Consultation documents

The International Pharmacopoeia

Atazanaviri sulfas
Atazanavir sulfate

This is a draft proposal for The International Pharmacopoeia (Working document QAS/13.566, December 2013).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

Molecular formula. C_{38}H_{52}N_{6}O_{7}•H_{2}SO_{4}

Relative molecular mass. 802.9

Chemical name. (3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl) phenyl]methyl]-2,5,6,10,13-pentaoazatetradecanedioic acid dimethyl ester, sulfate (1:1).

Description. A white or almost white powder.

Solubility. Freely soluble in methanol, practically insoluble in water.

Category. Antiretroviral (protease inhibitor).

Storage. Atazanavir sulfate should be kept in a tightly closed container at a temperature not exceeding 30°C.

Additional information. Atazanavir sulfate is slightly hygroscopic.

Requirements. Atazanavir sulfate contains not less than 98.0% and not more than 102.0% of C_{38}H_{52}N_{6}O_{7}•H_{2}SO_{4} calculated on the dried basis.

Identity tests

Either test A and D, or test B, C and D should be performed.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from atazanavir sulfate RS or with the reference spectrum of atazanavir sulfate.
B. Carry out test B.1, or where UV detection is not available, test B.2.

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 9.5 volumes of dichloromethane R and 0.5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 10 μl of each of 2 solutions in methanol R containing (A) 1 mg of the test substance per ml and (B) 1.0 mg of atazanavir sulfate RS per ml. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of air.

Examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described under test B.1, but using a plate containing silica gel R5 as the coating substance.

Spray the plate with potassium permanganate, basic (~5 g/l) TS. Examine the chromatogram in daylight. The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

C. The absorption spectrum of a 10 µg/ml solution in methanol R, when observed between 230 nm and 340 nm, exhibits two maxima at about 250 nm and 280 nm, respectively.

A 20 mg/ml solution yields Reaction A described under 2.1 General identification tests as characteristic of sulfates.

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to method A; not more than 20 µg/g.

**Sulfated ash (2.3).** Not more than 1.0 mg/g.

**Loss on drying.** Dry for 3 hours at 105 °C; it loses not more than 10.0 mg/g.

**pH value.** Apparent pH of a 10 mg/ml solution in carbon-dioxide-free water R and acetonitrile R (50:50, v/v), 2.0–2.5.¹

**Related substances.** Carry out the test as described under 1.14.4 High–performance liquid chromatography, using a column (150 mm x 4.6 mm) packed with end-capped base deactivated particles of silica gel the surface of which has been modified with chemically bonded octylsilyl groups (5 μm).² Use the following conditions for gradient elution:

Mobile phase A: 0.02 M phosphate buffer pH 3.5.

Mobile phase B: Acetonitrile R.

Prepare the phosphate buffer pH 3.5 by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate R in 800 ml of water R, adjust the pH to 3.5 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 ml with water R.

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¹ Value subject to confirmation.
² An Inertsil C8 column has been found suitable.
Prepare the following solutions using as diluent a mixture of equal volumes of water R and acetonitrile R. For solution (1) use 1 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 5 μg of Atazanavir sulfate per ml. For solution (3) mix 1 ml of solution (1) with 4.5 ml of water R and 0.5 ml of sodium hydroxide (10 g/l) TS and heat the mixture in a water-bath at 85°C for 15 min.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of 30°C.

Inject 20 μl of solution (3). The test is not valid unless the resolution between the peak due to atazanavir (retention time about 22 minutes) and the peak with a relative retention of about 1.2 is at least 4.

Inject alternatively 20 μl each of solutions (1) and (2).

In the chromatograms obtained with test solution (1), the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Assay**

Dissolve 0.150 g, accurately weighed, in 30 ml of methanol R and sonicate for 10 minutes. Then add 30 ml of water and titrate with sodium hydroxide (0.1 mol/l), carbonate-free, VS. Determine the end-point potentiometrically as described under 2.6 Non-aqueous titration *Method A*. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 40.145 mg of $C_{38}H_{52}N_6O_7\cdot H_2SO_4$.

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Atazanavir capsules

This is a draft proposal for The International Pharmacopoeia (Working document QAS/13.567, December 2013).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

Category. Antiretroviral (Protease Inhibitor).

Storage. Atazanavir sulfate capsules should be kept in a tightly closed container at a temperature not exceeding 30°C.

Additional information. Strength in the current WHO Model list of essential medicines: 100 mg, 150 mg, 300 mg of atazanavir (as sulfate). Strength in the current WHO Model List of essential medicines for children: 100 mg, 150 mg, 300 mg of atazanavir (as sulfate).

Each mg of atazanavir \((C_{38}H_{52}N_6O_7)\) is equivalent to 1.139 mg of atazanavir sulfate \((C_{38}H_{52}N_6O_7 \cdot H_2SO_4)\).

Requirements. Comply with the monograph for Capsules.

Definition. Atazanavir capsules contain atazanavir sulfate. They contain not less than 90.0% and not more than 110.0% of the amount of atazanavir, \(C_{38}H_{52}N_6O_7\), stated on the label.

Identity tests

A. Carry out test A.1, or where UV detection is not available, test A.2.

A.1 Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 9.5 volumes of dichloromethane R and 0.5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 10 µl of each of the following 2 solutions in methanol R. For solution (A) disperse a quantity of the contents of the capsules containing about 20 mg of atazanavir in 10 ml of methanol R, sonicate for 10 minutes, allow to cool to room temperature, dilute to 20 ml, filter and use the filtrate. For solution (B) use 1.1 mg of atazanavir sulfate RS per ml.

After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution (A) corresponds in position, appearance, and intensity to that obtained with solution (B).

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test A.1 but using a plate containing silica gel R5 as the coating substance. Spray with potassium permanganate, basic (~5 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

B. Disperse a quantity of the contents of the capsules containing about 20 mg of atazanavir in 10 ml of methanol R, sonicate for 10 min, allow to cool to room temperature, dilute to 20 ml and filter. Dilute 1 ml of the filtrate to 100 ml with methanol R. The absorption
spectrum (1.6) of the resulting solution, when observed between 230 and 340 nm, exhibits
two maxima at about 250 nm and 280 nm, respectively.

C. To a quantity of the contents of the capsules equivalent to 0.2 g of atazanavir add 10 ml
of a mixture of 1 volume of water R and 1 volume of acetonitrile R, shake and filter. The
filtrate yields Reaction A described under 2.1 General identification tests as characteristic of
sulfates.

Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage
forms, using as the dissolution medium, 900 ml of dissolution buffer pH 2.5 TS, and rotating the
paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium
through an in-line filter. Allow the filtered sample to cool to room temperature. Measure the
absorbance (1.6) of a 1 cm layer of the resulting solution, suitably diluted if necessary, at the
maximum at about 250 nm. Determine the content of atazanavir (C$_{38}$H$_{52}$N$_{6}$O$_{7}$) in the medium
from the absorbance obtained from a solution of known concentration of atazanavir sulfate RS.
The amount in solution for each capsule is not less than 75% (Q) of the amount stated on the
label.

Related substances. Carry out the test as described under 1.14.4 High–performance liquid
chromatography, using a stainless steel column (150 mm x 4.6 mm) packed with end-capped
base deactivated particles of silica gel the surface of which has been modified with chemically
bonded octylsilyl groups (5 μm). Use the following conditions for gradient elution:

Mobile phase A: 0.02 M phosphate buffer pH 3.5.

Mobile phase B: Acetonitrile R.

Prepare the phosphate buffer pH 3.5 by dissolving 2.72 g of anhydrous potassium dihydrogen
phosphate R in 800 ml of water R, adjust the pH to 3.5 by adding phosphoric acid (~105 g/l)
dilute to 1000 ml with water R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2 –10</td>
<td>70–60</td>
<td>30–40</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>10–30</td>
<td>60–50</td>
<td>40–50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–45</td>
<td>50–30</td>
<td>50–70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>45–50</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>50–52</td>
<td>30–70</td>
<td>70–30</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>52–60</td>
<td>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

Prepare the following solutions using as diluent a mixture of equal volumes of acetonitrile R
and water R. For solution (1) weigh and mix the contents of 20 capsules. Transfer a quantity
of the mixed contents equivalent to 20 mg of atazanavir into a 20 ml volumetric flask. Add
about 10 ml of the diluent, sonicate for 10 minutes, allow to cool to room temperature, make
up to volume and filter. For solution (2) dilute a suitable volume of solution (1) with the diluent
to obtain a concentration of 10 μg of atazanavir per ml. For solution (3) mix 1 ml of solution
(1) with 4.5 ml of water R and 0.5 ml of sodium hydroxide (10 g/l) TS and heat the mixture in a
water bath at 85°C for 15 min.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet
spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of
30°C.

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1 An Inertsil C8 column has been found suitable.
Inject 20 µl of solution (3). The test is not valid unless the resolution between the peak due to atazanavir (retention time about 22 minutes) and the peak with a relative retention of about 1.2 is at least 4.

Inject alternatively 20 µl each of solutions (1) and (2).

In the chromatograms obtained with test solution (1), the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with reference solution (2) (2.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Assay**

Either test A or test B may be applied.

Carry out the test as described under **1.14.4 High–performance liquid chromatography**, using a stainless steel column (150 mm x 4.6 mm) packed with end-capped base deactivated particles of silica gel the surface of which has been modified with chemically bonded octylsilyl groups (5 μm).²

As the mobile phase, use a solution prepared as follows: 60 volumes of acetonitrile R and 40 volumes of 0.02 M phosphate buffer pH 3.5. Prepare the phosphate buffer pH 3.5 by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate R in 800 ml of water R, adjust the pH to 3.5 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 ml with water R.

Prepare the following solutions using as diluent a mixture of equal volumes of acetonitrile R and water R. For solution (1) weigh and mix the contents of 20 capsules. Transfer a quantity equivalent to 20.0 mg of atazanavir, accurately weighed, into a 20 ml volumetric flask. Add about 10 ml of the diluent, sonicate for about 10 minutes, allow to cool to room temperature and make up to volume. Filter a portion of this solution, discarding the first few ml. Dilute 1.0 ml of the filtrate to 10.0 ml with the diluent. For solution (2) use 0.11 mg of atazanavir sulfate RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use a ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of 30°C.

Inject alternatively 20 µl each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of atazanavir.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of atazanavir, \( C_{38}H_{52}N_6O_7 \), using the declared content of \( C_{38}H_{52}N_6O_7 \) in atazanavir sulfate RS.

Weigh and mix the contents of 20 capsules. Transfer a quantity equivalent to 20 mg of atazanavir, accurately weighed, to a 20 ml volumetric flask. Add about 10 ml of methanol R, sonicate for about 10 minutes, allow to cool to room temperature and make up to volume. Filter a portion of this solution through a 0.45 µm filter, discarding the first few ml of the filtrate. Dilute 1.0 ml of the filtrate to 10.0 ml with methanol R. Measure the absorbance of this solution in a 1 cm layer at the maximum at about 250 nm against a solvent cell containing methanol R. Calculate the content of \( C_{38}H_{52}N_6O_7 \), using an absorptivity value of 15.9 (\( A_{1\text{cm}1\%} = 159 \)).³

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² An Inertsil C₈ column has been found suitable.

³ Value subject to confirmation.
Implementation of the revised general monograph on parenteral preparations in *The International Pharmacopoeia*: Limits for the test for bacterial endotoxins (3.4)

This is a revised draft proposal for *The International Pharmacopoeia* (Working document QAS/13.539/Rev.1, January 2014).

The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/). Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

**Limits for bacterial endotoxins in monographs on parenteral preparations**

During the forty-seventh meeting of the Expert Committee on Specifications for Pharmaceutical Preparations in October 2012 a revision of the general monograph on parenteral preparations was adopted.

One of the major changes to the monograph on parenteral preparations was the required compliance of all parenteral preparations with the test for bacterial endotoxins (or, where justified, pyrogens). As a consequence individual monographs on injectable dosage forms in *The International Pharmacopoeia* (Ph.Int.) were investigated with a view to add a limit for bacterial endotoxins to each monograph that currently does not include such a requirement. The endotoxin limits shown in Table 1 are proposed for inclusion in *The International Pharmacopoeia*. The limits are determined following the approaches listed in Annex 1.

**Table 1. Proposed limits for bacterial endotoxins in Ph.Int. monographs on parenteral preparations lacking such specification**

<table>
<thead>
<tr>
<th>Ph.Int. monographs on parenteral preparations currently lacking limits for the bacterial endotoxins test</th>
<th>Proposed limits for the bacterial endotoxins test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemether injection</td>
<td>Less than 1.56 IU of endotoxin per mg Artemether</td>
</tr>
<tr>
<td>Artemotil injection</td>
<td>Less than 1.04 IU of endotoxin per mg Artemotil</td>
</tr>
<tr>
<td>Ephedrine sulfate injection</td>
<td>Less than 1.7 IU of endotoxin per mg Ephedrine sulfate</td>
</tr>
<tr>
<td>Ergometrine hydrogen maleate injection</td>
<td>Less than 700 IU of endotoxin per mg of ergometrine hydrogen maleate</td>
</tr>
<tr>
<td>Melarsoprol injection</td>
<td>Less than 1.39 IU of endotoxin per mg Melarsoprol</td>
</tr>
<tr>
<td>Magnesium sulfate injection</td>
<td>Less than 0.18 IU of endotoxin per mg magnesium sulfate heptahydrate</td>
</tr>
<tr>
<td>Oxytocin injection</td>
<td>Less than 0.5 IU of endotoxin per IU of Oxytocin</td>
</tr>
<tr>
<td>Pentamidine isetionate powder for injections (^2)</td>
<td>Less than 1.25 IU of endotoxin per mg Pentamidine isetionate</td>
</tr>
<tr>
<td>Prednisolone sodium phosphate injection (^3)</td>
<td>Less than 4.09 IU of endotoxin per mg Prednisolone</td>
</tr>
<tr>
<td>Quinine dihydrochloride injection</td>
<td>Less than 1.0 IU of endotoxin per mg Quinine dihydrochloride</td>
</tr>
<tr>
<td>Zidovudine intravenous infusion</td>
<td>Less than 1.0 IU of endotoxin per mg Zidovudine</td>
</tr>
</tbody>
</table>

1. The complete phrase to be used in *The International Pharmacopoeia* should be: “Bacterial Endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains … (phrase of the table to be added).”

2. Title of the monograph to be changed to: Pentamidine isetionate for injection.

3. Title of the monograph to be changed to: Prednisolone phosphate injection.
Further changes to *The International Pharmacopoeia* following the implementation of the revised general monograph on parenteral preparations

The following additional changes to *The International Pharmacopoeia* are proposed:

In the new **general monograph on Parenteral preparations** the statement is made that:

“For powders and concentrates for injections and intravenous infusions, the amount of the preparation to be tested and the nature and volume of the liquid in which it is to be dissolved, suspended or diluted is specified in the individual monograph.”

It is proposed to delete this sentence since the preparation of the sample solution is described in Chapter 3.4 Test for bacterial endotoxins. The text requires that samples should be dissolved or diluted in aqueous solutions so that the final solutions do not exceed the maximum valid dilution (MVD).

In the **monograph on Metronidazole injection** the following provision for the test for bacterial endotoxins is made:

“Carry out the test as described under 3.4 Test for bacterial endotoxins. Dilute the injection, if necessary, with water LAL to give a solution containing 5 mg per ml (solution A). Solution A contains not more than 3.5 IU of endotoxin per ml. Carry out the test using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.”

Again, details of the sample solution preparation should be deleted and the sentence should be changed to read:

“Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 3.5 IU of endotoxin per ml.”

In general, two phrases are used to specify endotoxin limits in *The International Pharmacopoeia*:

“… not more that x IU of endotoxin per mg/ml …” ; and
“… not more that x IU of endotoxin RS per mg/ml …”.

All expressions using “IU of endotoxin RS” should be changed to “IU of endotoxin”. Chapter 3.4 Test for bacterial endotoxins already requires the use of the WHO International Standard for endotoxin when performing the test (or an endotoxin reference standard that has been calibrated against this standard; see section Preparation of standard endotoxin stock solution).

**Annex 1**

**Calculation of bacterial endotoxin limits**

The endotoxin limits proposed in Table 1 are calculated using the following approaches:

For the monograph on Ergometrine hydrogen maleate injection, the limit given in *The International Pharmacopoeia* for the active pharmaceutical ingredient (for parenteral use) was applied.

For the monographs on Ephedrine sulfate injection, Magnesium sulfate injection, Oxytocin injection, Prednisolone sodium phosphate injection and Zidovudine intravenous infusion, limits given in monographs of other pharmacopoeias were taken over.

For the monographs on Artemether injection, Artemotil injection, Melarsoprol injection, Pentamidine isethionate powder for injections and Quinine dihydrochloride injection, the endotoxin limits were calculated using recommendations given in Chapter 3.4 Test for bacterial endotoxins.
The endotoxin limit for parenteral preparations, defined on the basis of dose, is equal to:

\[
\text{Endotoxin limit} = \frac{K}{M}
\]

\( K = \) threshold pyrogenic dose of endotoxin per kilogram of body mass (i.e. 5.0 IU per kg body weight for any route of administration other than intrathecal; 2.5 IU per kg body weight for intravenous route of radiopharmaceuticals; 0.2 IU per kg body weight for intrathecal route).

\( M = \) maximum recommended bolus dose of product per kilogram of body mass. (When the product is to be injected at frequent intervals or infused continuously, \( M \) is the maximum total dose administered in a single hour period.)

Table 2 lists data used in the calculation of the proposed bacterial endotoxin limits.

### Table 2. Data used in the calculation of bacterial endotoxin limits

<table>
<thead>
<tr>
<th>Ph.Int. monographs lacking endotoxin limit</th>
<th>Endotoxin limits in pharmacopoeias</th>
<th>Information on dosage and route of application</th>
<th>K</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemether injection</td>
<td>&quot;Intramuscular injection for adults, artesunate 2.4 mg/kg BW IV or IM given on admission (time = 0), then at 12 h and 24 h, then once a day is the recommended treatment. Artemether, or quinine, is an acceptable alternative if parenteral artesunate is not available: artemether 3.2 mg/kg BW IM given on admission then 1.6 mg/kg BW per day. &quot;</td>
<td>5 IU/kg 3.2 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artemotil injection</td>
<td>&quot;Artecef® 150 must only be applied via the intramuscular route. Medical treatment consists of a 3-day course. The initial dose consists of an injection of 4.8 mg artemotil per kg body weight evenly divided over both anterior thighs. The follow-up doses consist of 1.6 mg per kg body weight after 6, 24, 48 and 72 hours in alternating thighs.&quot;</td>
<td>5 IU/kg 4.8 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ephedrine sulfate injection</td>
<td>Ephedrine sulfate injection (USP 36): NMT 1.7 USP Endotoxin Unit per mg of ephedrine sulfate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ergometrine hydrogen maleate injection</td>
<td>Ergometrine hydrogen maleate (Ph.Int. 4.3): NMT 700.0 IU of endotoxin RS per mg substance for parenteral use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melarsoprol injection</td>
<td>&quot;Treatment of T. brucei rhodesiense and T. brucei gambiense with meningoencephalitic involvement [ ], by slow intravenous injection, ADULT and CHILD, dose gradually increased from 1.2 mg/kg to maximum of 3.6 mg/kg daily in courses of 3–4 days with intervals of 7–10 days between courses; [ ].&quot;</td>
<td>5 IU/kg 3.6 mg/kg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued/
<table>
<thead>
<tr>
<th>Ph.Int. monographs lacking endotoxin limit</th>
<th>Endotoxin limits in pharmacopoeias</th>
<th>Information on dosage and route of application</th>
<th>K</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulfate injection</td>
<td>Magnesium sulfate injection (USP 36): NMT 0.09 USP Endotoxin Unit per mg of magnesium sulfate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxytocin injection</td>
<td>Oxytocin Injection (USP 36): NMT 35.7 Endotoxin Units per USP Oxytocin Unit Oxytocin (EP 7.8): less than 300 IU per mg Oxytocin, corresponding to less than 0.5 IU per IU of Oxytocin</td>
<td>&quot;Visceral leishmaniasis (unresponsive to, or intolerant of, antimonial compounds), by deep intramuscular injection or by intravenous infusion, ADULT and CHILD, 4 mg/kg 3 times a week for 5–25 weeks or longer, until 2 consecutive splenic aspirates taken 14 days apart are negative.&quot;3 (page 186)</td>
<td>5 IU/kg</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>Pentamidine isethionate powder for injections</td>
<td>Prednisolone sodium phosphate injection (USP 36): NMT 5.0 USP Endotoxin Units per mg of prednisolone phosphate</td>
<td>&quot;Treatment of multidrug-resistant <em>P. falciparum</em> malaria (in patients unable to take quinine by mouth), by slow intravenous infusion (over 4 hours), ADULT, initially 20 mg/kg (quinine dihydrochloride), followed by 10 mg/kg (quinine dihydrochloride) every 8 hours; CHILD, initially 20 mg/kg (quinine dihydrochloride), followed by 10 mg/kg (quinine dihydrochloride) every 12 hours; initial dose should be halved in patients who have received quinine, quinidine or mefloquine during the previous 12–24 hours.&quot; 3 (page 198)</td>
<td>5 IU/kg</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>Prednisolone sodium phosphate injection</td>
<td>Zidovudine injection (USP 36): NMT 1.0 USP Endotoxin Unit per mg of zidovudine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2 Product Information Leaflet, Artecef BV 150 mg/ml injection. See WHO Prequalification website at www.who.int/prequal – Dossier assessment – WHO Public Inspection Reports (WHOPARs) for MA027 and MA028.

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Revision of general monograph: Suppositories

This is a draft proposal for The International Pharmacopoeia (Working document QAS/14.571, February 2014).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. The proposed general monograph is part of the review of general monographs endorsed by the Expert Committee at its 42nd meeting. It is proposed to replace the current general monograph on suppositories with a general monograph including solid, liquid and semi-solid dosage forms intended for rectal application.]

RECTAL PREPARATIONS

Definition

Rectal preparations are liquid, semi-solid or solid preparations that may contain one or more active ingredients. They are intended for rectal application in order to obtain a systemic or local effect.

Rectal preparations may require the use of excipients of various types. Any excipient must be proven through product development studies not to adversely affect the stability of the final product, nor the availability of the active ingredient(s) at the site of action; incompatibility between any of the components of the dosage form should be avoided.

The different categories of rectal preparations include:

- suppositories;
- rectal capsules;
- rectal solutions, emulsions and suspensions;
- powders and tablets for rectal solutions and suspensions;
- semi-solid rectal preparations.

Manufacture

The following information is intended to provide broad guidelines concerning main steps to be followed during production.

Manufacturing and filling processes for rectal preparations should meet the requirements of good manufacturing practices (GMP).

During development, the effectiveness of any antimicrobial preservative present in the preparation shall be demonstrated to the satisfaction of the relevant regulatory authority.

During development it must be demonstrated that the nominal contents can be withdrawn from the container of liquid and semi-solid rectal preparations presented in single-dose containers.

In the manufacture, packaging, storage and distribution of rectal preparations, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are
Consultation documents

provided in the chapter Microbial examination of non-sterile products: acceptance criteria for pharmaceutical preparations, published in the Supplementary information section.

In the manufacture of rectal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size.

Throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate in-process controls. These should be designed to guarantee the effectiveness of each stage of production.

**Labelling**

Every rectal preparation must comply with the labelling requirements established under GMP.

The label should include:

1. name of the pharmaceutical product;
2. name(s) of the active ingredient(s); International Nonproprietary Names (INN) should be used whenever possible;
3. amount of active ingredient(s) in a dose unit and the number of dose units in the container or the amount of active ingredient(s) in suitable dose volume and the volume of the container;
4. where applicable, the name of any added antimicrobial agent;
5. batch (lot) number assigned by the manufacturer;
6. expiry date and, when required, the date of manufacture;
7. any special storage conditions or handling precautions that may be necessary;
8. directions for use, warnings and precautions that may be necessary;
9. name and address of the manufacturer or the person responsible for placing the product on the market.

**REQUIREMENTS FOR SPECIFIC TYPES OF RECTAL PREPARATIONS**

**Suppositories**

**Definition**

Suppositories are solid single-dose preparations intended for rectal application. They are prepared by moulding or compression. The shape, volume and consistency of suppositories are suitable for rectal application.

Suppositories contain one or more active ingredients dispersed or dissolved in a suitable basis that may be soluble or dispersible in water or may melt at body temperature. When prepared by moulding, suppository bases such as magrogols, gelatinous mixtures consisting of, for example, gelatin, water and glycerol, hydrogenated vegetable oils, hard fat or cocoa butter are usually employed.

Excipients such as diluents, adsorbents, surface-active agents preferably of nonionic type, lubricants, antimicrobial preservatives and colouring matter authorized by the appropriate national or regional authority, may be added when necessary.
**Manufacture**

It is common to use a suppository base in which the active ingredient(s) does not dissolve in order to avoid problems associated with partition between the molten or softened base and the rectal liquid. The release of the active ingredient(s) may in case of a suspension be dependent on sedimentation of the solid particles in the molten or softened base to the interface of the rectal liquid. The particle size of the active ingredient(s) should therefore be optimized to take both sedimentation and dissolution in the rectal liquid into account.

In the manufacture of suppositories containing dispersed active ingredient(s), measures are taken to ensure a suitable and controlled particle size.

When prepared by moulding, the medicated mass, sufficiently liquefied by heating, is poured into suitable moulds. The suppositories solidify on cooling. In certain cases, it is also possible to cold-mould by compression in a suitable press.

The softening time is determined according to the text *Softening time determination of lipophilic suppositories*, published in the Supplementary information section.

A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from suppositories.

Packaging must be adequate to protect suppositories from light, excessive heat, moisture, and damage due to handling and transportation. It is necessary to ensure that the suppositories can be released from the packaging easily and without damage.

**Visual inspection**

Suppositories are elongated, smooth and have a uniform texture and appearance.

Evidence of physical and/or chemical instability is demonstrated by noticeable changes in:

- surface texture or form, and
- colour and odour.

**Disintegration**

Suppositories comply with 5.4 *Disintegration test for suppositories and rectal capsules* unless intended for sustained release. For suppositories with a lipophilic base, examine after 30 minutes, and for suppositories with a water-soluble base, examine after 60 minutes.

**Uniformity of mass**

Suppositories comply with 5.2 *Uniformity of mass of single-dose preparations*.

**Uniformity of content**

Suppositories comply with 5.1 *Uniformity of content of single-dose preparations* when the content of active ingredient is 5 mg or less per suppository or 5% or less of the total mass. If the suppository has more than one active ingredient, the requirement applies only to those active ingredients that fall into the above category. If the test for uniformity of content is prescribed, the test for uniformity of mass is not required.

**Containers**

Suppositories should be supplied in a well-closed container. The container material should not adversely affect the quality of the preparation, nor should it allow diffusion into or across the container material or yield foreign substances into the preparation.
Rectal capsules

Definition

Rectal capsules are solid, single-dose preparations generally similar to soft capsules as defined in the monograph on Capsules, except that they may have a lubricating coating. The contents of rectal capsules are usually solutions or suspensions of the active ingredient(s) in non-aqueous liquids, e.g. vegetable oil, or in semi-solid mixtures of suitable excipients.

Manufacture

See the manufacturing instructions for soft capsules. Other considerations for soft capsule suppositories include the study of and suitable controls for pH, leakage and pellicle formation.

A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from rectal capsules.

Visual inspection

Rectal capsules are of elongated shape, smooth and have a uniform external appearance.

Unpack and inspect at least 20 rectal capsules. They should be smooth and undamaged. Evidence of physical instability is demonstrated by gross changes in physical appearance, including hardening or softening, cracking, swelling, mottling or discoloration of the shell.

Disintegration

Rectal capsules comply with 5.4 Disintegration test for suppositories and rectal capsules unless intended for sustained release. Examine the state of the rectal capsules after 30 minutes unless otherwise prescribed in the individual monograph.

Uniformity of mass

Rectal capsules comply with the requirements to capsules in 5.2 Uniformity of mass of single-dose preparations.

Uniformity of content

Rectal capsules comply with 5.1 Uniformity of content of single-dose preparations when the content of active ingredient is 5 mg or less per suppository or 5% or less of the total mass. If the rectal capsule has more than one active ingredient, the requirement applies only to those active ingredients that fall into the above category. If the test for uniformity of content is prescribed, the test for uniformity of mass is not required.

Rectal solutions, emulsions and suspensions

Definition

Rectal solutions, emulsions and suspensions (also called enemas) are liquid preparations intended for rectal application to obtain a local or systemic effect, or they may be intended for diagnostic purposes. They contain one or more active ingredients dissolved or dispersed in water, glycerol, macrogols, vegetable oil or mixtures thereof.

Rectal emulsions may show evidence of phase separation but are readily redispersed on shaking. Rectal suspensions may show a sediment that is readily dispersible on shaking to give a suspension that remains sufficiently stable to enable the correct dose to be delivered.
They may contain excipients, for example to adjust the viscosity of the preparation, to adjust or stabilize pH, to increase the solubility of the active ingredient(s) and to stabilize the preparation. The excipients do not, at the concentrations used, cause undue local irritation.

Rectal solutions, emulsions and suspensions are supplied in single-dose containers containing a volume in the range of 2.5 ml to 2000 ml. The container is adapted to deliver the preparation to the rectum or is accompanied by a suitable applicator.

**Powders and tablets for rectal solutions and suspensions**

**Definition**

Powders and tablets intended for the preparation of rectal solutions or suspensions are single-dose preparations that are dissolved or dispersed in water or other suitable solvents at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent aggregation of the particles.

After dissolution or suspension, the preparation complies with the requirements for rectal solutions or rectal suspensions as appropriate.

**Disintegration**

Tablets for rectal solutions or suspensions comply with the following test: Place one tablet in a 250 ml beaker containing 200 ml of water R at 15–25 °C. Repeat the operation on five additional tablets. The tablets comply with the test if each of the six tablets used in the test dissolves or disintegrates within 3 minutes, unless otherwise specified in the individual monograph.

**Labelling**

The label states:

- the method of preparing the rectal solution or suspension;
- when necessary, conditions and duration of storage of the final preparation.

**Semi-solid rectal preparations**

**Definition**

Semi-solid rectal preparations are ointments, creams or gels intended for local treatment in the rectum.

They are usually supplied as single-dose preparations in containers adapted to deliver the preparation to the rectum or are accompanied by a suitable applicator.

Semi-solid rectal preparations comply with the requirements for Topical semi-solid dosage forms.

**Manufacture**

When supplied in multidose containers, the expected reproducibility of the delivery of the intended volume must be ensured.

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Revision of method of analysis:
5.3 Disintegration test for tablets and capsules

This is a draft proposal for The International Pharmacopoeia (Working document QAS/14.572, February 2014).

The working document with line numbers and tracked changes is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

[Note from the Secretariat.
It is proposed to include a disintegration test for large tablets in the test for disintegration of tablets and capsules. The proposed method is reproduced with permission from The European Pharmacopoeia.

This test is provided to determine whether tablets or capsules disintegrate within the prescribed time when placed in a liquid medium under the experimental conditions presented below.

For the purposes of this test disintegration does not imply complete dissolution of the unit or even of its active constituent. Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the discs, if used, is a soft mass having no palpably firm core.

Use apparatus A for tablets and capsules that are not greater than 18 mm. For larger tablets and capsules use apparatus B.

Test A. Tablets and capsules of normal size

This text is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). Some editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia.

Apparatus. The apparatus (Figure 1) consists of a basket-rack assembly, a 1000 ml, low-form beaker, 138–160 mm in height and having an inside diameter of 97–115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid between 35 °C and 39 °C, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute, through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below the surface of the fluid and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke and the change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.
Basket-rack assembly. The basket-rack assembly consists of six open-ended transparent tubes, each 75.0–80.0 mm long and having an internal diameter of 20.70–23.00 mm and a wall 1.0–2.8 mm thick; the tubes are held in a vertical position by two plates, each 88–92 mm in diameter and 5.00–8.50 mm in thickness, with six holes, each 22–26 mm in diameter, equidistant from the centre of the plate and equally spaced from one another. Attached to the lower surface of the lower plate is a woven stainless steel wire mesh, which has a plain square weave with 1.8–2.2 mm apertures and with a wire diameter of 0.570–0.660 mm. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis.

The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained. The basket-rack assembly conforms to the dimensions shown in Figure 1.

Discs. The use of discs is permitted only where specified or allowed. Each tube is provided with a cylindrical disc 9.35–9.65 mm thick and 20.55–20.85 mm in diameter. The disc is made of a suitable, transparent plastic material having a specific gravity of 1.18–1.20. Five parallel 1.9–2.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered 5.8–6.2 mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centres of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.5–1.7 mm and its bottom edges lie at a depth of 1.50–1.80 mm from the cylinder’s circumference. The parallel side of the trapezoid on the top of the cylinder has a length of 9.2–9.6 mm and its centre lies at a depth of 2.5–2.7 mm from the cylinder’s circumference. All surfaces of the disc are smooth. If the use of discs is specified, add a disc to each tube and operate the apparatus as directed under procedure. The discs conform to the dimensions found in Figure 1.
The use of automatic detection employing modified discs is permitted where the use of discs is specified or allowed. Such discs must comply with the requirements of density and dimension given in this chapter.

**Procedure.** Place one dosage unit in each of the six tubes of the basket and if specified add a disc. Operate the apparatus using water as the immersion fluid unless another liquid is specified and maintain its temperature at 35–39 °C. At the end of the specified time, lift the basket from the fluid and observe the dosage units: all of the dosage units have disintegrated completely. If one or two dosage units fail to disintegrate, repeat the test on 12 additional dosage units. The requirements of the test are met if not less than 16 of the 18 dosage units tested are disintegrated.

**Test B. Large tablets and large capsules**

*This test is reproduced with permission from* The European Pharmacopoeia.

**Apparatus.** The main part of the apparatus (Figure 2) is a rigid basket-rack assembly supporting 3 cylindrical transparent tubes 77.5 ± 2.5 mm long, 33.0 mm ± 0.5 mm in internal diameter, and with a wall thickness of 2.5 ± 0.5 mm. Each tube is provided with a cylindrical disc 31.4 ± 0.13 mm in diameter and 15.3 ± 0.15 mm thick, made of transparent plastic with a relative density of 1.18–1.20. Each disc is pierced by 7 holes, each 3.15 ± 0.1 mm in diameter, 1 in the centre and the other 6 spaced equally on a circle of radius 4.2 mm from the centre of the disc. The tubes are held vertically by 2 separate and superimposed rigid plastic plates 97 mm in diameter and 9 mm thick, with 3 holes. The holes are equidistant from the centre of the plate and equally spaced. Attached to the under side of the lower plate is a piece of woven gauze made from stainless steel wire 0.63 ± 0.03 mm in diameter and having mesh apertures of 2.0 ± 0.2 mm. The plates are held rigidly in position and 77.5 mm apart by vertical metal rods at the periphery. A metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly at a constant frequency of between 29 and 32 cycles per minute, through a distance of 55 ± 2 mm.

The assembly is suspended in the specified liquid medium in a suitable vessel, preferably a 1 litre beaker. The volume of the liquid is such that when the assembly is in the highest position the wire mesh is at least 15 mm below the surface of the liquid, and when the assembly is in the lowest position the wire mesh is at least 25 mm above the bottom of the beaker and the upper open ends of the tubes remain above the surface of the liquid. A suitable device maintains the temperature of the liquid at 35–39 °C.

The design of the basket-rack assembly may be varied provided the specifications for the tubes and wire mesh are maintained.

**Method.** Test 6 tablets or capsules either by using 2 basket-rack assemblies in parallel or by repeating the procedure. In each of the 3 tubes, place 1 tablet or capsule and, if prescribed, add a disc; suspend the assembly in the beaker containing the specified liquid. Operate the apparatus using water as the immersion fluid unless another liquid is specified for the prescribed period, withdraw the assembly and examine the state of the tablets or capsules. To pass the test, all 6 of the tablets or capsules must have disintegrated.
Figure 2. Diagram for disintegration apparatus B  
(dimensions are expressed in millimeters)
Revision of method of analysis:

5.4 Disintegration test for suppositories and rectal capsules

This is a draft proposal for The International Pharmacopoeia (Working document QAS/14.573, February 2014).

The working document with tracked changes and line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat:
At its meeting in October 2012 the Expert Committee on Specifications adopted a general method for determination of the softening time of lipophilic suppositories to be placed in the Supplementary information section of The International Pharmacopoeia. Consequently, it is proposed to revise chapter 5.4 Disintegration test for suppositories and to replace the current Method 2 by the method for determination of the softening time of lipophilic suppositories.

This test measures the time elapsed for a suppository placed in water to disintegrate.

The disintegration test determines whether suppositories soften or disintegrate within a prescribed time when placed in an immersion fluid using the experimental conditions described below.

Disintegration is considered to be achieved when:
- dissolution is complete;
- the components of the suppositories have separated, e.g. melted fatty substances have collected on the surface of the liquid, insoluble powders have fallen to the bottom and soluble components have dissolved or are distributed in one or more of the ways described in Methods 1 and 2;
- there is softening of the test sample, usually accompanied by an appreciable change of shape without complete separation of the components. The softening process is such that a solid core no longer exists when pressure is applied with a glass rod; or
- rupture of the gelatin shell or rectal capsule occurs resulting in release of the contents.

Method 1 (for water-soluble, hydrodispersible and fat-based suppositories and rectal capsules):

This test measures the time elapsed for a suppository placed in water to disintegrate.

Apparatus

The apparatus (Figure 1) consists of a 60 mm long cylinder of glass or transparent plastic and a metal device consisting of two perforated stainless steel discs, held about 30 mm apart. These discs each have 39 holes, 4mm in diameter, which are evenly spaced in a concentric pattern. The diameter of the discs is marginally inferior to that of the interior of the cylinder. Once inserted into the cylinder, the metal device is attached to the rim of the cylinder by means of three spring clips. The test is carried out using three such apparatuses, each containing a single test sample. Each apparatus is placed in a beaker with a minimum capacity of 4 litres filled with water unless otherwise prescribed. The beaker is fitted with a slow stirrer and a support that holds the apparatus vertically 90 mm below the surface of the water so that it can be inverted without emerging from the water.
Figure 1. Apparatus for water-soluble, hydrodispersible, and fat-based suppositories

A. Horizontal view. B. Vertical view. Measurements in mm.

Procedure

Unless otherwise described in the individual monograph, use water maintained at a temperature of 36–37 °C as the immersion fluid. The test requires three suppositories and the procedure is applied to each of the suppositories.

Place the sample on the lower disc of the metal device and then insert it into the cylinder. Place the apparatus into the beaker and invert it every 10 minutes without removing it from the liquid. Repeat the operation with the remaining two suppositories. Record the time required for the disintegration of the suppositories.

Unless otherwise stated in the individual monograph, for each of the three suppositories or rectal capsules, examine the state of the sample after 30 minutes for fat-based suppositories and rectal capsules, and after 60 minutes for water-soluble suppositories.

Method 2 (alternative for fat-based suppositories):

This test measures the time elapsed for a suppository placed in water to soften to the extent that it no longer offers resistance when a defined weight is applied.

The method is described in the chapter Softening time determination of lipophilic suppositories, published in the Supplementary information section.

The test requires three suppositories and the procedure is applied to each of the suppositories. Unless otherwise stated in the individual monograph, for each of the three suppositories, examine the state of the sample after 30 minutes.

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**RADIOPHARMACEUTICALS:**

**Sodium iodide (131I) solution**

This is a revised draft proposal for *The International Pharmacopoeia* (Working document QAS/13.547/Rev.2, March 2014).

The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/). Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidt@who.int.

Monographs: Radiopharmaceuticals: Specific monographs: 

*Natrii iodidi (131I) solutio* - Sodium iodide (131I) solution

**Latin.** Natrii iodide (131I) solutio.
**English.** Sodium iodide (131I) solution.
**Structural formula.** Na⁺…………⑴⁻
**Relative molecular mass.** 153.895.
**Empirical formula.** Na⁹¹
**Chemical name.** Sodium [131I] iodide
**Other names.** Natrii radioiodidum, Iodotope Sodium iodide-I 131
**Description.** Sodium iodide (131I) solution is a clear colourless solution. Iodine-131 has a half-life of 8.02 days.
**Category.** Diagnostic or therapeutic.
**Storage.** Stored at room temperature in a single-dose or multiple-dose containers.
**Labelling.** The label complies with the General monograph, the monograph of Radiopharmaceuticals.
**Manufacture.** No carrier added iodine-131 may be obtained by neutron bombardment of tellurium or by extraction from uranium fission products. Sodium iodide (131I) solution may contain sodium thiosulfate, sodium hydrogen carbonate or other suitable reducing agents and may contain a suitable buffer.
**Additional information.** Wherever V is used within the tests of this monograph, V is the maximum recommended dose, in millilitres.

**Requirements**

Complies with the monographs for Liquid preparations for oral use, Parenteral Preparations and with that for Radiopharmaceuticals as and where appropriate.

**Definition.** Sodium iodide solution is an aqueous solution containing radioactive (131I) in the form of sodium iodide (131I), suitable for either oral or intravenous administration.

The solution contains not less than 90% and not more than 110% of the declared radioactivity due to iodine-131 stated on the label at the reference date and time. Not less than 99.9% of the total radioactivity is due to iodine-131. Not less than 95% of the total iodine-131 radioactivity is present as iodide. It contains minute amounts of naturally occurring iodine 127. The specific activity is not less than 185 MBq per microgram of iodine at the reference date and time stated on the label. The iodide content of maximum recommended dose should not be more than 20 µg.
Identity tests

• Either tests A and C or tests B and C may be applied.

  A. Record the gamma-ray and X-ray spectrum using a suitable instrument with a sample of iodine-131, suitably diluted if needed. The spectrum is concordant with the reference spectrum of a specimen of iodine-131 in that it exhibits a major peak of 365 keV. Standardized iodine-131 solutions are available from laboratories recognized by the relevant national or regional authority.

  B. The half-life determined using a suitable detector system is between 7.61 and 8.42 days.

  C. Examine the radiochromatogram obtained in the test for radiochemical purity. The principal peak in the chromatogram obtained with the test solution (a) is similar in retention time to the principal peak in the chromatogram obtained with the reference solution (c).

pH value. Carry out the test as described under 1.13 Determination of pH or R1.5 under the monograph for Radiopharmaceuticals. pH is between 7.0 and 10.0 with 3.2.1 Test for sterility of non-injectable preparations, modified as described in the monograph for Radiopharmaceuticals. If intended for intravenous administration, it complies with 3.2 Test for sterility for injectable preparations, modified as described in the monograph for Radiopharmaceuticals. The solution may be released for use before completion of the test.

Bacterial endotoxins

Carry out the test as described under 3.4 Test for bacterial endotoxins, for solution intended for intravenous use modified as described in the monograph for Radiopharmaceuticals. The injection contains not more than 175/V (I.U of endotoxins per millilitre).

Radionuclidic purity. Record the gamma-ray and X-ray spectrum using a suitable instrument and measure the half-life using a suitable method. Determine the relative amounts of iodine-131, iodine-133, iodine-135 and other radionuclidic impurities that may be present. Iodine-133 has a half-life of 20.8 hours and exhibits major peaks of 530 keV and 875 keV. Iodine-135 has a half-life of 6.57 hours and exhibits major peaks of 527 keV, 1132 keV and 1260 keV. Not less than 99.9% of the total radioactivity is due to iodine-131.

Chemical purity

Iodide. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (0.25 m x 4.0 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm), maintain the temperature constant between 20 °C and 30 °C. Dissolve 5.844 g of sodium chloride R in 1000 mL of water R, add 650 µL of octylamine R and adjust to pH 7.0 with phosphoric acid R, add 50 mL of acetonitrile R and mix. Use the mixture as the mobile phase. Use flow rate of 1.5 mL/min, and spectrophotometer detector at 220 nm and radioactivity detector (connected in series) for detection. Prepare the test solution (a) which is the preparation to be examined. Prepare the test solution (b) by diluting test solution (a) using 0.05 M sodium hydroxide until the radioactivity is equivalent to about 74 MBq/mL and add an equal volume of a solution containing 1 g/L of potassium iodide R, 2 g/L of potassium iodate R and 10 g/L of sodium hydrogen carbonate R and mix. The reference solution (c) is prepared by diluting 1 mL of a 26.2 mg/L solution of potassium iodide R to V with water R, (V being the maximum recommended dose in millilitres). Prepare the reference solution (d) by dilution 1 mL of a 24.5 mg/L solution of potassium iodate R to V with water R, (V being the maximum recommended dose in millilitres). Mix equal volumes of this solution and of reference solution (c). Prepare a solution containing 2 mg/mL of each of the components stated on the label, apart from iodide, used as blank solution. Inject 25 µL of test solution (a), the blank solution
and reference solutions (c) and (d). The run time is 12 minutes. The relative retention of iodate with reference to iodide (retention time of iodide is about 5 minutes): iodate is from 0.2 to 0.3.

**System suitability.** Regarding the chromatogram due to the blank solution, none of the obtained peaks shows a retention time similar to that of the peak due to iodide. The resolution is a minimum of 2 between the peaks due to iodide and iodate in the chromatogram obtained with reference solution (d) recorded with the spectrophotometer.

The limit of iodide is detected by studying the chromatogram obtained with the spectrophotometer and comparing the peak due to iodide with the chromatogram due to reference solution (c). The area of the peak due to iodide is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c).

**Radiochemical purity**

- Either test A, B, or C may be applied

  A. Carry out the test as described under 1.14.2 Paper chromatography and ascending conditions, using paper for chromatography R (25 × 300 mm). Place a measured volume of a solution containing 100 mg of potassium iodide, 200 mg of potassium iodate and 1 g of sodium bicarbonate, and 25 mm from one end of the chromatographic paper. Allow the paper to dry. To the same area of the paper add an equal volume of appropriately diluted solution such that it provides a count rate of about 20 000 counts per minute and allow the paper to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using dilute methanol (7:10, v/v). Allow the paper to dry in air and determine the radioactivity distribution by scanning with a suitable radiation detector: the radioactivity of the $[^{[131]}I]$iodide band is not less than 95% of the total radioactivity and its $R_f$ value falls within ±5% of the value found for sodium iodide when determined under parallel conditions. Confirmation of the identity of the iodide band is made by the addition to the suspected iodide band of 6 drops of acidified hydrogen peroxide solution (prepared by adding 6 drops of 1 N hydrochloric acid to 10 mL of hydrogen peroxide solution), followed by the dropwise addition of starch TS; the development of a blue color indicates presence of iodide.

  B. Carry out the test 1.14.4 High-performance liquid chromatography as described in the test for iodide with the following modification:
  - inject test solution (b),
  - using the chromatogram obtained with the radioactivity detector, determine the radioactivity of the peak for iodide as a percentage of the total radioactivity. Not less than 95% of the total radioactivity is due to $[^{[131]}I]$ iodide.

  C. Carry out the test as described under 1.15 Electrophoresis, Paper-electrophoresis. Prepare paper strips, type Whatman No. 3 MM for electrophoresis with dimensions of 65 cm × 3 cm. Apply 10–20 μL samples at a distance of 10–13 cm from the end of the stripes. Use borate buffer with a concentration of 9.0 g/L and pH 9.0 ± 0.1. Carry out the electrophoresis at a potential of 900 V for 50 minutes. The $R_f$ values for iodide are between 0.7 and 0.9. $R_f$ for iodate is 0.4, periodate from 0.0 to 0.1. The product can be accepted if the $^{[3]}I$ anion content is higher than 95% even on the expiry date.

**Radioactivity.** Measure the radioactivity using a suitable instrument as described under R.1.1 Detection and measurement of radioactivity.

**Impurities**

$[^{[3]}I]$ iodate ion.
Technetium (99mTc) exametazime complex injection

This is a revised draft proposal for *The International Pharmacopoeia* (Working document QAS/13.548/Rev.2, March 2014).

The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/). Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

Monographs: Radiopharmaceuticals: Specific monographs:

*Technetii (99mTc) exametazimi multiplex injectio - Technetium (99mTc) exametazime complex injection*

**Latin.** Technetii (99mTc) exametazimi multiplex injection.

**English.** Technetium (99mTc) exametazime complex injection.

**Structural formula**

![Structural formula image]

and enantiomer

C₁₃H₂₅N₄O₃ · ⁹⁹ᵐTc

**Relative molecular mass.** 384.269

**Chemical name.** Racemic mixture of (3RS,9RS)-4,8-diaza-3,6,6,9-tetramethylundecane-2,10-dione bisoxime complex with (99mTc) technetium.

**Other names.** (99mTc)-D,L-Hexamethylpropyleneamine oxime complex injection; (99mTc)-D,L-HMPAO injection.

**Description.** Technetium (99mTc) exametazime complex injection is a clear, colourless aqueous solution. Technetium-99m has a half-life of 6.01 hours.

**Category.** Diagnostic.

**Storage.** Technetium (99mTc) exametazime complex injection should be kept at a temperature between 2°C to 8°C. Technetium (99mTc) exametazime complex injection should be used within 30 minutes of reconstitution of the unlabelled kit with Technetium-99m, unless the preparation has been stabilized with cobalt chloride solution or methylene blue solution or any other stabilizer.

**Labelling.** The label complies with the General monograph, the monograph of Radiopharmaceuticals. The label includes the name stabilizer if added.
**Manufacture.** Technetium (99mTc) exametazime injection is prepared aseptically from sterile starting materials such as a sterile kit containing a mixture of (3RS, 9RS)-4, 8-diaza-3,6,6,9-tetramethylundecane-2,10-dione bisoxime and stannous salt with Sodium pertechnetate (99mTc) injection (Fission) or Sodium Pertechnetate (99mTc) injection (Non-fission). The injection may have the pH adjusted and may contain stabilizing agents. The injection may also be prepared under aseptic processing combined with sterilization by Filtration (see 5.8 Methods of sterilization).

**Additional information.** Wherever V is used within the tests of this monograph, V is the maximum recommended dose in millilitre.

**Requirements**

Complies with the monograph for Parenteral Preparations and with that for Radiopharmaceuticals.

**Definition.** Technetium (99mTc) exametazime injection is a sterile lipophilic solution of racemic mixture of (3RS, 9RS)-4, 8-diaza-3,6,6,9-tetramethylundecane-2,10-dione bisoxime (exametazime) complexes with sodium pertechnetate (99mTc) injection (fission or non-fission) in presence of stannous salt. The injection is suitable for intravenous administration and contains sufficient sodium chloride to make the solution isotonic with blood. The content of technetium-99m is not less than 90% and not more than 110% of the content of technetium-99m. Not less than 80% of the total technetium-99m radioactivity is present as lipophilic (99mTc) exametazime complex and its meso isomer.

**Identity tests**

- Either tests A and C or tests B and C may be applied.
  
  **A.** Record the gamma-ray spectrum using a suitable instrument with a sample of technetium-99m, suitably diluted if needed. The spectrum is concordant with the reference spectrum of a specimen of technetium-99m in that it exhibits a major peak of 142 keV.
  
  Standardized technetium-99m solutions are available from competent laboratories recognized by the relevant national or regional authority.
  
  **B.** The half-life determined using a suitable detector system is between 5.72 and 6.32 hours.
  
  **C.** Examine the chromatograms obtained in the test of Impurity A under Radiochemical purity. The principal peak in the chromatogram obtained with the test solution is similar in retention time to the peak due to lipophilic technetium-99m exametazime in the chromatogram obtained with the reference solution.

**pH value.** Carry out the test as described under 1.13 Determination of pH or R1.5 under the monograph for Radiopharmaceuticals. The pH of the injection is between 5.0 and 10.0.

**Sterility.** The injection complies with 3.2 Test for sterility, modified as described in the monograph for Radiopharmaceuticals. Test for sterility will be initiated on the day of manufacture. The injection may be released for use before completion of the test.

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins, modified as described in the monograph for Radiopharmaceuticals. The injection contains not more than 175/V I.U of endotoxins per millilitre.

**Radionuclidic purity.** Complies with the tests of radionuclidic purity under the monographs of Sodium pertechnetate (99mTc) injection (Fission) or Sodium Pertechnetate (99mTc) injection
(Non-fission) used for the preparation of Technetium (99mTc) exametazime injection. Not less than 99.9% of the total radioactivity is due to technetium-99m.

Radiochemical purity

**Impurity C.** Carry out the test described under 1.14.1 Thin-layer chromatography for impurity C use TLC silica gel plate R, a glass fiber plate and 9 g/L solution of sodium chloride as a mobile phase. Apply to the plate about 5 µl of the injection to be examined and develop immediately for a distance over 2/3 of the plate. Allow the plate to dry in air and determine the radioactivity distribution using a suitable detector. Impurity C has Rf value of 0.8 to 1.0; lipophilic technetium-99m exametazime and impurities A, B, D and E do not migrate. The maximum limit of impurity C is 10% of the total radioactivity.

**Total of lipophilic technetium-99m exametazime and impurity A.** Carry out the test under 1.14.1 Thin-layer chromatography. Use TLC silica gel plate R, a glass fibre plate and methyl ethyl ketone as a mobile phase. Apply to the plate about 5 µl of the injection to be examined and develop immediately for a distance over 2/3 of the plate. Allow the plate to dry in air and determine the radioactivity distribution using a suitable detector. The lipophilic technetium-99m exametazime, impurities A and C have Rf value of 0.8 to 1.0; for impurities B, D and E do not migrate.

Calculate the percentage of radioactivity due to impurities B, D and E from test C and the percentage of the radioactivity due to impurity C from test B. Calculate the total percentage of lipophilic technetium-99m exametazime and impurity A from the expression: 100 - A - B.

Not less than 80% of the total technetium-99m radioactivity is present as lipophilic technetium-99m exametazime and impurity A.

**Impurity A.** Carry out the test as descried under 1.14.4 High-performance liquid chromatography, using a stainless steel column (0.25 m x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm). As a mobile phase use a mixture of 33 volumes of acetonitrile R and 67 volumes of 0.1 M phosphate buffer solution R pH 3.0 to use as mobile phase. The flow rate is 1.5 mL/min, the detector is radioactivity detector with loop injector and the run time is 20 min. Prepare the reference solution by dissolving the contents of a vial of meso-rich exametazime CRS in 0.5 ml of a 9 g/L solution of sodium chloride and transfer to a lead-shielded, nitrogen-filled vial. Add 6 µL of a freshly prepared 1 g/L solution of stannous chloride R in 0.05 M hydrochloric acid and 2.5 mL of sodium pertechnetate (99mTc) injection (fission or non-fission) containing 370–740 MBq. Mix carefully and use within 30 min of preparation. The relative retention with reference to lipophilic technetium-99m exametazime to impurity A is about 1.2.

The produced chromatogram is similar to the chromatogram provided with meso-rich exametazime CRS. The resolution is minimum of 2 between the peaks due to lipophilic technetium-99m exametazime and to impurity A. Impurity A should not more than 5% of the radioactivity due to lipophilic technetium-99m exametazime and impurity A.

**Tin estimation.** Carry out the test as described under R2.1.4 Tin estimation by UV absorption, using 1.0 ml of a test solution prepared by diluting 1.5 ml of the injection to be examined to 25.0 ml with hydrochloric acid (103 g/L) VS and mixing thoroughly. Prepare the reference solution by dissolving 0.115 g of stannous chloride R using a solution in hydrochloric acid R (103 g/L HCl) and dilute to 1000.0 mL using the same acid. To the test solution and to 1 mL of each of the reference solutions add 0.05 mL of thioglycollic acid R, 0.1 mL of dithiol reagent R, 0.4 mL of a 20 g/L solution of sodium laurilsulfate R, 3 mL of 21g/L solution of hydrochloric acid R. Mix and measure the absorbance of each solution at 540 nm using 21g/L solution of...
hydrochloric acid as a compensation liquid. The absorbance of the test solution is not greater than that of the reference solution; not more than 0.6 µg of Sn per ml.

**Radioactivity.** Measure the radioactivity using a suitable instrument as described under R.1.1 Detection and measurement of radioactivity.

**Impurities**

A. Meso isomer of lipophilic technetium-99m exametazime,
B. Technetium-99m in colloidal form,
C. [99mTc]pertechnetate ion,
D. Non lipophilic technetium-99m exametazime complex,
E. Meso isomer of non-lipophilic technetium-99m exametazime complex.

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Thallous (\(^{201}\text{TI}\)) chloride injection

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Monographs: Radiopharmaceuticals: Specific monographs:

*thallosi (\(^{201}\text{TI}\)) chloridi injectio* - Thallous \(^{201}\text{TI}\) chloride injection

**Latin.** Thallosi \(^{201}\text{TI}\) chloridi injectio

**English.** Thallous \(^{201}\text{TI}\) chloride injection

**Structural formula.** \(^{201}\text{Tl}^{+} \ldots \ldots \text{Cl}^{-}\)

**Empirical formula.** \(^{201}\text{TlCl}\)

**Relative molecular mass.** 236.423

**Chemical name.** \(^{201}\text{Tl}\)Thallium chloride

**Other names.** Thallous \(^{201}\text{TI}\) chloride

**Description.** Thallous \(^{201}\text{TI}\) chloride injection is a clear colourless, aqueous solution. Thallium-201 has a half-life of 72.96 hours.

**Category.** Diagnostic.

**Storage.** After aseptic withdrawal of the first dose from a multidose container, the container should be stored at a temperature between 2°C to 8°C.

**Labelling.** The label complies with the General monograph, the monograph of Radiopharmaceuticals.

**Manufacture**

No-carrier-added thallium-201 radioisotope is produced by proton bombardment of enriched thallium-203 target followed by chemical separation of radioactive lead-201 isotope. The lead-201 isotope has a half-life of 9.4 hours and decays to thallium-201. Separation of thallium-201 may be done using anion-exchange resin chromatography or solvent extraction. Thallous \(^{201}\text{TI}\) chloride injection may be sterilized by “Heating in an autoclave” (see 5.8 Methods of Sterilization).

**Additional information**

Wherever V is used within the tests of this monograph, V is the maximum recommended dose in millilitres.

**Requirements**

Complies with the monograph for Parenteral Preparations and with that for Radiopharmaceuticals.
Definition. Thallous (\textsuperscript{201}Tl) chloride injection is a sterile, isotonic, aqueous solution of thallium-201 as thallous chloride, suitable for intravenous administration. It contains sufficient sodium chloride to make the solution isotonic with blood and may contain suitable antimicrobial preservatives such as benzyl alcohol or stabilizing agents. The injection contains not less than 90\% and not more than 110\% of the content of thallium-201 at the reference date and time stated on the label. Not less than 97\% of the total radioactivity is due to thallium-201. Not more than 2\% of the total radioactivity is due to thallium-202. The specific activity is not less than 3.7 GBq of thallium-201 per milligram of thallium at the reference date and time stated on the label.

Identity tests

- Either tests A and C or tests B and C may be applied.
  - A. Record the gamma-ray using a suitable instrument with a sample of thallium-201, suitably diluted if needed. The spectrum is concordant with the reference spectrum of a specimen of thallium-201 in that it exhibits major peaks of 135, 166 and 167 keV and X-rays of 69 and 83 keV.
  - B. The half-life determined using a suitable detector system is between 69.31 and 76.6 hours.
  - C. Examine the radiochromatogram obtained in the test for radiochemical purity. Not less than 95\% of the radioactivity present as \([\textsuperscript{201}Tl]\)Thallium chloride and migrates on the strip towards the cathode as a single peak.

pH value. Carry out the test as described under 1.13 Determination of pH or R1.5 under the monograph for Radiopharmaceuticals. The pH of the injection is between 4.0 and 7.0.

Sterility. The injection complies with 3.2 Test for sterility, modified as described in the monograph for Radiopharmaceuticals. Test for sterility will be initiated on the day of manufacture. The injection may be released for use before completion of the test.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins, modified as described in the monograph for Radiopharmaceuticals. The injection contains not more than 175/I (I.U. of endotoxins per millilitre). The injection may be released for use before completion of the test.

Radionuclidic purity. Record the gamma-ray and X-ray spectrum using a suitable instrument and measure the half-life using a suitable method. Determine the relative amounts of thallium-200, thallium-201, thallium-202, lead-201, lead-203 and other radionuclidic impurities that may be present. Thallium-202 has a half-life of 12.2 days and exhibits a main peak of 440 keV. Thallium-200 has a half-life of 1.09 days and exhibits main peaks of 368, 579, 828 and 1206 keV. Lead-201 has a half-life of 9.4 hours and exhibits a main peak of 331 keV. Lead-203 has a half-life of 2.17 days and exhibits a main peak of 270 keV. Not less than 97\% of the total radioactivity is due to thallium-201. Not more than 2\% of the total radioactivity is due to thallium-202.

Standardized solutions of thallium-201 and thallium-202 are available from laboratories recognized by the relevant national or regional authority.

Radiochemical purity. Carry out the test as described under 1.15 Electrophoresis, zone-electrophoresis. Prepare a suitable cellulose polyacetate strip as the supporting medium and soak the strip in a solution of disodium edetate R (18.6 g/l) as the electrolyte solution. Soak the strip in the electrolyte solution for 45–60 min. Remove the strip with forceps, taking care to handle the outer edges only. Place the strip between 2 absorbent pads and blot to remove excess solution. Apply not less than 5 µl of a mixture of equal volumes of the preparation to
be examined and the electrolyte solution to the centre of the blotted strip and mark the point of application. Attach the strip to the support bridge of an electrophoresis chamber containing equal volumes of disodium edetate R in each side of the chamber. Ensure that each end of the strip is in contact with the disodium edetate R. Apply an electric field of 250 volts per metre for 30 minutes. Allow the strip to dry in air. Determine the distribution of radioactivity using suitable detector.

Not less than 95% of the radioactivity on the strip migrates towards the cathode as a single peak.

**Chemical purity**

**Thallium.** Transfer 1.0 ml of the injection and 1.0 ml of thallium standard (2 µg/ml Tl) TS to separate screw-cap test tubes. To each tube add the following five solutions (A, B, C, D and E) and mix after each addition: 2 drops of a solution prepared by carefully mixing 18 ml of nitric acid (~1000 g/l) TS and 82 ml of hydrochloric acid (~250 g/l) TS (solution A); 1.0 ml of sulfosalicylic acid (0.1 mol/l) VS (solution B); 2 drops of hydrochloric acid (~250 g/l) TS (solution C); 4 drops of a solution prepared by dissolving 50 mg of rhodamine B R in hydrochloric acid (~250 g/l) TS and diluting to 100.0 ml (solution D); 1.0 ml of diisopropyl ether R (solution E). Screw the caps on tightly, shake the tubes by hand for exactly 1 minute, releasing any pressure build-up by loosening the caps slightly. Recap the tubes and allow the phases to separate. Transfer 0.5 ml of the ether layer from each tube to clean tubes. The colour of the ether layer obtained from the injection is not darker than that from the thallium standard (2 µg/ml Tl) TS.

**Iron.** Into separate cavities of a spot plate place 0.1 ml of the injection and 0.1 ml of iron standard TS diluted with water R to a concentration of 5 µg/ml. Add to each cavity 0.1 ml of a solution of hydroxylamine hydrochloride R (1 in 10), 1 ml of a solution of sodium acetate R (1 in 4) and 0.1 ml of a 0.5% dipyridyl solution prepared by dissolving 0.5 g of 2,2'-dipyridyl R in 100 ml of water R containing 0.15 ml of hydrochloric acid (~250 g/l) TS and mix. After 5 minutes the colour obtained from the injection is not darker than that from the iron standard solution.

**Copper.** Into separate cavities of a spot plate place 0.2 ml of the injection and 0.2 ml of copper standard (5 µg/ml Cu) TS. Add to each cavity the following 3 solutions (A, B and C) and mix after each addition: 0.2 ml of water R (solution A) and 0.1 ml of a solution of iron thiocyanate prepared by dissolving 1.5 g of ferric chloride R and 2 g of potassium thiocyanate R in water R and diluting to 100.0 ml with the same solvent (solution B); 0.1 ml of a solution of sodium thiosulphate R (1 in 100) (solution C). The time required for the injection to decolorize is equal to or longer than that observed for the copper standard solution.

Radioactivity. Measure the radioactivity using a suitable instrument as described under R.1.1 Detection and measurement of radioactivity.

**Impurities**

A. Lead-201,
B. Lead-203,
C. Thallium-200,
D. Thallium-202,
E. [\(^{201}\)Tl] Thallic (III) ion.

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