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

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Use of RS in specific monographs
RS for identification and RS for assay

2019 Training Session
"The European Pharmacopoeia"
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EDQM Laboratory Department

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

IDENTIFICATION

- **Identification methods that generally require comparison with an RS**
  - → infrared absorption spectrophotometry (IR)
  - → nuclear magnetic resonance spectrometry (NMR), if spectrum cannot be interpreted
  - → chromatographic separation techniques (LC, GC, TLC)
- **RS strategy**
  - → substance compliant with corresponding Ph.Eur. monograph
  - → in some justified cases, another form is used e.g. salt/hydrate (sample preparation needed)
  - → sometimes the identification RS is also used as external standard in the LC/GC assay
  - → other uses e.g. system suitability and peak identification are normally to be avoided

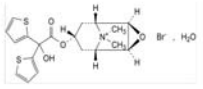
IDENTIFICATION

- **Example IR**

07/2010:2420

TIOTROPIUM BROMIDE MONOHYDRATE

Tiotropii bromidum monohydricum



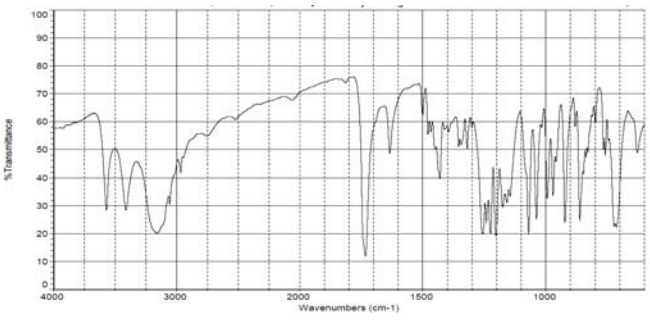
$C_{19}H_{22}BrNO_4S_2,H_2O$ M_r 490.4

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *tiotropium bromide monohydrate CRS*.

B. It gives reaction (a) of bromides (*2.3.1*).



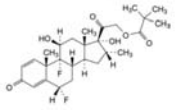
IDENTIFICATION

- **Example TLC**

01/2008:1327
corrected 6.0

FLUMETASONE PIVALATE

Flumetasoni pivalas



$C_{37}H_{46}F_2O_6$ [2002-29-1] M_r 494.6

IDENTIFICATION

First identification: A, B.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison: *flumetasone pivalate CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *acetone R*, evaporate to dryness on a water-bath and record new spectra using the residues.

B. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 10 mg of the substance to be examined in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (a). Dissolve 10 mg of *flumetasone pivalate CRS* in *acetone R* and dilute to 10 mL with the same solvent.

Reference solution (b). Dissolve 10 mg of *desoxycortone acetate CRS* in *acetone R* and dilute to 10 mL with the same solvent. Dilute 5 mL of this solution to 10 mL with reference solution (a).

Plate: *TLC silica gel F_{254} plate R*.

Mobile phase: add a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*.

Application: 5 µL.

Development: over a path of 15 cm.

Drying: in air.

Detection A: examine in ultraviolet light at 254 nm.

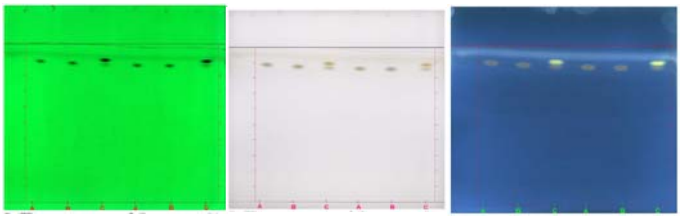
Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B: spray with *alcoholic solution of sulfuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results B: the principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability: reference solution (b):

– the chromatogram shows 2 clearly separated spots.



IDENTIFICATION

- **Example NMR**

07/2019:0828
corrected 10.0

HEPARINS, LOW-MOLECULAR-MASS

Heparina massae molecularis minoris

IDENTIFICATION

A. Nuclear magnetic resonance spectrometry (2.2.33).

Preparation: dissolve 0.200 g of the substance to be examined in a mixture of 0.2 mL of *deuterium oxide R* and 0.8 mL of *water R*.

Comparison: dissolve 0.200 g of the appropriate specific low-molecular-mass heparin reference standard in a mixture of 0.2 mL of *deuterium oxide R* and 0.8 mL of *water R*.

Operating conditions:

– *field strength*: 75 MHz;

– *temperature*: 40 °C;

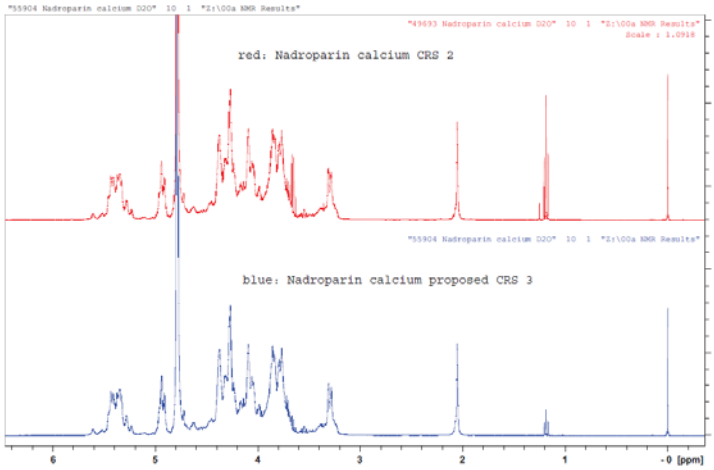
– *cell diameter*: 5 mm.

Processing:

– Fourier transformation;

– deuterated methanol reference signal set at 50.0 ppm.

Results: the ^{13}C NMR spectrum obtained is similar to that obtained with the appropriate specific low-molecular-mass heparin reference standard.



IDENTIFICATION

Technical guide for the elaboration of monographs (7th edition – 2015)

II.4.3. Infrared absorption spectrophotometry

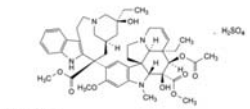
This method always necessitates the use of a **reference substance or a reference spectrum**. Reference substances are preferred to reference spectra. The latter are used where there are practical difficulties with providing a reference substance.

Note (not in technical guide): practical difficulties are e.g. toxicity, instability, risk for explosion.

01/2008:0748

VINBLASTINE SULFATE

Vinblastini sulfas



$C_{46}H_{58}N_4O_{10}S$ [143-67-9] M_r 909

IDENTIFICATION

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the *Ph. Eur. reference spectrum of vinblastine sulfate*.

IDENTIFICATION

European Directorate for the Quality of Medicines & HealthCare

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For any questions: www.edqm.eu (HelpDesk)

Ph. Eur. Reference Spectrum

VINBLASTINE SULFATE batch 1

Intended use

This official Reference Spectrum is to be used with the currently valid European Pharmacopoeia monograph(s) and/or general chapter(s) describing its suitable use.

Further information about the Reference Standards is available in the on-line catalogue currently at http://crs.edqm.eu.

Preparation

The spectrum has been recorded using 1.5 mg of substance prepared as a disc in 300 mg of potassium bromide R. The absorption maxima of polystyrene at 2850 cm^{-1} (3.51 µm), 1601 cm^{-1} (6.25 µm) and 1028 cm^{-1} (9.73 µm) have been superimposed on the spectrum.

Catalogue code: V0305000 Revision 01 Date of issue: 10/12/2007 Page 1/2

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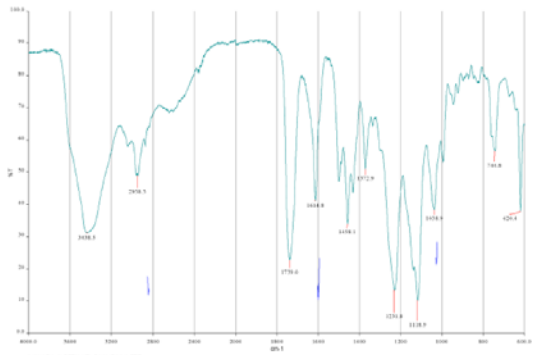


VINBLASTINE SULPHATE

Catalogue code: V0305000 Revision 01 Date of issue: 10/12/2007 Page 2/2

ASSAY

- **Assay methods that generally require the use of an RS**
  - → liquid chromatography (LC) / gas chromatography (GC)

  (→ direct UV absorption spectrophotometry = to be phased out for assay: use specific absorbance)
- **RS strategy**
  - → whenever possible, substance compliant with corresponding Ph.Eur. monograph
  - → higher amount of candidate material is required: extensive characterisation and increased amount per vial (sufficient for preparation of two solutions)
  - → content is assigned based on compendial tests + complementary tests (if needed)
  - → uncertainty of the assigned value shown to be negligible compared to content limits in the monograph

ASSAY

- **RS strategy (continued)**
  - → RS may also be used for identification
  - → other uses e.g. system suitability and peak identification are normally avoided
  - → in justified cases, a lyophilised RS or a different salt or hydrate is used e.g. due to instability, hygroscopicity, …

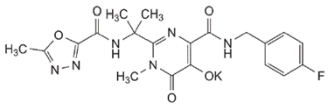
ASSAY

- **Example (assay RS = monograph substance)**

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*.

INFORMATION LEAFLET Ph. Eur. Reference Standard
Raltegravir potassium CRS batch 1

2.2 Analytical information related to intended use, when applicable
The "as is" content is : **99.1 % of C20H20FKN6O5**

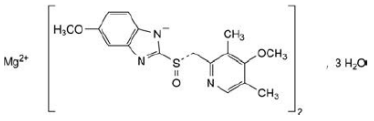
ASSAY

- **Example (assay RS ≠ monograph substance)**

07/2014:2372

ESOMEPRAZOLE MAGNESIUM TRIHYDRATE

Esomeprazolum magnesicum trihydricum



$C_{34}H_{36}MgN_6O_6S_2,3H_2O$ M_r 767.2

DEFINITION

Magnesium bis[5-methoxy-2-[(*S*)-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazol-1-ide] trihydrate.

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

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *esomeprazole magnesium trihydrate CRS*.

ASSAY

Liquid chromatography (*2.2.29*).

Calculate the percentage content of $C_{34}H_{36}MgN_6O_6S_2$ taking into account the assigned content of *omeprazole CRS*.

1 g of omeprazole is equivalent to 1.032 g of esomeprazole magnesium.

INFORMATION LEAFLET Ph. Eur. Reference Standard
Omeprazole CRS batch 5

2.2 Analytical information related to intended use, when applicable
The "as is" content is : **99.9 % of C17H19N3O3S (for 2787, 2372 and 2374)**

Thank you for your attention

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