Consultation Documents

The International Pharmacopoeia

Aciclovirum
Aciclovir

This is a draft proposal for The International Pharmacopoeia (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidth@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

Molecular formula. C₈H₁₁N₅O₃

Relative molecular mass. 225.20

Chemical name. 2-Amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6H-purin-6-one. CAS Reg. No. 59277-89-3.

Description. White or almost white, crystalline powder.

Solubility. Slightly soluble in water; freely soluble in dimethyl sulfoxide; very slightly soluble in ethanol (96%). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

Category. Antiviral (purine nucleoside analogue).

Storage. Preserve in well-closed containers. Protect from light and moisture.

Additional information. Aciclovir may exhibit polymorphism.

Requirements

Definition. Aciclovir contains not less than 98.5% and not more than 101.0% of C₈H₁₁N₅O₃ calculated with reference to the dried substance.

Identity tests

Either test A alone, or test B and D or test C and D may be applied.
A. Carry out the test as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from aciclovir RS or with the reference spectrum of aciclovir.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions given under Guanine and related substances test A1. The principal spot in the chromatogram obtained with solution (B) corresponds in position, appearance and intensity to the spot due to aciclovir in the chromatogram obtained with solution (C).

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Guanine and related substances test B. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the aciclovir peak in the chromatogram obtained with solution (4).

D. Dissolve about 10 mg of the test substance in 5.0 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100.0 ml with water R. Dilute 5.0 ml of this solution to 50.0 ml with water R. The absorption spectrum (1.6) of the resulting solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 255 nm and the absorption at 255 nm is about 0.5.

Clarity and colour of solution. A solution, containing 0.25 g of the test substance in 25 ml of sodium hydroxide (0.1 mol/l) TS, is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

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

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 60 mg/g.

Guanine and related substances

Either test A or test B may be applied.

A. Carry out test A.1 and A.2.

A.1 Guanine. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R1 as the coating substance (Merck cellulose F plate has been found suitable) and a mixture of 10 volumes of propan-1-ol, 30 volumes of ammonia (260 g/l) TS and 60 volumes of ammonium sulfate (50 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following four, freshly prepared solutions in sodium hydroxide (0.1 mol/l) TS. For solution (A) use 5 mg of the test substance per ml. For solution (B) dilute 1 volume of solution (A) to 10 volumes. For solution (C) use a solution of 0.5 mg of aciclovir RS and 0.5 mg of guanine R per ml. For solution (D) use 35 µg of guanine R per ml. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). In the chromatogram obtained with solution (C) guanine is eluted with a Rf value of 0.5 and aciclovir with a Rf value of 0.7. The test is not valid unless this chromatogram shows two clearly separated spots. Any secondary spot corresponding to guanine in the chromatogram obtained with solution (A) is not more intense than the principal spot in the chromatogram obtained with solution (D) (0.7%).
A.2 Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 2 volumes of ammonia (260 g/l) TS, 20 volumes of methanol R and 80 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 2 µl of each of the following three, freshly prepared solutions in dimethyl sulfoxide R. For solution (A) use 25 mg of the test substance per ml. For solution (B) dilute 1 volume of solution (A) to 200 volumes. For solution (C) use a mixture of 0.5 mg of aciclovir RS and 0.5 mg of aciclovir impurity A RS per ml. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots. Any spot with an Rf value greater than that of the principal spot in the chromatogram obtained with solution (A) is not more intense than the principal spot in the chromatogram obtained with solution (B) (0.5%).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl group (5 µm). (Dionex C18 column and Shiseido MG C18 column have been found suitable.)

Use the following conditions for gradient elution:

<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>0–5</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5–27</td>
<td>100 to 80</td>
<td>0 to 20</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>27–42</td>
<td>80</td>
<td>20</td>
<td>Isocratic</td>
</tr>
<tr>
<td>40–42</td>
<td>80 to 100</td>
<td>20 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>42–52</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 30 °C.

Prepare the following solutions. For solution (1) dissolve 25 mg of the test substance in 5.0 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 25.0 ml with water R. For solution (2) dilute 1.0 ml of solution (1) to 100.0 ml with water R. Dilute 1.0 ml of this solution to 10.0 ml with water R. For solution (3) dissolve 10 mg of guanine R in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100.0 ml with water. Dilute 5.0 ml of this solution to 50.0 ml with water R. For solution (4) dissolve 5 mg of aciclovir RS, 5 mg of guanine R and 10 mg of aciclovir impurity C RS in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100 ml with water R.

Inject separately 20 µl each of solutions (1), (2), (3) and (4). Record the chromatograms for about 45 minutes.
In the chromatogram obtained with solution (4) the peak of aciclovir impurity C is eluted with a relative retention time of 0.94 with reference to the peak of aciclovir. The test is not valid unless the resolution factor between the peak due to aciclovir impurity C and the peak due to aciclovir is at least 1.5.

In the chromatogram obtained with solution (1):

- The area of any peak corresponding to guanine is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.7%).
- The area of any other peak, other than the principal peak and the peak due to guanine, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).
- The sum of all other areas, other than the principal peak and the peak due to guanine, is not greater than 8 times the area of the principal peak obtained with solution (2) (0.8%).

Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Assay**

Dissolve about 0.150 g, accurately weighed, in 60 ml of anhydrous acetic acid R. Titrate with perchloric acid (0.1 mol/l) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titrations. Carry out a blank titration. Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 22.52 mg of acyclovir (C₁₈H₁₁N₅O₃).

**Impurities**

A. 2-[[2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl]methoxy]ethyl acetate,

B. 2-amino-1,7-dihydro-6H-purin-6-one (guanine),
C. 2-amino-7-[(2-hydroxyethoxy)methyl]-1,7-dihydro-6H-purin-6-one,

F. N-[9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1H-purin-2-yl]acetamide,

G. 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-9H-purin-9-yl]methoxy]ethyl acetate,

I. 2-amino-7-[[2-[2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl]methoxy]ethoxy]methyl]-
1,7-dihydro-6H-purin-6-one,

J. 9,9′-[ethylenebis(oxyethylene)]bis(2-amino-1,9-dihydro-6H-purin-6-one),
K. 2,2′-[methylenediimino]bis[9-[(2-hydroxyethoxy)methyl]1,9-dihydro-6H-purin-6-one],

L. N-(9-acetyl-6-oxo-6,9-dihydro-1H-purin-2-yl)acetamide (N2,9-diacetylguanine),

M. 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-7H-purin-7-yl]methoxy]ethyl acetate,

N. unknown structure

O. unknown structure

P. 2-amino-9-(2-hydroxyethyl)1,9-dihydro-6H-purin-6-one.
**New reference substances**

Aciclovir RS  
Aciclovir impurity A RS  
Aciclovir impurity C RS

**New reagents**

**Guanine R**  
\(\text{C}_5\text{H}_5\text{N}_5\text{O}, 2\text{-Amino-1,7-dihydro-6H-purin-6-one.}\)

Amorphous white or almost white powder, practically insoluble in water, slightly soluble in ethanol (96 per cent). It dissolves in ammonia and in dilute solutions of alkali hydroxides.

**Test Solutions to be added**

**Ammonium sulfate (50 g/l) TS**  
Transfer 50 g ammonium sulfate R in a 1000 ml volumetric flask and make up to volume with water R.

**Phosphate buffer, pH 2.5, TS**  
Dissolve 3.48 g of dipotassium hydrogen phosphate R in 1000 ml of water R and adjust to pH 2.5 with phosphoric acid R.

**Phosphate buffer, pH 3.1, TS**  
Dissolve 3.48 g of dipotassium hydrogen phosphate R in 1000 ml of water R and adjust to pH 3.1 with phosphoric acid R.

---

**Acicloviri ad injectionem**

**Aciclovir for injection**

This is a draft proposal for *The International Pharmacopoeia* (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidth@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

**Description.** A white powder or loose lumps; odourless or almost odourless.

**Category.** Antiviral (Purine nucleoside analogue).

**Storage.** Preserve in well-closed containers. Protect from light and moisture.

**Labelling.** The label should state that the active ingredient is Aciclovir.

**Additional information.** Strength in the current WHO Model List of Essential Medicines: 250 mg in vial. Strength in the current WHO Model List of Essential Medicines for Children: 250 mg in vial.
**Requirements**

The powder for injections and the reconstituted solution for injection comply with the monograph on Parenteral preparations.

**Definition.** Aciclovir for injection is a sterile powder prepared from Aciclovir with the aid of a suitable alkali. The container of Aciclovir for injection contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir (C₈H₁₁N₅O₃).

**Identity tests**

Either test A alone or test B and D, or test C and D may be applied.

A. To a quantity of the test substance, containing the equivalent of about 100 mg of aciclovir, add 10 ml water R, adjust to pH 4–7 with hydrochloric acid (0.1 mol/l) TS and allow to stand for 30 minutes. Filter, use 20 ml water R to wash the precipitate and dry it at 105 °C for 3 hours. Carry out the test with the precipitate as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from aciclovir RS or with the reference spectrum of aciclovir. If the spectra thus obtained are not concordant repeat the test by separately adding 10 ml of water R to the test substance and aciclovir RS and preceding as described. The infrared absorption spectrum is concordant with the spectrum obtained from aciclovir RS.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions given under Guanine and related substances test A1. The principal spot in the chromatogram obtained with solution (B) corresponds in position, appearance and intensity to the spot due to aciclovir in the chromatogram obtained with solution (C).

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay test A. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the aciclovir peak in the chromatogram obtained with solution (2).

D. The absorption spectrum (1.6) of the solution, prepared as described under Assay test B, when observed between 230 nm and 350 nm, exhibits a maximum at 255 nm.

**Clarity and colour of solution.** A solution, containing the equivalent to 0.10 g of aciclovir in 10 ml of water R, is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under 1.11 Colour of liquids.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. Use 0.5 g of the test substance. The water content is not more than 55 mg/g.

**pH value.** pH of a solution containing the equivalent to 25 mg of aciclovir per ml of water R, 10.7–11.7.

**Guanine and related substances**

Either test A or test B may be applied.

A. Carry out test A.1 and A.2.
A.1 Guanine. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R1 as the coating substance (Merck cellulose F plate has been found suitable.) and a mixture of 10 volumes of propan-1-ol, 30 volumes of ammonia (260 g/l) TS and 60 volumes of ammonium sulfate (50 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following four, freshly prepared solutions in sodium hydroxide (0.1 mol/l) TS. For solution (A) dissolve a quantity of the powder to obtain a solution containing 5 mg of Aciclovir per ml. For solution (B) dilute 1 volume of solution (A) to 10 volumes. For solution (C) use a solution of 0.5 mg of aciclovir RS and 0.5 mg of guanine R per ml. For solution (D) use 35 µg of guanine R per ml. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). In the chromatogram obtained with solution (C) guanine is eluted with a Rf value of 0.5 and aciclovir with a Rf value of 0.7. The test is not valid unless this chromatogram shows two clearly separated spots. Any secondary spot corresponding to guanine in the chromatogram obtained with solution (A) is not more intense than the principal spot in the chromatogram obtained with solution (D) (0.7%).

A.2 Related substances. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 2 volumes of ammonia (260 g/l) TS, 20 volumes of methanol R and 80 volumes of dichloromethane R as a mobile phase. Apply separately to the plate 2 µl of each of the following three, freshly prepared solutions in dimethyl sulfoxide R. For solution (A) dissolve a quantity of the powder for injection to obtain a solution containing 25 mg of aciclovir per ml. For solution (B) dilute 1 volume of solution (A) to 200 volumes. For solution (C) use a mixture of 0.5 mg of aciclovir RS and 0.5 mg of aciclovir impurity A RS per ml. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots. Any spot with an Rf value greater than that of the principal spot in the chromatogram obtained with solution (A) is not more intense than the principal spot in the chromatogram obtained with solution (D) (0.5%).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm), packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl group (5 µm). (DionexC18 and Shiseido MG C18 column have been found suitable.)

Use the following conditions for gradient elution:

<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>0–5</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5–27</td>
<td>100 to 80</td>
<td>0 to 20</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>27–40</td>
<td>80</td>
<td>20</td>
<td>Isocratic</td>
</tr>
<tr>
<td>40–42</td>
<td>80 to 100</td>
<td>20 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>42–52</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>
Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 30 °C.

Prepare the following solutions. For solution (1) dissolve a quantity of the powder for injection, equivalent to 25 mg of aciclovir in 5.0 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 25.0 ml with water R. For solution (2) dilute 1.0 ml of solution (1) to 100.0 ml with water. Dilute 1.0 ml of this solution to 10.0 ml with water. For solution (3) dissolve 10 mg of guanine R in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100.0 ml with water R. Dilute 5.0 ml of this solution to 50.0 ml with water R. For solution (4) dissolve 5 mg of aciclovir RS, 5 mg of guanine R and 10 mg of aciclovir impurity C RS in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100 ml with water R.

Inject separately 20 μl each of solutions (1), (2), (3) and (4). Record the chromatograms for about 45 minutes.

In the chromatogram obtained with solution (4) the peak of aciclovir impurity C is eluted with a relative retention time of 0.94 with reference to the peak of aciclovir. The test is not valid unless the resolution factor between the peak due to aciclovir impurity C and the peak due to aciclovir is at least 1.5.

In the chromatogram obtained with solution (1):
• The area of any peak corresponding to guanine is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.7 %).
• The area of any other peak, other than the principal peak and the peak due to guanine, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%).
• The sum of all other areas, other than the principal peak and the peak due to guanine, is not greater than 8 times the area of the principal peak obtained with solution (2) (0.8%).

Disregard any peak with an area less than 0.5 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.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm), packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl group (5 μm). (Dionex C18 column and Shiseido MG C18 column have been found suitable.)

As the mobile phase, use a mixture of 90 volumes of Mobile phase A, as described under Guanine and related substances test B, and 10 volumes of acetonitrile R.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 30 °C.
Prepare the following solutions. For solution (1) dissolve a quantity of the powder for injection, equivalent to about 20 mg of aciclovir, accurately weighed, in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100 ml with water. Dilute 5.0 ml of this solution to 50 ml with water. For solution (2) dissolve 20 mg of aciclovir RS in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100 ml with water. Dilute 5.0 ml of this solution to 50 ml with water.

Inject separately 20 µl each of solution (1) and (2). Record the chromatograms for about 20 min.

Measure the areas of the peak responses obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of aciclovir ((C₈H₁₁N₅O₃)) per sealed container, using the declared content of (C₆H₁₁N₅O₃) in aciclovir RS.

B. Mix the contents of 5 containers. Transfer a quantity of the powder for injection, equivalent to 150 mg of Aciclovir, accurately weighed, to a 100 ml volumetric flask and dilute to volume with hydrochloric acid (0.1 mol/l) TS, mix and filter. Dilute 1.0 ml of the resulting solution to 100.0 ml with hydrochloric acid (0.1 mol/l) TS. Measure the absorbance of this solution in a 1 cm layer at 255 nm, using hydrochloric acid (0.1 mol/l) TS as the blank. Calculate the percentage content of aciclovir (C₆H₁₁N₅O₃) per sealed container, using an absorptivity value of 56.0. i.e., $A = \frac{560}{1\text{cm}} = 560$

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins. Contains not more than 0.17 IU of endotoxin per mg of aciclovir.

---

**Acicloviri compressi**  
**Aciclovir tablets**

This is a draft proposal for *The International Pharmacopoeia* (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidt@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

**Category.** Antiviral (purine nucleoside analogue).

**Storage.** Preserve in well-closed containers. Protect from light and moisture.

**Additional information.** Strength in the current WHO Model List of Essential Medicines: 200 mg. Strengths in the current WHO Model List of Essential Medicines for Children: 200 mg.

**Requirements**

Complies with the monograph on Tablets.

**Definition.** Aciclovir tablets contain not less than 90.0% and not more than 110.0% of the labelled amount of aciclovir (C₆H₁₁N₅O₃).
Identity tests

Either test A and C or test B and C may be applied.

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions given under Guanine and related test A1. The principal spot in the chromatogram obtained with solution (B) corresponds in position, appearance and intensity to the spot due to aciclovir in the chromatogram obtained with solution (C).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay test A. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the aciclovir peak in the chromatogram obtained with the solution (2).

C. The absorption spectrum (1.6) of the solution, prepared as described under Assay test B, when observed between 230 nm and 350 nm, exhibits a maximum at 255 nm.

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 Hydrochloric acid (~4 g/l) TS and rotating the paddle at 75 revolutions per minute. At 30 minutes withdraw a sample of 10 ml of the medium through an in-line filter. Measure the absorbance (1.6) of the filtered sample, suitably diluted if necessary, at a wavelength of 255 nm. At the same time measure the absorbance of a suitable solution of aciclovir RS in hydrochloric acid (~4 g/l) TS, using the same buffer as the blank.

For each of the six tablets tested calculate the total amount of aciclovir ($C_9H_11N_5O_3$) in the medium from the absorbances obtained, using the declared content of $C_9H_11N_5O_3$ in aciclovir RS. Use the requirements as described under 5.5 Dissolution test for solid oral dosage forms, Acceptance criteria to evaluate the results: the amount in solution is not less than 75% (Q) of the amount declared on the label.

Guanine

Either test A or test B may be applied.

A. Carry out the test as described under 1.14.1 Thin-layer chromatography, using cellulose R1 as the coating substance (Merck cellulose F plate has been found suitable) and a mixture of 10 volumes of propan-1-ol, 30 volumes of ammonia (260 g/l) and 60 volumes of ammonium sulfate (50 g/l). Apply separately to the plate 10 µL of each of the following four, freshly prepared solutions in sodium hydroxide (0.1 mol/l) TS. For solution (A) shake a quantity of the powdered tablets, containing about 25 mg of aciclovir, with 5 ml of sodium hydroxide (0.1 mol/l) TS, filter and use the filtrate. For solution (B) dilute 1 volume of (A) to 10 volumes. For solution (C) use a solution of 0.5 mg of aciclovir RS and 0.5 mg of guanine RS per ml. For solution (D) use 50 µg of guanine R per ml. After removing of the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). In the chromatogram obtained with solution (C) guanine is eluted with a Rf value of 0.5 and aciclovir with a Rf value of 0.7. The test is not valid unless this chromatogram shows two clearly separated spots. Any secondary spot corresponding to guanine in the chromatogram obtained with solution (A) is not more intense than the principal spot in the chromatogram obtained with solution (D) (1.0%).

B. Carry out the test as described under 1.14.4 High performance liquid chromatography, using the conditions given under Assay test A.
Prepare the following solutions. For solution (1) shake a quantity of the powdered tablets, containing 25 mg of aciclovir, with 5.0 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 25.0 ml with water, filter and use the filtrate. For solution (2) dissolve 10 mg of guanine R in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100.0 ml with water R. Then dilute 5.0 ml of this solution to 50.0 ml with water. For solution (3) dissolve 5 mg of aciclovir RS, 5 mg of guanine R in 10 ml of sodium hydroxide (0.1 mol/l) TS and dilute to 100 ml with water R.

Inject 20 μl of solution (3). The test is not valid unless the resolution between the peak due to aciclovir and the peak due to guanine is at least 3.0.

Inject separately 20 μl each of solutions (1) and (2). In the chromatogram obtained with solution (1) the area of any peak corresponding to guanine is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Assay

Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm), packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl group (5 μm). (Dionex C18 column and Shiseido MG C18 column have been found suitable.)

As the mobile phase, use a mixture of 90 volumes of phosphate buffer, pH 3.1, TS and 10 volumes of acetonitrile R.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 30 °C.

Prepare the following solutions. For solution (1) shake a quantity of the powdered tablets, equivalent to about 20 mg of aciclovir, accurately weighed, with 10 ml of sodium hydroxide (0.1 mol/l) and dilute to 100 ml with water. Dilute 5.0 ml of this solution to 50 ml with water. For solution (2) dissolve 20 mg of aciclovir RS in 10 ml of sodium hydroxide (0.1 mol/l) and dilute to 100 ml with water. Dilute 5.0 ml of this solution to 50 ml with water.

Inject separately 20 μl each of the solutions (1) and (2). Record the chromatograms for about 20 min.

Measure the areas of the peak responses obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of aciclovir (C₈H₁₁N₅O₃) in the tablets, using the declared content of C₈H₁₁N₅O₃ in aciclovir RS.

B. Weigh and powder 20 tablets. Transfer a quantity of the powder, equivalent to about 0.1 g of Aciclovir, accurately weighed, to a 100 ml volumetric flask, add 60 ml of sodium hydroxide (0.1 mol/l), sonicate for about 15 minutes, allow to cool to room temperature and make up to volume with the same solvent, shake and filter. Transfer 15.0 ml of the filtrate to a 100 ml volumetric flask, add 50 ml of water and 5.8 ml of hydrochloric acid (70g/l) TS and dilute to volume with water R. Dilute 5.0 ml of the solution to 50.0 ml with hydrochloric acid (0.1 mol/l) TS. Measure the absorbance of
the resulting solution in a 1 cm layer at 255 nm, using hydrochloric acid (0.1 mol/l) TS as the blank. Calculate the percentage content of aciclovir (C₈H₁₁N₅O₃) in the tablets, using an absorptivity value of using an absorptivity value of 56.0, i.e., \[ A \frac{\%}{1\text{cm}} = 560 \]

Radiopharmaceuticals: general monograph

This is a draft general monograph revision for The International Pharmacopoeia (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidt@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

This general monograph is intended to be read in conjunction with the individual monographs on radiopharmaceutical preparations. A radiopharmaceutical preparation that is the subject of an individual monograph in The International Pharmacopoeia complies with the general requirements stated below and with the general monograph for the relevant dosage form (most commonly that for parenteral preparations) as modified by any of the requirements given below and by any specific instruction included in the individual monograph.

Requirements

Definition

Radiopharmaceutical preparation or radiopharmaceutical. A radiopharmaceutical preparation or radiopharmaceutical is a medicinal product in a ready-to-use form suitable for human use that contains a radionuclide. The radionuclide is integral to the medicinal application of the preparation, making it appropriate for one or more diagnostic or therapeutic applications.

For the purpose of this general monograph radiopharmaceuticals also cover:

Radionuclide generator. A system in which a daughter radionuclide (short half-life) is separated by elution or by other means from a parent radionuclide (long half-life) and later used for production of a radiopharmaceutical preparation.

Radionuclide precursor. A “radionuclide precursor” means any radionuclide not being a radiopharmaceutical or generator or radionuclide kit which is produced for the radiolabelling of another substance for administration.

Kit for radiopharmaceutical preparation. In general a vial containing the non-radionuclide components of a radiopharmaceutical preparation, usually in the form of a sterilized, validated product to which the appropriate radionuclide is added or in which the appropriate radionuclide is diluted before medical use. In most cases the kit is a multidose vial and production of the radiopharmaceutical preparation may require additional steps such as boiling, heating, filtration and buffering.

Chemical precursor. Non-radioactive substances in combination with radionuclide.
**Manufacture**

The manufacturing process for radiopharmaceutical preparations should meet the requirements of good manufacturing practice (GMP).

The manufacturer is responsible for ensuring the quality of his products and especially for examining preparations of short-lived radionuclides for long-lived impurities after a suitable period of decay. In this way, the manufacturer ensures that the manufacturing processes employed are producing materials of appropriate quality. In particular, the radionuclide composition of certain preparations is determined by the chemical and isotopic composition of the target material (see "Target materials") and pilot preparations are advisable when new batches of target material are employed.

When the size of a batch of a radiopharmaceutical preparation is limited to one or few units (for example, certain therapeutic preparations or very short-lived preparations) release of the product must rely on the process control rather than product quality control tests. Therefore validation and revalidation of manufacture process must be fully implemented as well as the product quality control tests.

**Radiation Protection.** The relevant premises and equipment must be designed, built and maintained so that they do not bear any negative impact on or represent any hazard to the product, personnel or immediate surroundings. The corresponding supporting materials are provided by various IAEA publications.


*See also Regulations for the Safe Transport of Radioactive Materials. Safety Requirements. No TS-R-1 (IAEA, Vienna, 2009).* For further details. Consult the IAEA website (http://www-ns.iaea.org/standards/) for current guidance.

**Radionuclide production.** In general ways of manufacturing radionuclides for use in radiopharmaceutical preparations are:

**Nuclear fission.** Nuclides with high atomic number are fissionable and a common reaction is the fission of uranium-235 by neutrons in a nuclear reactor. For example, iodine-131, molybdenum-99 and xenon-133 can be produced in this way. Radio-nuclides from such a process must be carefully controlled in order to minimize the radionuclidic impurities.

**Charged particle bombardment.** Radionuclides may be produced by bombarding target materials with charged particles in particle accelerators such as cyclotrons. The isotopic composition and purity of the target material will influence the radionuclidic purity of irradiated target.

**Neutron bombardment.** Radionuclides may be produced by bombarding target materials with neutrons in nuclear reactors. The rate of the nuclear reaction
depends on the energy of the incident particle, neutron flux and nuclear cross-section. The isotopic composition and purity of the target material will influence the radionuclidic purity of irradiated target.

**Radionuclide generator systems.** Radionuclides of short half-life may be produced by means of a radionuclide generator system involving separation of the daughter radionuclide from a long-lived parent by chemical or physical separation. Care must be taken to avoid contamination of daughter radionuclide with parent radionuclide and decay products.

**Starting materials (including chemical precursors and excipients).** In the manufacture of radiopharmaceutical preparations, measures shall be taken to ensure that all ingredients are of appropriate quality, including those starting materials, such as chemical precursors for synthesis, that are produced on a small scale and supplied by specialized producers or laboratories for use in the radiopharmaceutical industry. The actual quantity of radioactive material compared with quantities of excipients is normally very small therefore excipients can greatly influence the quality of the radiopharmaceutical preparation.

**Target materials.** The composition and purity of the target material and the nature and energy of the incident particle will determine the relative percentages of the principal radionuclide and other potential radionuclides (radionuclidic impurities) and thus ultimately the radionuclidic purity. Strict control of irradiation parameters such as beam energy, intensity and duration is also essential. For very short lived radionuclides including the ones present in most positron emission tomography (PET) tracers the determination of radiochemical and radionuclidic purity of radiopharmaceutical preparation before patient use is difficult. Therefore before clinical use of these radionuclides, strict operational conditions and extensive validations are essential. Any subsequent change in operational conditions should be revalidated.

Where applicable (e.g., cyclotron irradiation of solid targets) each new batch of target material must be tested and validated in special production runs before its use in routine radionuclide production and manufacture of radiopharmaceutical preparation. This will ensure that under specified conditions, the target yields a radionuclide in the desired quantity and quality.

**Carriers.** A carrier, in the form of inactive material, either isotopic with the radionuclide, or non-isotopic, but chemically similar to the radionuclide, may be added during radionuclide production and radiopharmaceutical preparation. In some situations it may be added to enhance chemical, physical or biological properties of the radiopharmaceutical preparation. The amount of carrier added must be controlled and sufficiently small for it not to cause undesirable physiological effects.

**Carrier-free preparation.** It is a preparation free from stable isotope of the same element as the radionuclide concerned present in the preparation in the stated chemical form or at the position of the radionuclide in the molecule concerned. When appropriate, specific radioactivity must be measured in the radiopharmaceutical preparation.

**No-carrier added preparation.** It is a preparation to which no stable isotopes of the same element as the radionuclide concerned are intentionally added in the stated
chemical form or at the position of the radionuclide in the molecule concerned. When appropriate specific radioactivity must be measured in the radiopharmaceutical preparation.

**Production of radiopharmaceutical preparation.** Radiopharmaceutical preparations may contain the types of excipients permitted by the general monograph for the relevant dosage form.

**Sterilization.** Radiopharmaceutical preparations intended for parenteral administration are sterilized by a suitable method (see 5.8 Methods of sterilization). Whenever possible, steam sterilization is recommended.

All sterilization processes must be validated.

**Addition of antimicrobial preservatives.** Radiopharmaceutical injections are commonly supplied in multidose containers. The nature of the antimicrobial preservative, if present, is stated on the label or, where applicable, that no antimicrobial preservative is present.

Radiopharmaceutical injections for which the shelf-life is greater than one day and that do not contain an antimicrobial preservative should preferably be supplied in single-dose containers. If, however, such a preparation is supplied in a multidose container, requirements of the general monograph for Parenteral Preparations should apply.

Radiopharmaceutical injections for which the shelf-life is greater than one day and that do contain an antimicrobial preservative may be supplied in multidose containers. After aseptic withdrawal of the first dose, the container should be stored at a temperature between 2° and 8° C and the contents used within 7 days unless otherwise specified.

**Radiation protection**


**Identity tests**

Tests for identity of the radionuclide are included in the individual monographs for radiopharmaceutical preparations. The radionuclide is generally identified by its half-life or by the nature and energy of its radiation or by both as stated in the monograph.

**Other tests**

**Half-life measurement.** The half-life is a characteristic of the radionuclide that may be used for its identification. The half-life is calculated by measuring the variation of radioactivity of a sample to be tested as a function of time. Perform the measurements in the linearity range of a calibrated instrument. Measurements should comply with the R 1.1 Detection and measurement of radioactivity. Approximate half-life can be determined over a relatively short period of time to allow release for use of radiopharmaceutical preparations. The calculated approximate half-life is within the range of the values stated in the individual monograph.
Radionuclidic purity
Radionuclidic impurities may arise during the production and decay of a radionuclide. Potential radionuclidic impurities may be mentioned in the monographs and their characteristics are described in the general monograph: Annexes: Table of physical characteristics. In most cases, to establish the radionuclidic purity of a radiopharmaceutical preparation, the identity of every radionuclide present and its radioactivity must be known.

Technical details of radionuclide identification and radionuclidic purity determination are described in R1.2 Radiation spectrometry and R1.3 Determination of radionuclidic purity. Because the level of radionuclidic impurities, expressed as a percentage of each impurity, may increase or decrease with time, the measured radioactivity of each impurity must be recalculated to the activity during the period of validity of the preparation.

The individual monographs prescribe the radionuclidic purity required and may set limits for specific radionuclidic impurities (for example, molybdenum-99 in technetium-99m). While these requirements are necessary, they are not in themselves sufficient to ensure that the radionuclidic purity of a preparation is sufficient for its clinical use. The manufacturer must examine the product in detail and especially must examine preparations of radionuclides with a short half-life for impurities with a long half-life after a suitable period of decay. In this way, information on the suitability of the manufacturing processes and the adequacy of the testing procedures is obtained. In cases where two or more positron-emitting radionuclides need to be identified and/or differentiated, for example the presence of 18F-impurities in 13N-preparations, half-life determinations need to be carried out in addition to gamma-ray spectrometry.

Radiochemical purity
A radioactive preparation may contain the radionuclide in different chemical forms other than the intended one. Therefore it is necessary to separate the different substances containing the radionuclide and determine the percentage of radioactivity due to the radionuclide concerned associated with the stated chemical form and the contribution to the total radioactivity due to the radionuclide concerned coming from other substances. For this purpose instruments for the detection and measurement of radioactivity are used in combination with a physic-chemical separation technique. Radiochemical purity is assessed by a variety of analytical techniques such as 1.14.4 High-performance liquid chromatography, 1.14.2 Paper Chromatography, 1.14.1 Thin-layer Chromatography and 1.15 Electrophoresis combined with suitable radioactivity measurement described in R1.1 Detection and measurement of radioactivity.

In all cases the radioactivity of each analyte is measured after the separation has been achieved using the stated method.

The radiochemical purity section of an individual monograph may include limits for specified radiochemical impurities, including isomers.

In some cases, it is necessary to determine the physiological distribution of the radiopharmaceutical in a suitable test animal.

Specific radioactivity. Specific radioactivity is defined as radioactivity of a radionuclide per unit mass of the element or of the chemical form concerned. Specific radioactivity is usually calculated taking into account the radioactivity concentration
and the concentration of the chemical substance being studied. Specific radioactivity changes with time. The statement of the specific radioactivity therefore includes reference to a date and, if necessary, time.

Specific radioactivity must be measured in carrier added preparations. For some non-carrier added radiopharmaceutical preparations (for example, receptor ligands) it is important to state specific radioactivity. Individual monographs might state the range of specific radioactivity.

**Chemical purity**

Chemical purity refers to the proportion of the preparation that is in the specified chemical form regardless of the presence of radioactivity; it may be determined by accepted methods of analysis.

In general, limits should be set for chemical impurities in preparations of radiopharmaceuticals if they are toxic or if they modify the labelling process or alter physiological uptakes that are under study or if they result in undesirable interactions (e.g. aluminium can induce flocculation of Tc-99m sulphur colloid). Special attention is necessary for impurities with a pharmacologically active or pharmacodynamic effect even for very low amounts (for example, receptor ligands). Where appropriate, the stereo-isomeric purity has to be verified. In general, the type of limit for inorganic impurities such as arsenic and heavy metals that are specified in monographs for pharmaceutical substances are also valid for radiopharmaceuticals.

Characterize impurities as much as possible. Generic limits can be set for unidentified impurities. The limits has to be chosen carefully considering amounts and toxicity based upon toxicities of starting materials, precursors, possible degradation products and the final product.

**pH**

When required, measure the pH of non-radioactive solutions as described under 1.13 Determination of pH. For radioactive solutions the pH may be measured using a pH indicator strip R.

**[Note from Secretariat:** Add **pH indicator strip R** to the section on Reagents using the following:**

**pH indicator strip. R.**

*Plastic or paper strip containing multiple segments of different dye-impregnated papers allowing visual determination of pH in the prescribed range by comparison with a master chart.*]

**Sterility**

A number of monographs for radiopharmaceuticals contain the requirement that the preparation is sterile. Such preparations comply with 3.2 Test for sterility. The special difficulty arises with the radiopharmaceuticals because of the short half-life of the radionuclide, the small size of batches and the radiation hazards. In the case that the monograph states that the preparation can be released for use before completion of the test for sterility, the sterility test must be started as soon as practically possible in relation to the radiation. If not started immediately, samples are stored under conditions that are shown to be appropriate in order to prevent false negative result.
When the size of the batch of a radiopharmaceutical is limited to one or few samples (e.g., therapeutic or very short-lived radiopharmaceutical preparations), sampling the batch may not be possible. In this case, reliance is on process control rather than the final product control.

**Bacterial endotoxins/pyrogens**

Where appropriate, an individual monograph for a radiopharmaceutical preparation requires compliance with 3.4 Test for bacterial endotoxins. Validation of the test is necessary to exclude any interference or artefact due to the nature of the radiopharmaceutical. The pH of some radiopharmaceutical preparations will require to be adjusted to pH 6.5–7.5 to achieve optimal results.

Where it is not possible to eliminate interference with the test for bacterial endotoxins due to the nature of the radiopharmaceutical, compliance with 3.5 Test for pyrogens may be specified.

**Labelling**

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

*Note from Secretariat: Check that the text is consistent with current GMP text needs to be undertaken in final version.*

The label on the primary container should include:

- A statement that the product is radioactive or the international symbol for radioactivity;
- Name of the radiopharmaceutical preparation;
- Where appropriate, that the preparation is for diagnostic or for therapeutic use;
- Route of administration;
- Total radioactivity present at a stated date and, where necessary, time; for solutions, a statement of the radioactivity in a suitable volume (for example, in MBq per ml of the solution) may be given instead;
- Expiry date and, where necessary, time;
- Batch (lot) number assigned by the manufacturer;
- For solutions, the total volume.

The label on the outer package should include:

- Statement that the product is radioactive or the international symbol for radioactivity;
- Name of the radiopharmaceutical preparation;
- Where appropriate, that the preparation is for diagnostic or for therapeutic use;
- Route of administration;
• Total radioactivity present at a stated date and, where necessary, time; for solutions, a statement of the radioactivity in a suitable volume (for example, in MBq per ml of the solution) may be given instead;

• Expiry date and, where necessary, time;

• Batch (lot) number assigned by the manufacturer;

• For solutions, the total volume;

• Any special storage requirements with respect to temperature and light;

• Where applicable, the name and concentration of any added microbial preservatives or, where necessary, that no antimicrobial preservative has been added.

Note: The shipment of radioactive substances is subject to special national and international regulations as regards to their packaging and outer labelling. (Regulations for the Safe Transport of Radioactive Materials. Safety Requirements. No.TS-R-1 (IAEA, Vienna, 2009). For further details and the current guidance consult the IAEA web site at http://www-ns.iaea.org/standards/)

**Storage**
Radiopharmaceuticals should be kept in well-closed containers and stored in an area assigned for the purpose. Storage conditions should be such that the maximum radiation dose rate to which persons may be exposed is reduced to an acceptable level.

Care should be taken to comply with national regulations for protection against ionizing radiation.

Radiopharmaceutical preparations that are intended for parenteral use should be kept in a glass vial, ampoule or syringe that is sufficiently transparent to permit the visual inspection of the contents. Glass containers may darken under the effect of radiation.

**Annexes: Terminology**

**Biological half-life**
The biological half-life ($T_{1/2b}$) of a radiopharmaceutical is the time taken for the concentration of the pharmaceutical to be reduced 50% of its maximum concentration in a given tissue, organ or whole body, not considering radioactive decay.

**Critical organ**
The critical organ is the organ or tissue which is the most vulnerable to radiation damage This may not be the target tissue or the tissue that receives the highest dose and therefore the dose to the critical organ will determine the maximum safe dose which can be administered.

**Effective half-life**
The effective half-life ($T_{1/2e}$) is the actual half-life of a radiopharmaceutical in a given tissue, organ or whole body and is determined by a relationship including both the physical half-life and biological half-lives. The effective half-life is important in calculation of the optimal dose of radiopharmaceutical to be administered and in monitoring the amount of radiation exposure. It can be calculated from the formula:

$$T_{1/2e} = \frac{T_{1/2p} \times T_{1/2b}}{T_{1/2p} + T_{1/2b}}$$
Where $T_{\frac{1}{2}}^{p}$ and $T_{\frac{1}{2}}^{b}$ are the physical and biological half-lives respectively.

**Half-life**  
The time in which the radioactivity decreases to one-half its original value.

**Explanatory note.** The rate of radioactive decay is constant and characteristic for each individual radionuclide. The exponential decay curve is described mathematically by the equation:

$$ N = N_0 e^{-\lambda t} $$

where $N$ is the number of atoms at elapsed time $t$, $N_0$ is the number of atoms when $t = 0$, and $\lambda$ is the disintegration constant characteristic of each individual radionuclide. The half-life period is related to the disintegration constant by the equation:

$$ T_{\frac{1}{2}} = \frac{0.693}{\lambda} $$

Radioactive decay corrections are calculated from the exponential equation, or from decay tables, or are obtained from a decay curve plotted for the particular radionuclide involved (see Figure 1).

**Figure 1. Master decay chart**

![Master decay chart](image-url)
Isotopes
Atoms of the same element with different atomic mass numbers are called isotopes.

Nuclide
Nuclide is defined as species of atom as characterized by the number of protons, the number of neutrons, and the energy state of the nucleus.

Radioactive concentration
The radioactive concentration of a solution refers to the amount radioactivity per unit volume of the solution. As with all statements involving radioactivity, it is necessary to include a reference date and time of standardization. For radionuclides with a half-life of less than one day, a more precise statement of the reference time is required. Units for radioactive concentration are megaBecquerels per millilitre (MBq/ml).

Since the radioactive concentration will change with time due to decrease in the nuclide radioactivity it is always necessary to provide a reference time. For short-lived radionuclides the reference time will be more precise including time of day in addition to date.

Radioactive decay
The property of unstable nuclides during which they undergo a spontaneous transformation within the nucleus. This change results in the emission of energetic particles or electromagnetic energy from the atoms and the production of an altered nucleus.

Explanatory note. The term “disintegration” is widely used as an alternative to the term “transformation”. Transformation is preferred as it includes, without semantic difficulties, those processes in which no particles are emitted from the nucleus.

Radioactivity
Generally the term “radioactivity” is used both to describe the phenomenon of radioactive decay and to express the physical quantity of this phenomenon. The radioactivity of a preparation is the number of nuclear disintegrations or transformations per unit time. In the International System (SI), the term “activity” is used, which corresponds to radioactivity in the context of this general monograph.

It is expressed in becquerel (Bq), which is 1 nuclear transformation per second.

Explanatory note. The term “disintegration” is widely used as an alternative to the term “transformation”. Transformation is preferred as it includes, without semantic difficulties, those processes in which no particles are emitted from the nucleus.

Radiochemical purity
The ratio expressed as a percentage of radioactivity of radionuclide concerned which is present in the radiopharmaceutical preparation in the stated chemical form, to the total radioactivity of that radionuclide present in the radiopharmaceutical preparation. Relevant potential radiochemical impurities are listed with their limits in the individual monographs. (Note: Source of information: European Pharmacopoeia.)

As radiochemical purity may change with time, mainly because of radiolysis or chemical decomposition, the result of the radiochemical purity test should be started at given date and if necessary hour indicating when the test was carried out. The radiochemical purity limit should be valid during the whole shelf-life.
Radionuclidic purity
The radionuclidic purity is the ratio expressed as a percentage of radioactivity of the radionuclide concerned to the total radioactivity of the radiopharmaceutical preparation. The relevant potential radionuclidic impurities are listed with their limits in their individual monographs.

Specific radioactivity
The specific radioactivity of a radionuclide corresponds to the SI term “specific activity” in the context of this monograph and is defined as radioactivity of radionuclide per unit mass of the element or of the chemical form concerned, e.g., Bq/g or Bq/mole.

The term employed in radiochemical work is “specific activity”. As the word “activity” has other connotations in a pharmacopoeia, the term should, where necessary, be modified to “specific radioactivity” to avoid ambiguity.

Units of radioactivity
The activity of a quantity of radioactive material is expressed in terms of the number of spontaneous nuclear transformations taking place in unit time. The SI unit of activity is the becquerel (Bq), a special name for the reciprocal second (s⁻¹). The expression of activity in terms of the becquerel therefore indicates the number of transformations per second.

The historical unit of activity is the curie. The curie (Ci) is equivalent to 3.7 x 10¹⁰ Bq. The conversion factors between becquerel and curie and its submultiples are given in Table 1.

<table>
<thead>
<tr>
<th>Number of atoms transforming per second</th>
<th>SI unit: becquerel (Bq)</th>
<th>historical unit: curie (Ci)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 Bq</td>
<td>27 picocurie (pCi)</td>
</tr>
<tr>
<td>1000</td>
<td>1 kilobecquerel (kBq)</td>
<td>27 nanocurie (nCi)</td>
</tr>
<tr>
<td>1 x 10⁶</td>
<td>1 megabecquerel (MBq)</td>
<td>27 microcurie (μCi)</td>
</tr>
<tr>
<td>1 x 10⁹</td>
<td>1 gigabecquerel (GBq)</td>
<td>27 millicurie (mCi)</td>
</tr>
<tr>
<td>37</td>
<td>37 Bq</td>
<td>1 (nCi)</td>
</tr>
<tr>
<td>37,000</td>
<td>37 kBq</td>
<td>1 (μCi)</td>
</tr>
<tr>
<td>3.7 x 10⁷</td>
<td>37 MBq</td>
<td>1 (mCi)</td>
</tr>
<tr>
<td>3.7 x 10¹⁰</td>
<td>37 GBq</td>
<td>1 Ci</td>
</tr>
</tbody>
</table>

Annex: Table of physical characteristics

Physical characteristics of clinically relevant radionuclides
Information on the physical characteristics of key radionuclide used in nuclear medicine is provided in Table 2.

Note from Secretariat: Table 2 will be updated by IAEA.
Radiopharmaceuticals

Safety considerations

This is a draft proposed text for the Supplementary Information section of The International Pharmacopoeia (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidt@who.int. Working documents are available for comment on-line at http://www.who.int.

Safety considerations
Radiopharmaceuticals are radioactive and can pose a risk to the personnel involved in handling them during inter alia manufacture, storage, transport, compounding, testing, dispensing and administration, to the patients to whom they are administered and to the environment.

All personnel involved in any part of the above operations are required to have appropriate specific additional training. All personnel with access to the areas where these operations are carried out, for example, maintenance and support staff such as cleaners should receive specific instruction and appropriate supervision whilst in the operational areas. Risk to patients should be minimized. It is essential to ensure that reproducible and clinically reliable results will be obtained. All operations should be carried out or supervised by personnel who have received expert training in handling radioactive materials.

Specialized techniques are required to minimize the risks to personnel. All procedures in which radiopharmaceuticals are handled must be designed and carried out in compliance with the ALARA principle, that is to ensure that exposure to radiation is as low as reasonably applicable. Three key components of the ALARA principle are time (reduce time of exposure), distance (the greater the distance, the lower the risk) and shielding (appropriate shielding is essential at all stages of handling).

Airborne radioactive contamination is a risk factor. Protection of staff requires a negative pressure environment which is conflicting with the general GMP requirement to protect the product in a positive pressure environment. These issues should be balanced according to risk-based approach and rationalized.

Radiation shielding. Adequate shielding must be used to protect all personnel from ionizing radiation. Additionally, when testing radiopharmaceuticals instruments must be suitably shielded from background radiation.

References

List of IAEA publications (safety, facility design, etc.)

Note from the Secretariat: IAEA will provide a list of publications.
Radiopharmaceuticals

Testing: additional guidance

This is a draft proposed text for the Supplementary Information section of The International Pharmacopoeia (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidth@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

Note from the Secretariat: It is suggested that the section on “end-user testing/rapid quality control tests” be replaced with the text below.

End-user testing

An end-user test is meant to be the simplified quality control test provided by the respective kit manufacturer in the package leaflet.

The end-user test may be used provided that it can be demonstrated that the preparation is fully traceable to a batch certified to comply with all the other requirements of the related monograph.

End-user testing is an important step in the quality management of radiopharmaceutical preparation and for the safety of patients, especially for those radiopharmaceutical preparations that are dispensed or compounded in the end-user facility (for example, nuclear medicine clinics). Application of the test is specified in the relevant monograph may not be possible at this stage either because of the short half-life of the radioisotope or due to other analytical limitations. The use of alternative, simple tests that adequately identify this radiopharmaceutical preparation is therefore advisable.


Radiopharmaceuticals

Methods of analysis: R3, biological methods

This is a draft proposed text for the Supplementary Information section of The International Pharmacopoeia (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidth@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

Note from the Secretariat: In principle, the Secretariat will aim to avoid conclusion of this test in order to avoid tests on animals. The following text is nevertheless proposed to replace the previous version as a general method of analysis.
R3.1 Biodistribution

A physiological distribution test is prescribed for certain radiopharmaceutical preparations. Specific requirements are set out in individual monographs. The distribution pattern of radioactivity observed in specified organs, tissues or other body compartments of an appropriate animal species (usually small animals such as rats or mice) can be a reliable indication of the expected distribution in humans and thus of the suitability of the intended purpose. The individual monograph prescribes the details concerning the performance of the test and the physiological distribution requirements, which must be met for the radiopharmaceutical preparation. A physiological distribution conforming to the requirements will assure appropriate distribution of the radioactive compounds to the intended biological target in humans and limits its distribution to non-target areas. Determination of the biodistribution pattern is usually done in the development phase of a kit, radiopharmaceutical or revalidation of known compound.

Selection of animals

Usually healthy animals are used, except for certain special circumstances such as cancer models, which are drawn from a uniform stock that have not previously been treated with any material which will interfere with the test. If relevant, the species, sex, strain and weight and/or age of the animals are specified in the monograph. Unless otherwise stated, mice weigh not less than 20 g and not more than 30 g; rats weigh not less than 150 g and not more than 250 g; and guinea pigs (especially for cardiac radiopharmaceuticals) weigh not less than 250 g.

Method

Prepare the test radiopharmaceutical, draw required radioactivity in a small volume (e.g. 0.2 mL) into a 1 mL syringe. Inject the specified radioactivity (x) of the radiopharmaceutical preparation into the tail vein of animals (usually three animals). Weight of the animals is measured in advance. Measure the radioactivity in the syringe before (y) and after the injection (z). Swab the injection site with cotton wool and retain the cotton wool and the residual dose in the syringe after injecting for counting (y) and (z), respectively.

Actual injected dose (a) = x-(y+z).

Immediately after injection, place each animal in a separate cage that is designed to allow collection of excreta and to prevent contamination of the body surface of the animal. After the time period specified in the monograph (uptake time), euthanize the animals. Collect a sample of blood by cardiac puncture and record the weight of the sample. Harvest the required organs, e.g., gall bladder, liver, stomach, intestines, bones and kidneys, and place in separate labelled counting tubes. Remove the tail above the injection site and place in a labelled counting tube. Determine the injected dose by an appropriate method depending on the activity.

Standard solutions of the radiopharmaceuticals are prepared. Draw 0.2 mL of the radiopharmaceutical solution in a syringe and estimate its weight by weighing the empty syringe and the syringe with solution and calculating the difference. Dispense this radiopharmaceutical solution into a clean 100 mL glass beaker and add 20 mL of distilled water. This solution is taken as the standard for estimation of the total activity that is injected into the animals. Corrections for different sample geometries are applied when necessary. Decay correction needs to be applied and times of
measurement are recorded. Measurements are done for three times and averaged. Background counts should be subtracted for each measurement.

The activity in the organs, tail and carcass is measured either in an isotope dose calibrator or in a NaI(Tl) crystal scintillation counter which is regularly calibrated.

Biodistribution can be calculated by the following methods.

**Method A**

The percentage activity in the organ is calculated as follows:

If using an isotope dose calibrator, the activity retained in the organs is calculated as:

\[
\frac{\text{activity obtained in the organ} \times 100}{\text{total activity injected}}
\]

If using a NaI(Tl) scintillation counter, the activity retained in the organs is calculated as:

\[
\frac{\text{counts in organ}}{\text{counts in standard (Wi/Ws) x 10}} \times 100
\]

where Wi is the weight of injection and Ws is the weight of the standard.

The percentage of radioactivity in blood is determined according to the formula:

\[
[100 \times (C/Ws) \times 0.07 \times (Wr) / a]
\]

where

- C = Radioactivity in specimen of blood;
- Ws = weight in grams of blood specimen;
- Wr = weight in grams of animal. (Normally, blood is approx. 7% of total body weight.)

**Method B**

\[
(ID/g) \text{ injected dose per gram of tissue}
\]

\[
\% \ ID/g = C_t \frac{V_t \cdot \frac{1}{D_{inj}} \times 100}{W_t} \quad \text{(%/g)}
\]

Where

- \( C_t \) = tissue concentration = activity / volume
- \( V_t \) = tissue volume
- \( W_t \) = tissue weight
- \( D_{inj} \) = dose injected

**Specification**

The preparation meets the requirements of the test if the distribution of radioactivity in at least two of the three animals complies with the criteria specified in the monograph. Disregard the results from any animal showing evidence of extravasation of the injection (observed at the time of injection or revealed by subsequent assay of tissue radioactivity).

Biodistribution studies by organ counting can be supplemented by the gamma camera imaging.
In the development of new radiopharmaceuticals, repeat studies should be done for different time points of organ harvesting (e.g., 1-hour, 3-hour, 6-hour or 24 hour post injection) with a similar number of animals for same cohort group.

Radiopharmaceuticals: specific monograph

**Natriiodidi (\(^{131}\)I) solutio**
**Sodiumiodide (\(^{131}\)I) solution**

This is a draft revised proposal for *The International Pharmacopoeia* (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidth@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

---

**Monograph.** Radiopharmaceuticals: Specific monographs: Natriiiodidi (\(^{131}\)I) solutio - Sodium iodide (\(^{131}\)I) solution

**Latin.** Natrii iodidi (\(^{131}\)I) solutio.

**English.** Sodium iodide (\(^{131}\)I) solution.

**Structural formula.** Na⁺ I⁻

**Relative molecular mass.** 153.895.

**Empirical formula.** Na \(^{131}\)I

**Chemical name.** Sodium (\(^{131}\)I) iodide

**Other names.** Natrii radioiodidum, IodotopeSodium iodide I 131

**Description.** Sodium iodide (\(^{131}\)I) solution is a clear colourless solution. Iodine-131 has a half-life of 8.08 days.

**Category.** Diagnostic or therapeutic.

**Storage.** Stored at room temperature. Preserve in single-dose or multiple-dose containers that previously have been treated to prevent adsorption.

**Labelling.** State the date and the time of calibration; the amount of \(^{131}\)I as iodide expressed as total MBq and the concentration expressed as MBq/ml, the expiration date, the name of any excipient, the name and quantity of any added preservative or stabilizer. The label states a statement of the intended use, whether oral or intravenous; a statement of whether the contents are intended for diagnostic or therapeutic use and the statement “Caution-Radioactive material”. The labelling indicates that in making dosage calculations, correction has to be made for radioactive decay, also indicates that the radioactive half-life of 131I is 8.08 days.
**Manufacture**

Iodine-131 may be obtained by neutron bombardment of tellurium or by extraction from uranium fission products. No carrier iodide is added.

Sodium iodide (131I) solution may contain sodium thiosulfate, sodium hydrogen carbonate or other suitable reducing agents and may contain a suitable buffer. Sodium iodide (131I) solution 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 “Liquid preparations for oral use”, “Parenteral Preparations” and with that for “Radiopharmaceuticals” as appropriate.

**Definition.** Sodium iodide solution is an aqueous solution containing of radioactive (131I) processed in the form of sodium iodide, 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 radioactivity is not less than 185 MBq (5 mCi) per microgram of iodine at the reference date and time stated on the label. The iodide content should not more than 20 µg in maximum recommended dose.

**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 364 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 184 and 203 hours.

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 (a).

**pH value.** Carry out the test as described under 1.13 Determination of pH or R1.5 under the monograph for “Radiopharmaceuticals”. pH between 7.5 and 9.0 of the solutions intended for parenteral administration and between 7.5 and 10.0 of the solutions intended for oral administration.

**Sterility.** The solution complies 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 preparation, modified as described in the monograph for "Radiopharmaceuticals”. Test for sterility will be initiated on the day of manufacture. 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 $175/V$ (μg 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 6.55 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 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 (a) 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 (b) 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 (a). Prepare a solution containing 2 mg/ml of each of the components stated on the label, apart from iodide, used as blank solution. Use the column with ($l = 0.25$ m, $Ø = 4.0$ mm). The stationary phase is spherical base-deactivated end-capped octadecylsilyl silica gel for chromatography R (5 μm), maintain the temperature constant between 20 °C and 30 °C. Use stainless steel tubing.

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. The flow rate is 1.5 ml/min, the detector is spectrophotometer at 220 nm and radioactivity detector connected in series. Inject 25 μl of test solution (a), the blank solution and reference solutions (a) and (b). The run time is 12 minutes.

The relative retention with reference to iodide is 5 and to iodate is 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 (b) 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 (a).

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 (a).

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 to dry. Develop the chromatogram over a period of about 4 hours by ascending chromatography, using dilute methanol (7.0 in 10). Allow the paper to dry in air, and determine the radioactivity distribution by scanning with a suitable radiation detector: the radioactivity of the iodide $^{131}$I 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)

- Detect iodide limit by examination of the radioactivity detector, not less than 95 per cent 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 × 3 cm.

Apply 10–20 μl samples in a distance of 10-13 cm from the end of the stripes. Use borate buffer with a concentration of 9 g/l and pH 9 ± 0.1. Carry out the electrophoresis on a potential of 900 V and time is 50 minutes.

The $R_F$ value for iodide is between 0.7 and 0.9, $R_F$ for iodate is 0.4, periodate from 0 to 0.1. Product can be accepted if the $^{131}$I anion content is higher than 95% even on the expiration date.
Radioactivity. Measure the radioactivity as described under R.1.1 Detection and measurement of radioactivity in suitable calibrated counting equipment by comparison with a standardized iodine-131 solution or by measurement in an instrument calibrated with the aid of such a solution.

Standardized iodine-131 solutions are available from laboratories recognized by the relevant national or regional authority.

Impurities

$[^{131}\text{I}]$ iodate ion.

Radiopharmaceuticals: specific monograph

Technetii ($^{99}\text{Mtc}$) exametazimi multiplex injectio
Technetium ($^{99}\text{Mtc}$) exametazime complex injection

This is a draft revised proposal for The International Pharmacopoeia (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidth@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

Additional or amended text.

Monograph. Radiopharmaceuticals: Specific monographs: Technetii ($^{99}\text{mTc}$) exametazimi multiplex injectio – Technetium ($^{99}\text{mTc}$) exametazime complex injection

Latin. Technetii ($^{99}\text{mTc}$) exametazimi multiplex injection.

English. Technetium ($^{99}\text{mTc}$) exametazime complex injection.

Structural formula

![Structural formula](image)

Empirical formula. $C_{13}H_{25}N_{4}O_{3}\cdot^{99}\text{mTc}$

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 6.02 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 either cobalt chloride solution or methylene blue solution.

Labelling. State the date and the time of calibration; the amount of 99mTc as labelled exametazime expressed as total MBq and the concentration expressed as MBq/ml; the expiration date; and the statement “Caution — Radioactive material”. The labelling indicates that in making dosage calculations, correction is to be made for radioactive decay, and indicates that the half-life of 99mTc is 6.02 hours. The label states that upon constitution with Sodium Pertechnetate 99mTc injection, beyond use time is 30 minutes for the unstabilized injection, and between 4 hours and 6 hours for the stabilized injections.

Manufacture. Technetium-99m is a radioactive nuclide formed by the radioactive decay of molybdenum-99. Molybdenum-99 is a radioactive isotope of molybdenum and may be produced by neutron irradiation of natural molybdenum or of molybdenum enriched in molybdenum-98 or it may be produced by uranium fission.

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 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 racemic mixture of (3RS, 9RS)-4, 8-diaza-3,6,6,9-tetramethylundecane-2,10-dione bisoxime (exametazime)
complexes with sodium pertechnetate ($^{99m}$Tc) 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 ($^{99m}$Tc) exametazime complex.

**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 140 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 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”. pH of the injection, between 5.0 to 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. The injection may be released for use before completion of the test.

**Radionuclidic purity.** Record the gamma-ray spectrum using a suitable instrument and measure the half-life using a suitable method. Determine the relative amounts of technetium-99m and radionuclidic impurities that may be present.

**Radiochemical purity**

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

A. Carry out three separate tests as described under 1.14.2 Paper chromatography and ascending conditions. Use suitable cellulose paper strips and methyl ethyl ketone R (system A) or sodium chloride (9 g/l) TS (system B) as the mobile phases.
Use suitable silica gel impregnated glass fiber paper strips and a mixture of equal volumes of acetonitrile R and water R as the mobile phase (system C). Apply to the paper about 5 μl of the injection to be examined, suitably diluted to give an optimum count rate and develop for a distance of about 15 cm. Allow the paper to dry in air and determine the radioactivity distribution by a suitable method. In system (A), the secondary exametazime complex and reduced hydrolysed technetium-99m have RF value of 0, and the lipophilic exametazime complex and the pertechnetate ion have RF value of 0.8 to 1.0. In system (B), reduced hydrolysed technetium-99m has RF value of 0, and the lipophilic exametazime complex, the secondary exametazime complex and the pertechnetate ion have an RF value of 0.8 to 1.0. In system (C), the pertechnetate ion has an RF value of 0.8 to 1.0, and the lipophilic exametazime complex, the secondary exametazime complex and reduced hydrolysed technetium-99m have RF value of 0. The sum of the percentages of radioactivity corresponding to the pertechnetate ion in system (C) and reduced hydrolysed technetium-99m in system (B) is less than 10%. Not less than 80% of the total technetium-99m radioactivity is present as lipophilic exametazime complex.

B. 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 per cent of the total radioactivity.

C. 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 fiber 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. 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 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 size of the column used is (l = 0.25 m, Ø = 4.6 mm). The stationary phase is spherical base-deactivated end-capped octadecylsilyl silica gel for chromatography R (5 μm) with a pore size of 13 nm.
and a carbon loading of 11 per cent. Mix 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 the run time is 20 min. The relative retention with reference to lipophilic technetium-99m exametazime to impurity A is about 1.2.

**System suitability: reference solution**
The produced chromatogram is similar to the chromatogram provided with meso-rich exametazime CRS. The resolution is a minimum of 2 between the peaks due to lipophilic technetium-99m exametazime and to impurity A. Impurity A should not more than 5 per cent of the radioactivity due to lipophilic technetium-99m exametazime and impurity A.

**Chemical purity**

**Tin.** 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 (1 mol/l) VS and mixing thoroughly. Prepare the reference solution by dissolving 0.115 g of stannous chloride R in hydrochloric acid (1 mol/l) VS, diluting to 1000 ml with the same solvent and mixing thoroughly. 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 as described under R.1.1 Detection and measurement of radioactivity in a suitable calibrated counting equipment by comparison with a standardized technetium-99m solution or by measurement in an instrument calibrated with the aid of such a solution (a good approximation may be obtained using an ionization chamber and employing a standardized solution of cobalt-57 provided that correction for the differences in the radiations emitted are made).

Standardized technetium-99m and cobalt-57 solutions are available from laboratories recognized by the relevant national or regional authority.

**Impurities**
A. Meso isomer of lipophilic technetium-99m exametazime,
B. Technetium-99m in colloidal form,
C. $[^{99m}\text{Tc}]$ pertechnetate ion,
D. Non lipophilic technetium-99m exametazime complex,
E. Meso isomer of non-lipophilic technetium-99m exametazime complex.

**Biodistribution.** Carry out the test as described under R3.1 Biological distribution using a set of three mice. At 5 to 10 minutes post injection not less than 1.5% of the injected radioactivity should be found in the brain and not more than 20% of the injected radioactivity should be found in the intestine. Not more than 15% of the injected radioactivity should be found in the liver.

---

**Radiopharmaceuticals: specific monograph**

**thallosi (201Tl) chloridi Injectio**

**thallous (201Tl) chloride injection**

This is a draft revised proposal for *The International Pharmacopoeia* (June 2013). Please address any comments to Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4730 or e-mail to schmidt@who.int. Working documents are available for comment on-line at http://www.who.int/medicines.

### Additional or amended text.

**Monograph.** Radiopharmaceuticals: Specific monographs: Thallosi (201Tl) chloridi injectio – Thallous (201Tl) chloride injection

**Latin.** Thallosi (201Tl) chloridi injectio

**English.** Thallous (201Tl) chloride injection

**Structural formula.** Tl+  Cl-

**Empirical formula.** $201\text{TlCl}$

**Relative molecular mass.** 236.423

**Chemical name.** Thallium (201Tl) chloride

**Other names.** Thallous (201Tl) chloride

**Description.** Thallous (201Tl) chloride injection is a clear colourless, aqueous solution. Thallium-201 has a half-life of 73.1 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 and the contents used within 7 days.

Labelling. State the date of withdrawal of the first dose for multidose containers. State the time and date of calibration; the amount of $^{201}\text{TI}$ as labeled thallous chloride expressed as total MBq and concentration as expressed as MBq/ml at the time of calibration; the expiration date; and the statement “Caution—Radioactive Material.” The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of $^{201}\text{TI}$ is 73.1 hours.

Manufacture
No-carrier-added thallium-201 radioisotope is produced by proton bombardment of enriched thallium 203 target followed by chemical separation of lead 201 radioactive isotopes. The separated lead isotope decay in optimum 32 hours into thallium 201 by electron capture or positron emission 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 ($^{201}\text{TI}$) 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 radioactivity is not less than 3.7 GBq (100 mCi) 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 167keV and X-rays of 69 and 83keV.

Standardized thallium-201 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 70 and 75 hours.

C. Examine the electropherogram obtained in the test for radiochemical purity. The distribution of the radioactivity contributes to the identification of the preparation. pH value. Carry out the test as described under 1.13 Determination of pH or R1.5 under the monograph for “Radiopharmaceuticals”. pH of the injection, 4.0 to 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/V (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 for at 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 color 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 of 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 as described under R.1.1 Detection and measurement of radioactivity in suitable calibrated counting equipment by comparison with a standardized thallium-201 solution or by measurement in an instrument calibrated with the aid of such a solution.

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

**Impurities**

A. Lead-201

B. Lead-203

C. Thallium-200

D. Thallium-202

E. [²⁰¹Tl] Thallic (III) ion.

**Biodistribution.** Carry out the test as described under R3.1 Biological distribution using a set of three guinea pigs. At 1 hour post injection not less than 4% of the injected radioactivity should be found in the heart.