant requirements of the Ph. Eur. monograph

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Assay RS

Example: Pemetrexed disodium heptahydrate CRS 3

Test	Result	%RSD	n
Appearance	White powder	n/a	1
Infrared absorption spectrophotometry 2.2.24.	Concordant with CRS 2	n/a	1
Mass spectrometry (in-house method) 2.2.43.	m/z found in accordance with sum formula	n/a	1
Identification reactions of ions and functional groups 2.3.1.	Positive identification reaction a) for Na	n/a	1
Nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	NMR spectra of CRS 2 and proposed CRS 3 are concordant	n/a	1
Enantiomeric purity, Liquid chromatography 2.2.29. / 2.2.46.	Baseline separation between impurity E and pemetrexed	n/a	1
	Symmetry factor: 1.1	n/a	1
	Impurity E: 0.08%	n/a	2
Related substances by liquid chromatography 2.2.29. / 2.2.46.	Peak to valley ratio imp. B / imp. C: 7.8	n/a	1
	No impurity above reporting threshold	n/a	6
	Reporting threshold: 0.03%	-	-

Test	Result		%RSD	n
Semi-micro determination of water 2.5.12.	See inter-laboratory study		-	-
Residual solvents by headspace gas chromatography (in-house method) 2.2.28. / 2.4.24.	<0.10%		n/a	2
Assay by liquid chromatography 2.2.29. / 2.2.46.	78.7% (as is) External standard: CRS 2		0.41%	3
Quantitative nuclear magnetic resonance spectrometry (in-house method) 2.2.33.	78.4% $C_{20}H_{19}N_5Na_2O_6$		0.37%	3
	Internal standard: dimethylmalonic acid		-	-
Elemental analysis (contracted out to SGS France)	Atom	Theoretical value[1]	Experimental value	-
	C	40.0 %	40.2 %	-
	H	5.6 %	5.5 %	-
	N	11.7 %	11.6 %	-
	O	35.1 %	34.6 %	-

[1] Theoretical values corrected for water content.

Assay RS

Example: Pemetrexed disodium heptahydrate CRS 3

★ Results of the inter-laboratory study for water content

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Mean
Result	**21.29 %** (n = 3) sd: 0.02 RSD: 0.1 %	**21.93 %** (n = 3) sd: 0.16 RSD: 0.7 %	**21.03 %** (n = 3) sd: 0.19 RSD: 0.9 %	**21.49 %** (n = 3) sd: 0.09 RSD: 0.4 %	**21.55 %** (n = 3) sd: 0.00 RSD: 0.0 %	**21.46 %** (n = 5) sd: 0.33
Acceptance criterion fulfilled? **(RSD ≤ 1.5 %)**	Yes	Yes	Yes	Yes	Yes	-

Assay RS

Example: Pemetrexed disodium heptahydrate CRS 3

Content assignment

[100% (m/m) - water% (m/m) by semi-micro determination of water - residual solvents% (m/m)] x [100% - sum of impurities by relative%] / 100%

=

78.5 % of $C_{20}H_{19}N_5Na_2O_6$

Confirmation by orthogonal techniques: **quantitative NMR** and **LC assay**

78.7%

« as is »: 78.4%

Assay RS

Information provided on the leaflet

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INFORMATION LEAFLET Ph. Eur. Reference Standard

Pemetrexed disodium heptahydrate CRS batch 3

1. **Identification**

Catalogue code: Y0001539

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): 2637, 3046.

2.2 Analytical information related to intended use, when applicable

The "as is" content is : **78.5 % of C20H19N5Na2O6**

2.3 Uncertainty of the assigned value, when applicable

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.

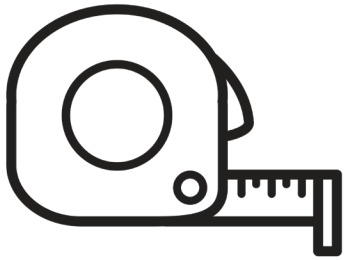
Ph. Eur. monograph 07/2023:2637 for pemetrexed disodium heptahydrate (LC assay)

Ph. Eur. monograph 07/2023:3046 for pemetrexed disodium 2.5-hydrate (LC assay)

Use of Ph. Eur. RS

★ Qualitative use:

- ★ For identification of the main substance,
- ★ For peak identification of impurities,
- ★ For system suitability evaluation.



★ **Quantitative use:**

- ★ For physico-chemical assay,
- ★ For microbiological assay of antibiotics,
- ★ For quantification of impurities (purity test).

RS for control of related substances (quantitative)

Use

- **Quantitative determination** of an impurity,
- Mostly in a **test for related substances** by chromatographic methods,
- Quantification usually performed versus a dilution of the test solution and correction factor is given if response factor is outside 0.8 – 1.2,
- Otherwise, external standard for impurities with a **response very different** from that of substance subject of the monograph.

RS for control of related substances (quantitative)

Description

- ★ Single substance RS,
- ★ Materials obtained via processes that do not guarantee the required degree of purity and homogeneity,
- ★ Content of RS is critical: ≥ 95.0 % or not?

4-2-2. **Related substances test**. A CRS corresponding to an impurity is characterised for identity and purity. Where a CRS is used to determine the content of a given impurity, the preferred minimum content is 95.0 per cent; where this is achieved the assigned content of the CRS is not given and it is considered to be 100.0 per cent; this approximation is acceptable since there will be no appreciable effect on the determination of impurities. When this minimum content cannot be obtained, an assigned content is given to the CRS.

- ★ Salt form has impact on procurement and use:
  - ★ Handling, hygroscopicity, volatility,
  - ★ Solubility,
  - ★ Need for stoichiometric conversion factor (to be evaluated).

RS for control of related substances (quantitative)

Stoichiometric conversion factor

★ In a monograph, specification limit given for impurity in **same (salt) form,**

★ What about the reference standards?

EUROPEAN PHARMACOPOEIA 07/2018:51200
5.12. Reference standards **corrected 11.3**

CRSs used to determine the content of a given impurity are normally in the same acid, base or salt form as the substance that is the subject of the corresponding monograph. Where this is not the case, unless otherwise justified, a corresponding stoichiometric conversion factor is applied.

★ Need to **check the presence and the identity of counter-ion,**

★ If **different** from the monograph form, stoichiometric conversion factor to be given,

★ Exception: if impurity cannot form the salt of the monograph.

RS for control of related substances (quantitative)

Establishment

- ★ Key quality attributes: **identity and content**
- ★ **Identity**: Full structural elucidation (IR, NMR, MS), if possible
- ★ **Identity of counter-ion**: specific tests 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

RS for control of related substances (quantitative)

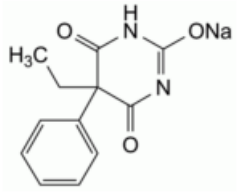
Establishment (continued)

- ★ Quantitative NMR
- ★ Homogeneity (e.g. water, content, volatiles)
- ★ Content assignment by mass balance or quantitative NMR
- ★ Checked by orthogonal methods (qNMR, mass balance, other)
- ★ If below 95.0%, content is assigned / provided

RS for control of related substances (quantitative)

Example 1: Phenobarbital impurity A CRS 2

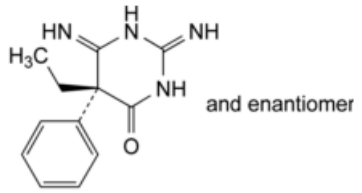
PHENOBARBITAL SODIUM



★ Analytical results:

- ★ Identity: confirmed
- ★ Loss on drying: 0.3%
- ★ LC purity: 99.8%

Mass balance:
99.5 % ≥ 95.0%
⇨ no need for assigned content



Phenobarbital impurity A

- ★ Confirmed as the free base:
  - ★ **Use in the monograph for phenobarbital (04/2012:0201):** provided in the same form as the monograph substance, a stoichiometric conversion factor is not required.
  - ★ **Use in the monograph for phenobarbital sodium (07/2016:0630)**: cannot form a sodium salt, a stoichiometric conversion factor is not required.

RS for control of related substances (quantitative)

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INFORMATION LEAFLET Ph. Eur. Reference Standard

PHENOBARBITAL IMPURITY A CRS batch 2

1. **Identification**

Catalogue code: Y0001350 Unit Quantity: ca 15 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): 0201, 0630.

2.2 Analytical information related to intended use, when applicable

2.3 Uncertainty of the assigned value, when applicable

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.

No assigned value as content ≥ 95.0%
No stoichiometric conversion factor

RS for control of related substances (quantitative)

Example 2: Captopril impurity J CRS 2

Analytical results:

- ★Identity: confirmed
- ★Water: 6.8%
- ★LC purity: 99.9%
- ★Residual solvents : <0.10%

Mass balance: 93.1 %
< 95.0% ⇨Need for assigned content

- ★Estimated content by qNMR (expressed « as is », as free acid): **94.3%**
- ★Content by LC assay versus CRS 1 (expressed « as is », as free acid): **93.9%**
- ★Elemental analysis: matches the theoretical composition

- ★**Free acid**:
  - ★**Use in the monograph for captopril (07/2021:1079):** provided in the same form as the monograph substance (not a salt) ,

  ⇨ no stoichiometric conversion factor required.

RS for control of related substances (quantitative)

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INFORMATION LEAFLET Ph. Eur. Reference Standard

CAPTOPRIL IMPURITY J CRS batch 2

1. **Identification**

Catalogue code: Y0001450 Unit Quantity: ca 10 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): 1079.

2.2 Analytical information related to intended use, when applicable

The "as is" content is : **93.1% C11H17NO4S**

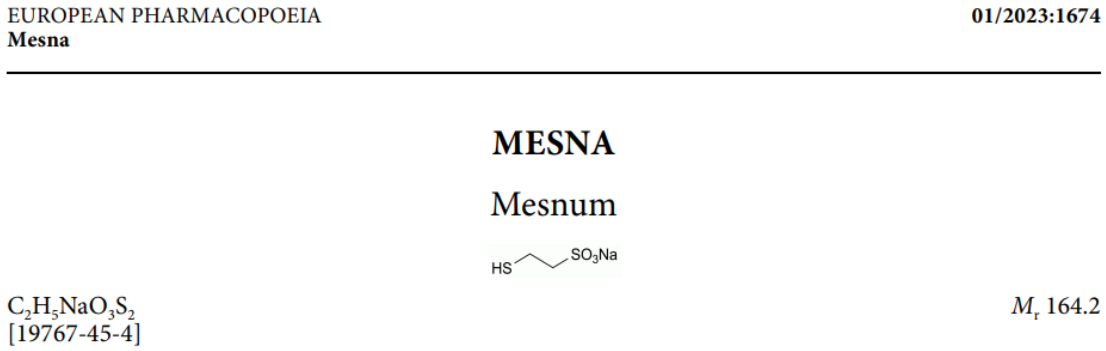
Assigned value as content < 95.0%
No stoichiometric conversion factor

2.3 Uncertainty of the assigned value, when applicable

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.

RS for control of related substances (quantitative)

Example 3: Mesna impurity C CRS 3



EUROPEAN PHARMACOPOEIA
Mesna
01/2023:1674

MESNA

Mesnum

$C_2H_5NaO_3S_2$
[19767-45-4]

M_r 164.2

★ **Analytical results:**

- ★ Identity: confirmed
- ★ Water: <0.1%
- ★ LC purity: 100.0%
- ★ Residual solvents: < 0.10%

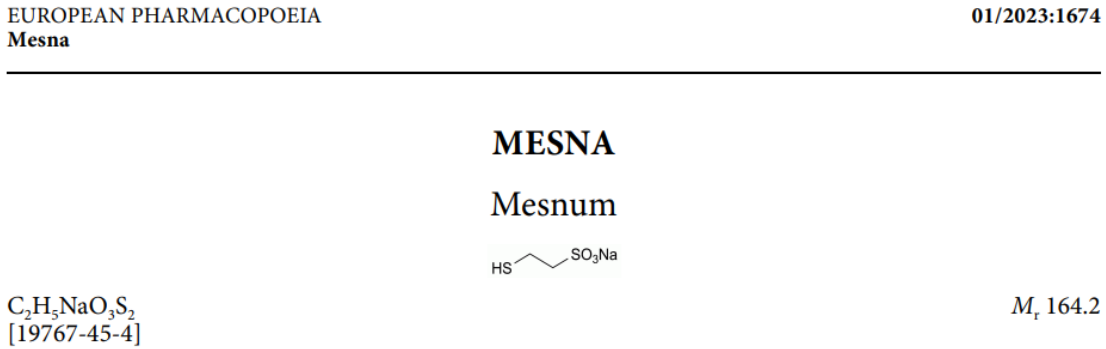
} **Mass balance: 100.0 %**

- ★ **Certificate of analysis: sodium as counter-ion**
- ★ Content by qNMR (expressed 'as is', as sodium salt): **91.8 %**
- ★ Content by LC assay versus CRS 2 (expressed 'as is', as sodium salt): **91.5%**
- ★ Elemental analysis: **does not match** the theoretical composition

⇨ **Investigation required**

RS for control of related substances (quantitative)

Example 3: Mesna impurity C CRS 3



EUROPEAN PHARMACOPOEIA 01/2023:1674
Mesna

MESNA

Mesnum

$C_2H_5NaO_3S_2$
[19767-45-4]

M_r 164.2

- **Analytical results:**
  - Mass balance: **100.0%**
- **Investigation**:
  - Identification of counter-ions (LC-CAD, MS, ^{23}Na-NMR): **Identification of potassium, only traces of sodium**
  - Content by qNMR (expressed 'as is', as potassium salt 1:1 stoichiometry): **99.0%**,
  - Content by LC assay versus CRS 2 (expressed 'as is', as potassium salt 1:1 stoichiometry): **98.6%**

RS for control of related substances (quantitative)

Information provided on the leaflet

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INFORMATION LEAFLET Ph. Eur. Reference Standard

MESNA IMPURITY C CRS batch 3

1. **Identification**

Catalogue code: Y0000316

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): 1674.

2.2 Analytical information related to intended use, when applicable

Mesna impurity C CRS 3 is supplied as the potassium salt.
For the calculation of the amount of mesna impurity C in the monograph for mesna, multiply the peak area of mesna impurity C obtained with reference solution (a) by a stoichiometric conversion factor of Mr A / Mr B = 1.1
Note: Molecular masses used for the calculation of the stoichiometric conversion factor in this leaflet:
Mr A: mesna impurity C as potassium salt: C4H7O4S2K --- 222.3 g/mol
Mr B: mesna impurity C as sodium salt: C4H7O4S2Na --- 206.2 g/mol

→ ***No assigned value as content ≥ 95.0%***
Stoichiometric conversion factor

2.3 Uncertainty of the assigned value, when applicable

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 standards in Ph. Eur. General Chapters

Reference standards in Ph. Eur. General Chapters

- ★ Use described in Ph. Eur. General methods,
- ★ CRS for equipment qualification.

Reference standards in Ph. Eur. General Chapters

Ph.Eur. Chapter 2.5.42. N-Nitrosamines in Active Substances

- ★ Analytical procedures for the detection of various N-nitrosamines in particular active substances: NDMA, NDEA, NDBA, NMBA, NDiPA, NEiPA and NDPA.
- ★ Procedures A and B: limit tests (30 ppb)
- ★ Procedure C: a quantitative test.

Last update : 10/11/2025

Available since	Cat. No.	Name	Batch No.	Unit Quantity	Price	SDS Product Code
	Y0002258	N-NITROSO-DIETHYLAMINE CRS	1	1 ML	79 EUR	202000237
	Y0002259	N-NITROSO-DIMETHYLAMINE CRS	2	1 ML	79 EUR	202000236
	Y0002260	N-NITROSO-N-METHYL-4-AMINOBUTYRIC ACID CRS	1	1 ML	79 EUR	202000239
	Y0002261	N-NITROSO-DIBUTYLAMINE CRS	1	1 ML	79 EUR	202000238
	Y0002262	N-NITROSO-ETHYL-ISOPROPYLAMINE CRS	1	1 ML	79 EUR	202000241
	Y0002263	N-NITROSO-DIISOPROPYLAMINE CRS	1	1 ML	79 EUR	202000242
	Y0002264	N-NITROSO-DIPROPYLAMINE CRS	1	1 ML	79 EUR	202000240

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INFORMATION LEAFLET Ph. Eur. Reference Standard

N-nitroso-diethylamine CRS batch 1

1. **Identification**

Catalogue code: Y0002258

2. **Scientific Information**

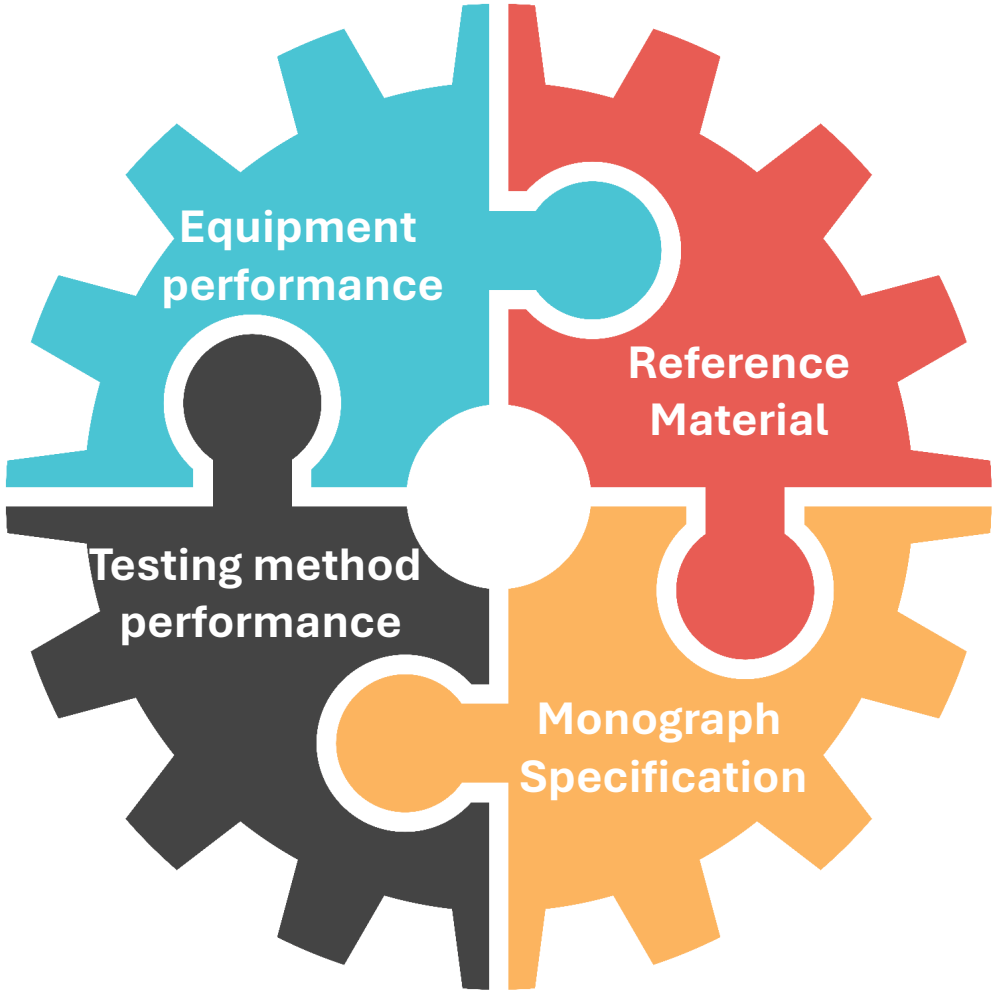
2.1 Intended use

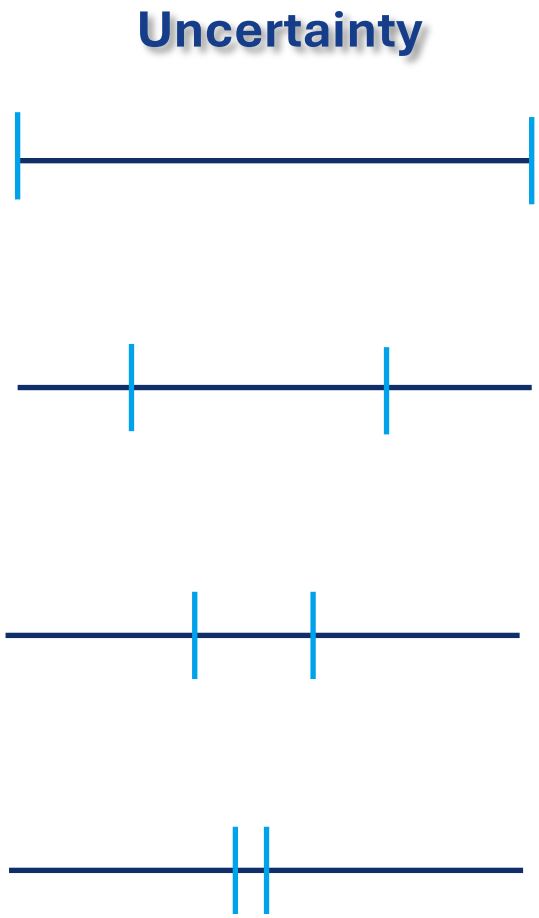
Reference Standard for laboratory tests as prescribed in the European Pharmacopoeia only.
Established for use with the monograph(s): 20542.

2.2 Analytical information related to intended use, when applicable

500 µg/mL solution of N-Nitroso-diethylamine (C4H10N2O) in methanol

CRS for equipment qualification





Specification range

Measurement method

Measuring equipment

Reference material used for equipment qualification

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CRS for equipment qualification

- **Ph. Eur. Chapter 2.2.48. Raman spectroscopy (04/2022:20248)**
  - Paracetamol for equipment qualification CRS (verification of the wavelength scale)
  - Calcium carbonate for equipment qualification CRS (spectral resolution)

- **Ph. Eur. Chapter 2.2.25. Absorption spectrophotometry, ultraviolet and visible (07/2024:20225)**
  - Nicotinic acid for equipment qualification CRS

- **Ph. Eur. Chapter 2.2.32. Loss on drying (07/2019:20232)**
  - Sodium aminosalicylate dihydrate for equipment qualification CRS

- **Ph. Eur. Chapter 2.5.12. Water: semi-micro determination (04/2018:20512)**
  - Sodium aminosalicylate dihydrate for equipment qualification CRS

- **Ph. Eur. Chapter 2.2.34. Thermal analysis (01/2016:20234)**
  - Calcium oxalate monohydrate CRS

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Frequently asked questions

Frequently Asked Questions (FAQ)

EDQM FAQ: https://faq.edqm.eu/display/FAQS/EDQM+FAQs

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Frequently Asked Questions (FAQ)

★ What is the intended use of EDQM standard?

★ Established **exclusively** for the intended use(s) described in the official texts of the Ph. Eur.

★ They are **officially valid** for the uses prescribed in the Ph. Eur. and represent an integral and essential part of the corresponding monograph(s).

★ Their **suitability for any other use is not guaranteed** and is the sole responsibility of the user.

★ EDQM reference standards are not intended for human or animal use.

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Frequently Asked Questions (FAQ)

Where can I find the content assigned to a reference standard?

- **Use in an assay:** given in the leaflet available in the Ph. Eur. reference standards database.

- **Use for quantification in the related substances test of a Ph. Eur. monograph**:
  - Content ≥ 95.0%: the content can be assumed to be 100% - No value provided in the leaflet
  - Content < 95.0%: "as is" content indicated in the leaflet

- **Use for qualitative use**: no value is assigned and no assumption can be made on the purity or content of the reference standard.

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

Frequently Asked Questions (FAQ)

Can secondary standard be established from EDQM reference standard ?

- **Not intended**, but possible for **the same assigned property and under given conditions**:
  - Identification by IR (qualitative) → Identification by IR (qualitative)
  - Identification by IR (qualitative) ≠ External standard in an assay (quantitative)

- **Traceability** to EDQM reference standard to be demonstrated

- **Suitability** for the intended use to be demonstrated (compatibility with the specification limits)

- **Establishment strategy** to be carefully chosen

⇨ **Under the full responsibility of the user**

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Frequently Asked Questions (FAQ)

Can secondary standard be established from EDQM reference standard ?

More info: https://www.edqm.eu/en/-/joint-edqm-usp-webinar-on-secondary-standards-considerations-in-traceability-to-pharmacopeial-standards-

- ★ Definitions,
- ★ Understanding uncertainty and risks,
- ★ Potential approach for establishment,
- ★ Study case.

Online training

Joint EDQM-USP Webinar on “Secondary standards - Considerations in traceability to pharmacopeial standards"

EUROPEAN PHARMACOPOEIA & REFERENCE STANDARDS | 10/10/2023 | ON-DEMAND WEBINAR

Conclusions

- **Establishment adapted to intended use** according to key quality attributes.
- Suitability for **off-label use** to be **demonstrated by user**.
- **Information** required for the **intended use** of a RS provided in the corresponding **monograph and leaflet.**
- Reference standards described in the **Ph. Eur. General methods** are a highly relevant tool to ensure **reliability** of measurement results.
- **Secondary standards**: possible, but **under the responsibility of the user**.

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