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

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Ph. Eur. Reference Standards: establishment and use

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Introduction

Introduction and background information:

→Reference standards – General aspects (Andrea Lodi)

→Ph. Eur. 5.12.

Outline:

→Establishment and use of CRS

→Medicinal product monographs

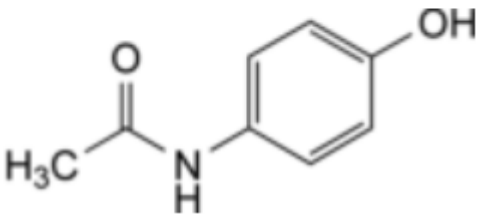
→Reference Standards for general chapters

Introduction

Overview:

PARACETAMOL

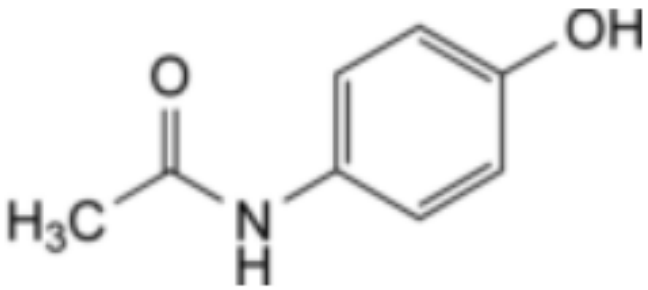
Paracetamolum



RS for identification

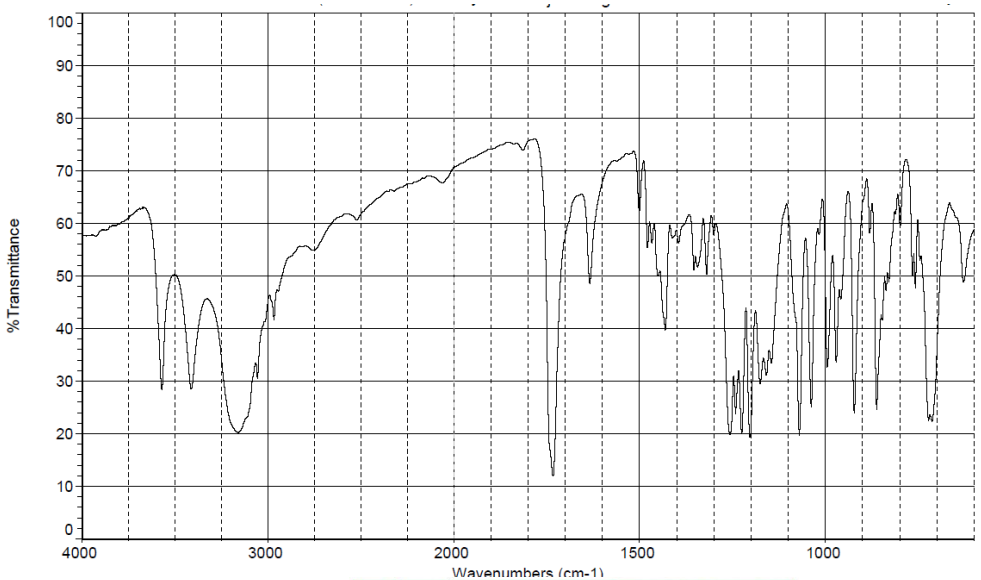
PARACETAMOL

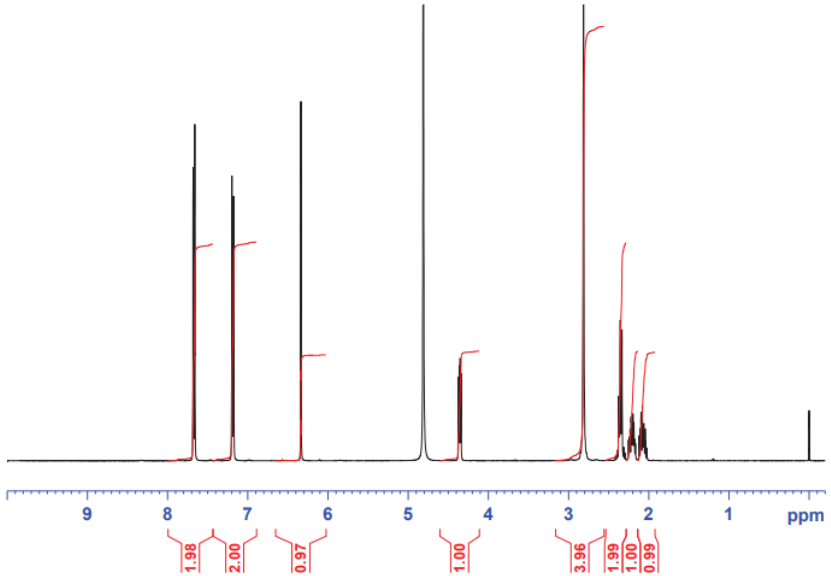
Paracetamolum

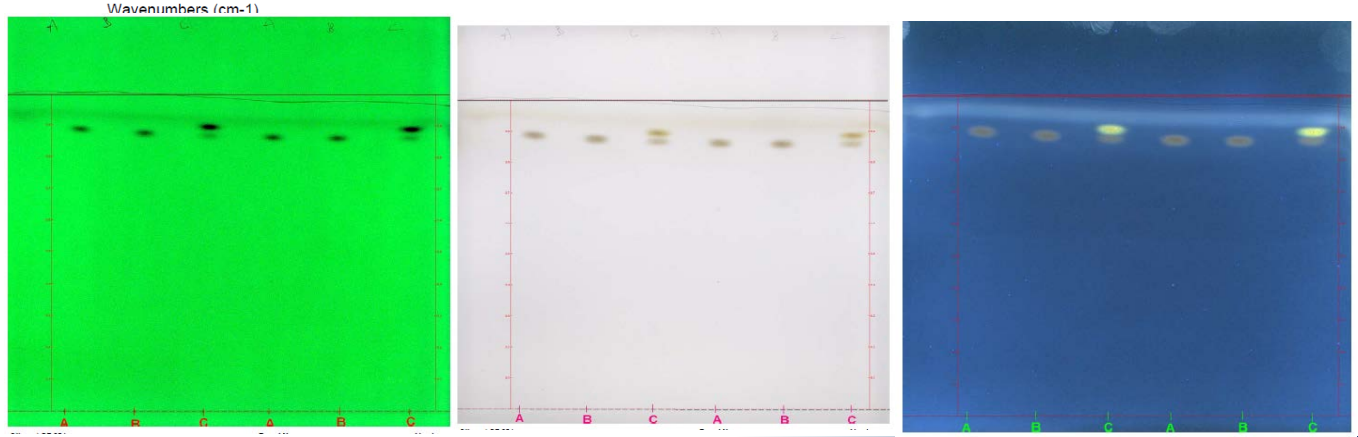


RS for identification

Identification of substances subject of a Ph. Eur. monograph







RS for identification

Establishment

→Key quality attribute= identity

→Identity: full structural elucidation (NMR, MS), whenever possible

→Compliance with relevant requirements of the monograph

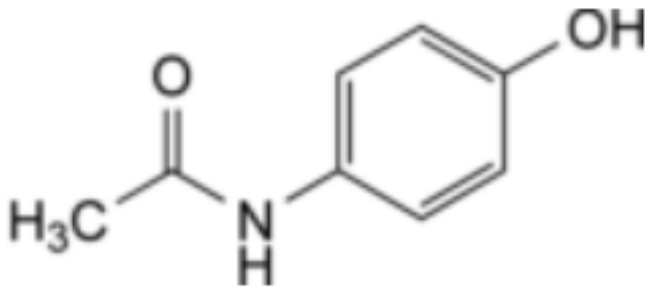
→Intended use

→Characterisation focussed on the substance itself rather than its impurities

Assay RS

PARACETAMOL

Paracetamolum



Assay RS

→ Quantitative benchmarks in assay procedures such as LC, GC, microbiology

→ Substance compliant with relevant requirements of corresponding Ph.Eur. monograph

→ Exceptional cases: other salt form, other hydrate, lyophilised RS

→ 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

Assay RS

Establishment

→Key quality attributes: Identity and content (qualitative and quantitative)

→Characterisation focused on substance and its impurities

→Identity

→Compliance with relevant requirements of the monograph

→Volatile impurities (LOD, residual solvents (HS-GC) and water)

→Inorganic impurities (sulfated ash for screening, further testing may be required)

Assay RS

Establishment (continued)

→Homogeneity (LOD or water, residual solvents in specific cases)

→Confirmation of assigned content/ purity by orthogonal methods (qNMR, elemental analysis, 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

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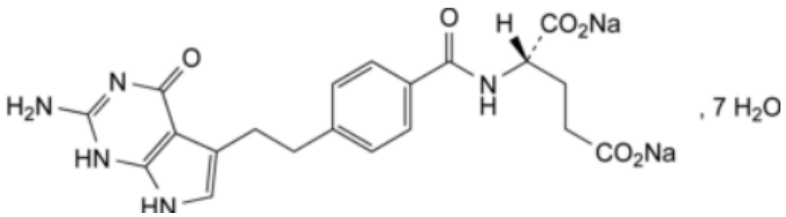
corrected 10.0



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 for chromatography R and dilute to 200.0 mL with the same solvent.

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

Column:

- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: base-deactivated 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.

Example: Pemetrexed disodium heptahydrate CRS 3

Characterisation EDQM Lab

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
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 collaborative 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)	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	-	-

Test	Atom	Theoretical value[1]	Experimental value	%RSD	n
Elemental analysis (contracted out to SGS France)	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.

Example: Pemetrexed disodium heptahydrate CRS 3

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Result
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	-

Example: Pemetrexed disodium heptahydrate CRS 3

Content assignment

[100% (m/m) - water% (m/m) by KF – inorganic impurities% (m/m) - residual solvents% (m/m)] x [100% - sum of impurities by relative%] / 100%

=

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

RS for control of related substances (qualitative)

RS for control of related substances (qualitative)

Qualitative RS for impurity control

→Chromatographic separation techniques (LC, GC, TLC)

→Batch testing: identification of signals (specified impurities or CF)

→System suitability testing

→Similarities in establishment

RS for control of related substances (qualitative)

RS strategy

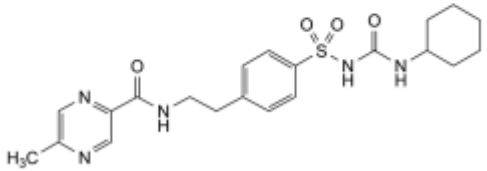
→ Single substance <-> mixtures

RS for control of related substances (qualitative)

04/2019:0906

GLIPIZIDE

Glipizidum



$C_{21}H_{27}N_5O_4S$ [29094-61-9] M_r 445.5

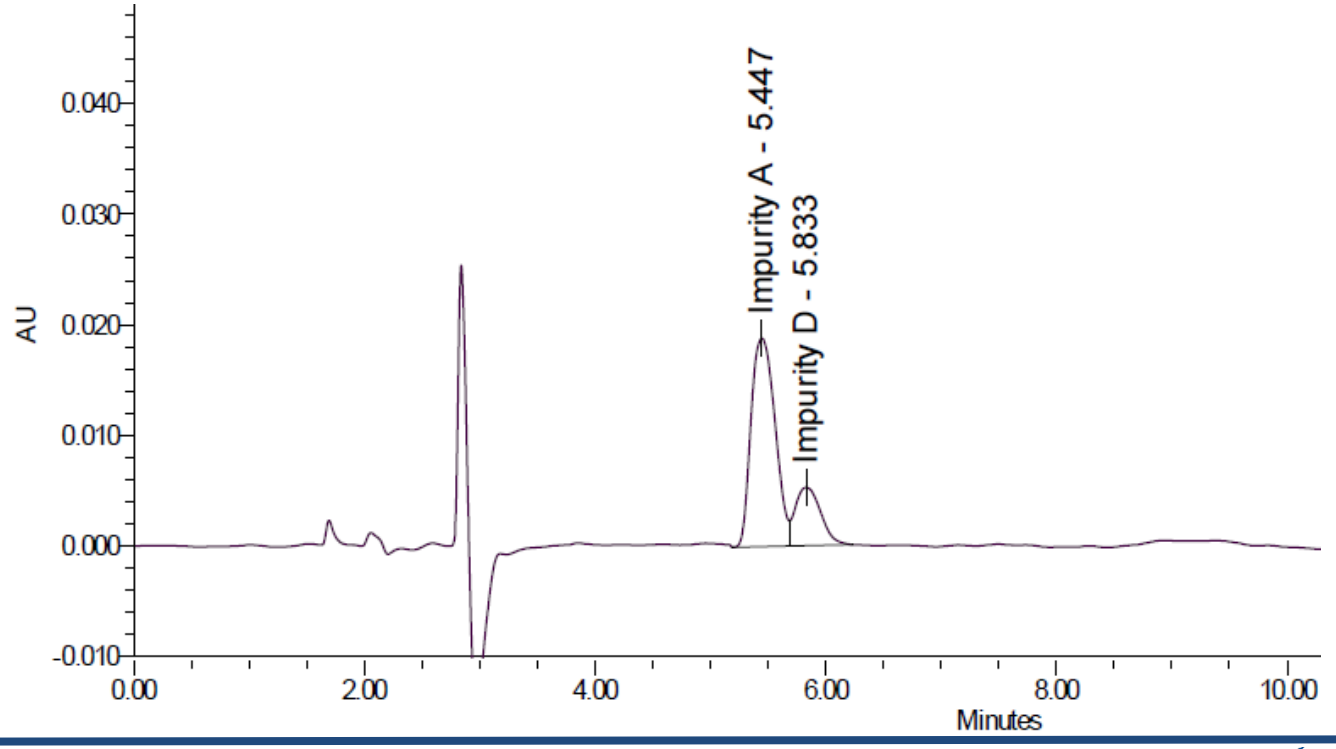
Related substances. Liquid chromatography (*2.2.29*).

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 (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).

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.



RS for control of related substances (qualitative)

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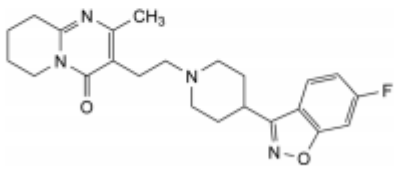
corrected 7.4

RISPERIDONE

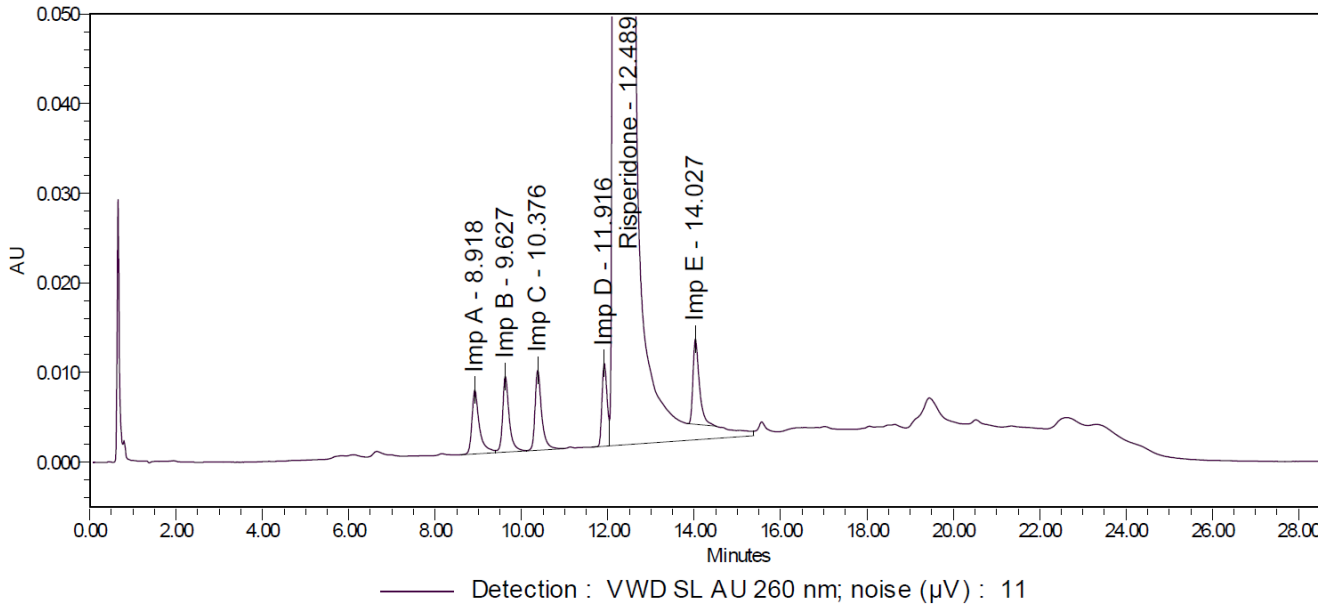
Risperidonum



$C_{23}H_{27}FN_4O_2$ [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*.

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.



RS for control of related substances (qualitative)

RS strategy

→ Single substance <-> mixtures

→ 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)

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

→Key quality attribute: identity (qualitative)

→Full structural elucidation, when possible

→Intended use

→Characterisation is less elaborated than for RS used quantitatively

RS for control of related substances (qualitative)

Establishment: mixture RS

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

→Identity of impurity peaks

→Spiking with authentic samples

→Homogeneity

→Intended use

RS for control of related substances (quantitative)

RS for control of related substances (quantitative)

Use

→ Mostly in chromatographic methods

→ External standard for impurities with a response very different from that of substance subject of the monograph

→Otherwise, correction factor is given (see 2.2.46.)

RS for control of related substances (quantitative)

→Content of RS is critical: ≥ 95.0 % or not?

→Single substance RS

→Materials obtained via processes that do not guarantee the required degree of purity and homogeneity

→Salt form has impact on use

- →easier to handle, less hygroscopic, volatile, procurement issues...
- →solubility?
- →need for stoichiometric conversion factor?

RS for control of related substances (quantitative)

→Stoichiometric conversion factor:

→Specification limit for impurity in same salt form

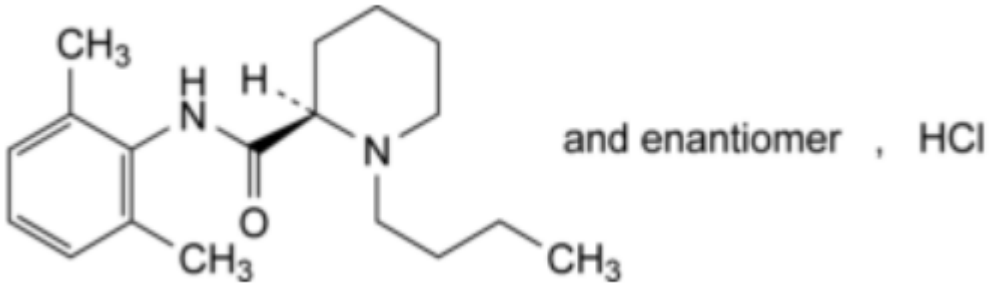
→Need to identify presence and identity of counter-ion

→Different from the monograph form

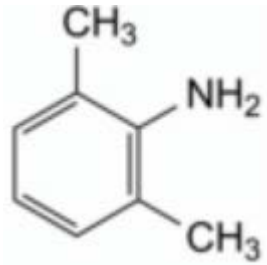
→Exception: if impurity cannot form the salt

RS for control of related substances (quantitative)

→Stoichiometric conversion factor: example



Bupivacaine hydrochloride



Bupivacaine impurity F

2.2 Analytical information related to intended use, when applicable

Bupivacaine impurity F CRS 3 is supplied as the free base.

For the calculation of the amount of bupivacaine impurity F in the monograph for bupivacaine hydrochloride, mepivacaine hydrochloride or xylazine hydrochloride for veterinary use, multiply the peak area of bupivacaine impurity F obtained with reference solution (a) by a stoichiometric conversion factor of Mr A /Mr B = 0.8

Note: Molecular masses used for the calculation of the stoichiometric conversion factor in this leaflet:
Mr A: Bupivacaine impurity F as free base: C8H11N --- 121.2 g/mol
Mr B: Bupivacaine impurity F as hydrochloride-salt: C8H11N * HCl --- 157.6 g/mol

RS for control of related substances (quantitative)

Establishment

→Key quality attributes: identity and content

→Identity: Full structural elucidation, if possible

→Identity of counter-ion: specific or screening

→Related substances: method of intended use (LC/GC)

→Volatile impurities: Loss on drying, thermogravimetry or water (+ residual solvents)

RS for control of related substances (quantitative)

Establishment

→Inorganic impurities: Sulfated ash (if amount allows), total ash or screening

→qNMR

→Homogeneity

→Content assignment (when <95.0%): mass balance or qNMR

→Orthogonal methods

RS for control of related substances (quantitative)

Example: Phenobarbital impurity A CRS 1

→Analytical results

→Identity: confirmed

→Loss on drying: 0.1 %

→LC-purity: 99.7 %

} Mass balance: 99.6 %
No need for assigned content

→Content by qNMR (expressed ‘as is’, as free base): 79 %

→Elemental analysis: does not match the theoretical composition

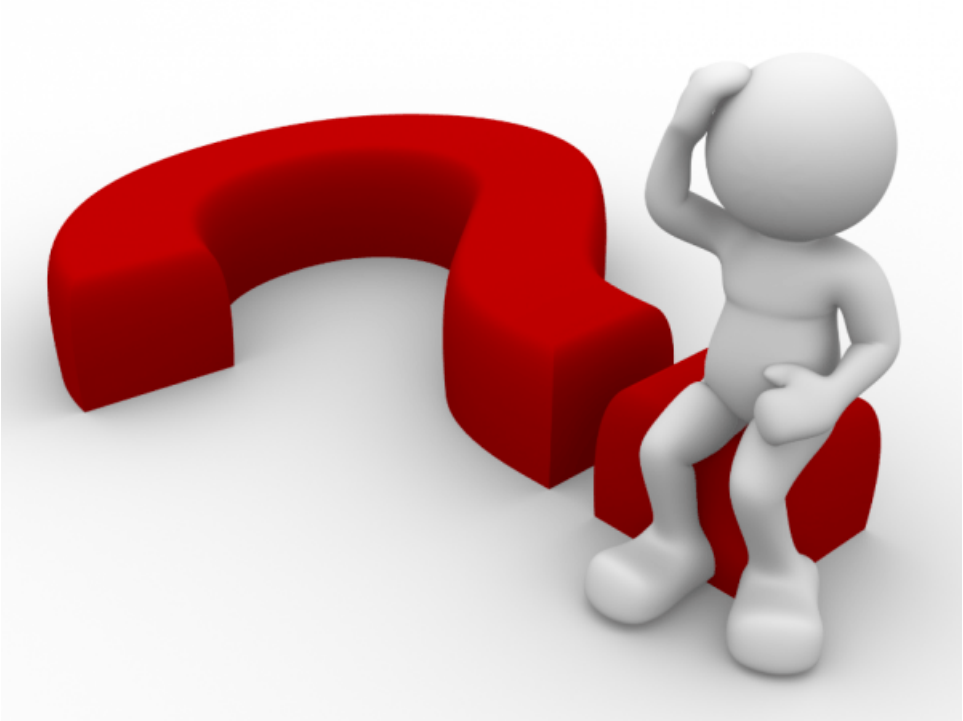
Investigation

→Identification of nitrate (not on CoA)

→Quantification of nitrate by ion-exchange chromatography: 20.6 %

RS for medicinal product monographs

Can we use the RS used in the corresponding monograph for the API?



RS for medicinal product monographs (identification)

RS for identification

→Identity already certified

→Independent of technique

→Use existing RS

07/2018:2938

RALTEGRAVIR TABLETS

Raltegraviri compressi

IDENTIFICATION

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

A. Record the UV spectrum of the principal peak in the chromatograms obtained with the solutions used in the assay with a diode array detector in the range of 190-400 nm.

Results: the UV spectrum of the principal peak in the chromatogram obtained with the test solution is similar to the UV spectrum of the principal peak in the chromatogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

Reference solution (a). Dissolve 22.0 mg of *raltegravir potassium CRS* in the solvent mixture and dilute to 200.0 mL with the solvent mixture.

RS for medicinal product monographs (assay)

RS suitability depends on the assay and related substances methods

→Same methods → Use existing assay RS with its assigned content

→Different methods (assay and/or related substances)

→Compare selectivity

→Similar: Use existing assay RS and its assigned content

→Not similar: Use a different RS

One assay RS = one assigned content!

RS for medicinal product monographs (impurity control)

RS presented as mixtures

→Specific related substances for MP (degradation products): separate RS

→Same related substances : use existing RS (Different methods→ add chromatogram to leaflet)

Impurity RS (quantification)

→RS for quantification of related substances in substances for pharmaceutical use are generally suitable also for the corresponding medicinal products

Reference Standards for general chapters

Reference Standards for general chapters

Only few examples:

→CRS used in limit tests

→CRS for quantitative use

→RS for equipment qualification

CRS USED IN LIMIT TESTS

Ph. Eur. 2.4.24. Identification and control of residual solvents

2.4.24. IDENTIFICATION AND CONTROL OF RESIDUAL SOLVENTS

The test procedures described in this general method may be used:

i. for the identification of the majority of Class 1 and Class 2 residual solvents in an active substance, excipient or medicinal product when the residual solvents are unknown;

ii. as a limit test for Class 1 and Class 2 solvents when present in an active substance, excipient or medicinal product;

iii. for the quantification of Class 2 solvents when the limits are greater than 1000 ppm (0.1 per cent) or for the quantification of Class 3 solvents when required.

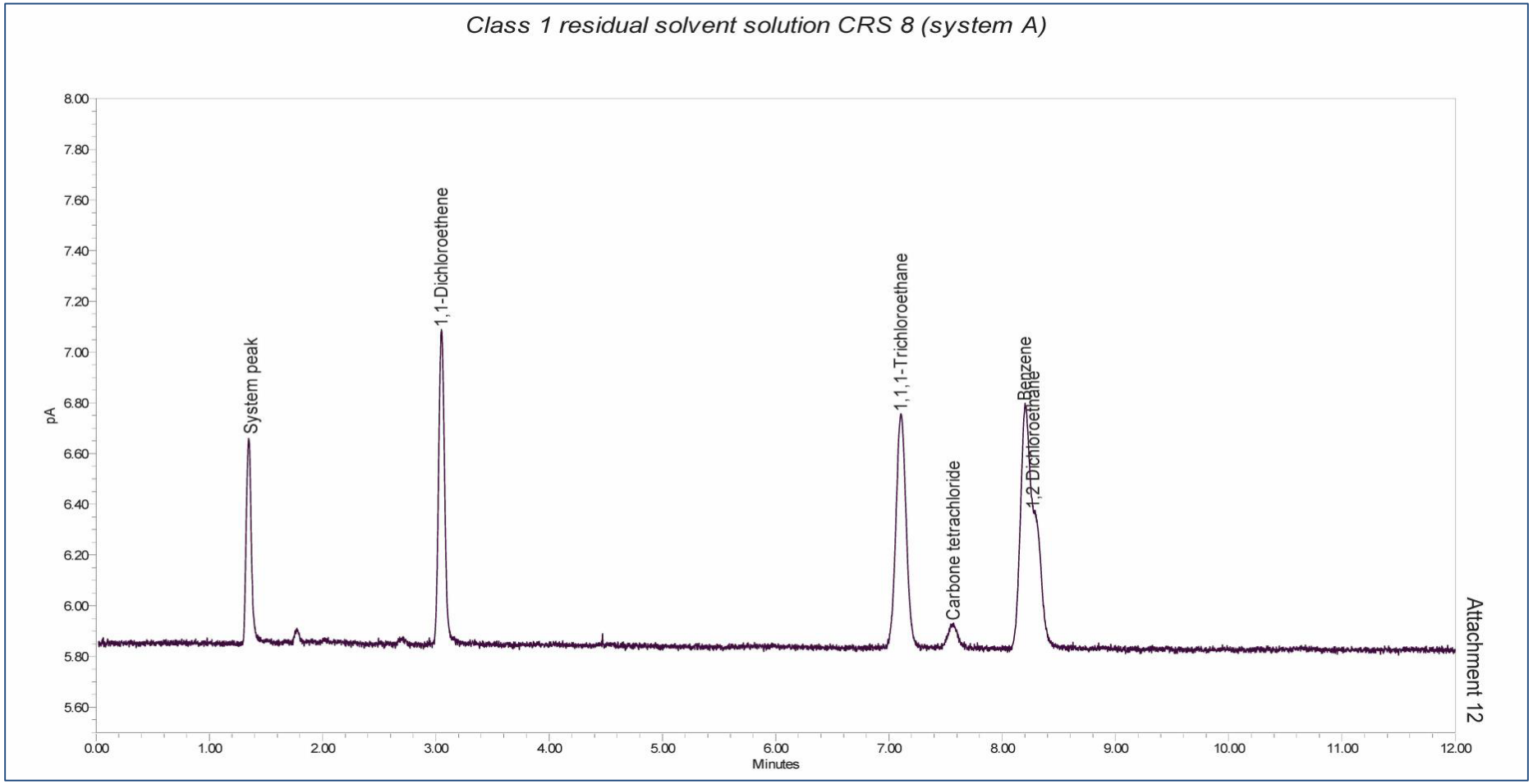
Solvent solution (a). To 1.0 mL of *Class 1 residual solvent solution CRS*, add 9 mL of *dimethyl sulfoxide R* and dilute to 100.0 mL with *water R*. Dilute 1.0 mL of this solution to 100 mL with *water R*. Dilute 1.0 mL of this solution to 10.0 mL with *water R*.

The reference solutions correspond to the following limits:

- benzene: 2 ppm;
- carbon tetrachloride: 4 ppm;
- 1,2-dichloroethane: 5 ppm;
- 1,1-dichloroethene: 8 ppm;
- 1,1,1-trichloroethane: 10 ppm.

CRS USED IN LIMIT TESTS

Class 1 residual solvent solution CRS



CRS FOR QUANTITATIVE USE

2.4.20. DETERMINATION OF ELEMENTAL IMPURITIES

VALIDATION REQUIREMENTS:

ACCURACY

Verify the accuracy using a certified reference material or by performing a test for recovery. *Elemental impurity solutions CRS* may be used.

The recovery may be determined on a sample of the substance to be examined, spiked with a known quantity of a reference standard of the element of interest (3 concentration levels in the range of 50-150 per cent of the intended specification limit, even if the original concentration of the reference standard is at the specified value), in triplicate.

CRS for quantitative use

Elemental impurity chemical reference substances (CRS) :

→Class 1

→Lead solution CRS (0.9996 mg/g)

→Cadmium solution CRS (1.0012 mg/g)

→Mercury solution CRS (0.999 mg/g)

→Arsenic solution CRS (1.001 mg/g)

→Class 2

→Nickel solution CRS (1.001 mg/g)

→Palladium solution CRS (0.996 mg/g)

→Platinum solution CRS – coming soon

CRS for quantitative use

Elemental impurity chemical reference substances (CRS) :

→Potential sources of elemental contamination

→Traceable to the SI (International System of units of measurements)

→Enable metrologically sound determination

→In relation with Ph. Eur. Chapter 2.4.20. describing the determination of elemental impurities.

CRS FOR QUANTITATIVE USE

INFORMATION LEAFLET Ph. Eur. Reference Standard

Arsenic solution CRS batch 1

1. Identification

Catalogue code: Y0002004 Unit Quantity: ca 10 mL

2. Scientific Information

2.1 Intended use

Reference Standard for laboratory tests as prescribed in the European Pharmacopoeia.
Established for use with chapter: 20420.

2.2 Analytical information

Mass fraction of arsenic in the solution:	1.001 mg/g
Associated expanded uncertainty:	U = 0.015 mg/g, k = 2
Density of the solution:	1.015 g/mL at 20.0 °C
Solvent composition:	about 2.5 % m/m nitric acid

Traceability to the SI base units kilogram and mole is achieved through an uninterrupted chain of calibration measurements that link arsenic solution CRS 1 to a primary material characterised by a National Metrology Institute at the highest metrological level (High purity copper BAM-Y001).
The IUPAC standard atomic weight for arsenic shall be applied.
Dilutions of arsenic solution CRS 1 should be made with 2.5 % nitric acid.

RS for equipment verification / calibration

→No measurement processes devoid of error

→Dispersion

→Conformity assessment is focused on determining actual product errors

→Ensure that measurement capabilities of equipment are appropriate

→Dispersion small

RS for equipment verification / calibration

Sodium aminosalicylate dihydrate for equipment qualification:

→Used for various techniques (LOD, (semi-)micro determination of water, TGA)

Ph.Eur. 2.5.12. Water: Semi-micro determination

> ... Instrument qualification is carried out according to established quality system procedures, for example using a suitable certified reference material (**sodium aminosalicylate dihydrate for equipment qualification CRS** may be used).

→Qualify the instrument

SODIUM AMINOSALICYLATE DIHYDRATE FOR EQUIPMENT QUALIFICATION CRS

ESTABLISHMENT:

→ Test for compliance with the Ph. Eur. Monograph for Sodium aminosalicylate dihydrate

→ Inter-laboratory study

→ Homogeneity assessment on a representative number of randomly sampled containers (n=37)

→ Calculation of the assigned property value as mean result of the inter-laboratory study

SODIUM AMINOSALICYLATE DIHYDRATE FOR EQUIPMENT QUALIFICATION CRS

ESTABLISHMENT:

→ Calculation of the associated expanded uncertainty

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

Where:

$U_{exp.}$ = expanded uncertainty

u_{IS} = standard uncertainty from inter-laboratory study

u_{hom} = standard uncertainty from homogeneity study

k = 2 (coverage factor at 95% confidence level)

SODIUM AMINOSALICYLATE DIHYDRATE FOR EQUIPMENT QUALIFICATION CRS

Extract of the leaflet accompanying the CRS:

2.1 Intended use

Reference Standard for laboratory tests as prescribed in the European Pharmacopoeia only.
Established for use with the monograph(s): 2.2.32., 2.5.12.,2.5.32.

2.5.12. – Semi-micro determination of water

Certified water content[1)]: 171.6 mg/g
Uncertainty[2)]: 1.0 mg/g

Test procedure: Carry out the test in triplicate using 100 mg of substance per determination.

Hydranal composite 5 was found suitable. If other solvents/titrants are used, carry the suitability test described in Ph. Eur. 2.5.12.

1) Unweighted mean value of means of accepted sets of results, each set having being obtained in a different laboratory with the method described above.
2) Estimated expanded uncertainty U with a coverage factor k = 2, corresponding to a level of confidence of about 95 % as defined in ISO/IEC Guide 98-3:2008 - Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995). Uncertainty contributions arising from characterisation as well as homogeneity assessments were taken into account.

SODIUM AMINOSALICYLATE DIHYDRATE FOR EQUIPMENT QUALIFICATION CRS

Additional leaflet info:

Suggested acceptance criteria:
Taking into account inter-laboratory standard deviation as well as the mean intra-laboratory standard deviation obtained 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):	167.3 mg/g to 173.7 mg/g

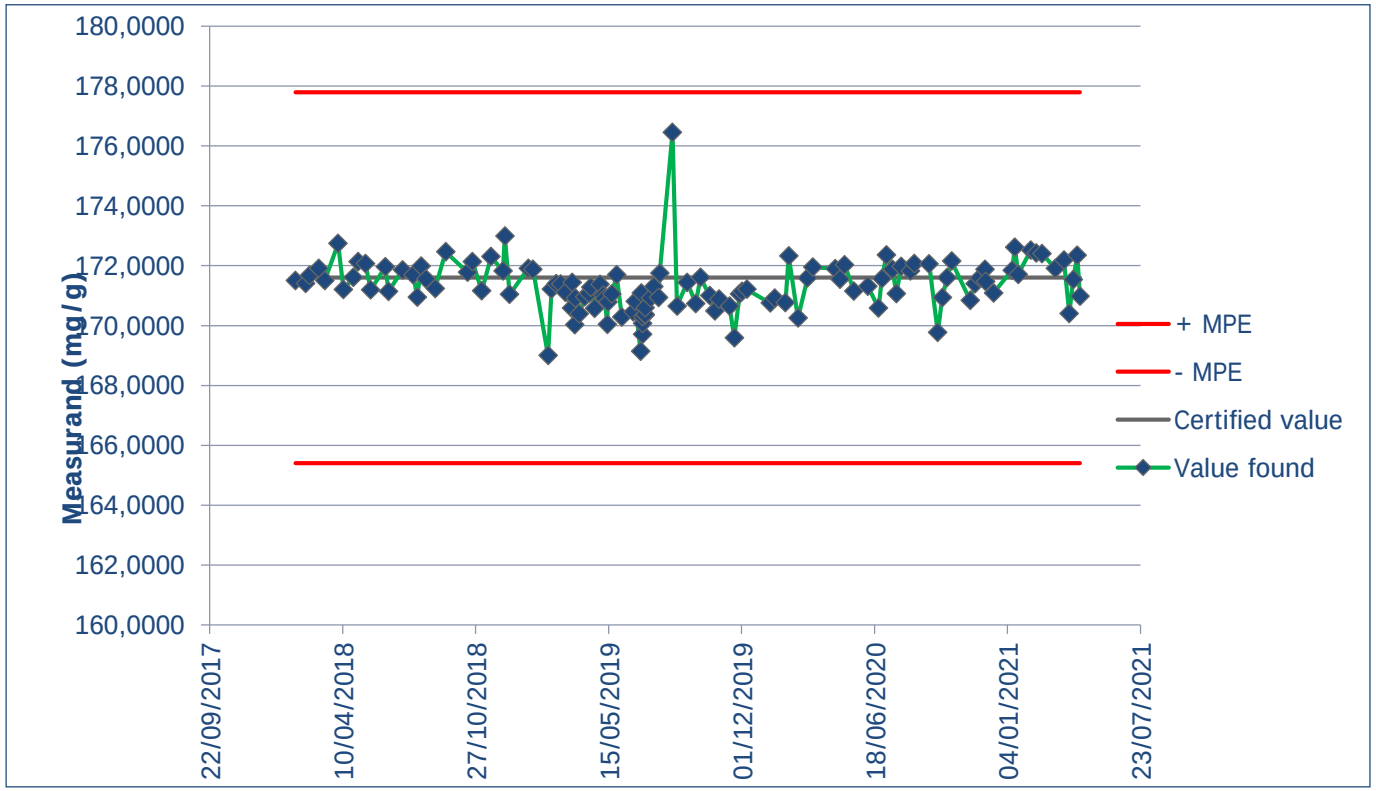
It is understood that a laboratory may apply a different approach to set acceptance criteria.

→ Other criteria may be chosen

→ Demonstrate suitability to competent authorities

SODIUM AMINOSALICYLATE DIHYDRATE FOR EQUIPMENT QUALIFICATION CRS

KF metrological equipment control chart



Check of systematic bias:

- Mean of 121 measurements: 171.3 mg/g
- Assigned value: 171.6 mg/g; U= 1mg/g

Measurement uncertainty ($U_{exp.}$, k=2): ± 1.3 mg/g

u= √ (u² trueness + u² precision), where:

u trueness= $\sqrt{bias^2 + (\frac{sd\ bias}{\sqrt{n}})^2 + u^2\ certif}$

u precision= sd deviation of all values, i.e. sd of values from control chart

Expanded U = 2u

Conclusions

→Establishment adapted to intended use according to key quality attributes

→Suitability for off-label use to be demonstrated by user

→In medicinal product monograph: use of same or additional CRS

→Reference standards described in the Ph. Eur. General methods are a highly relevant tool to ensure reliability of measurement results.

→Reference standards for equipment qualification are specifically characterised specimens that may be employed for several purposes.

Thank you for your attention

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