PROPOSED TEXT FOR THE SUPPLEMENTARY INFORMATION SECTION OF THE INTERNATIONAL PHARMACOPOEIA

RADIOPHARMACEUTICALS: METHODS OF ANALYSIS: R3, BIOLOGICAL METHODS
(June 2013)

DRAFT FOR DISCUSSION

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THE INTERNATIONAL PHARMACOPOEIA

RADIOPHARMACEUTICALS: METHODS OF ANALYSIS: R3, BIOLOGICAL METHODS

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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 texts is nevertheless proposed to replace the previous version as a general method of analysis.

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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 few 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:

\[
\text{% injected activity in the organ} = \frac{\text{Activity obtained in the organ}}{\text{Total activity injected}} \times 100
\]
If using a NaI(Tl) scintillation counter, the activity retained in the organs is calculated as:

\[
\text{% injected activity in the organ} = \left( \frac{\text{counts in organ}}{\text{counts in standard} \times (\text{Wi}/\text{Ws})} \right) \times 100
\]

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

\[
\left[ 100 \times \left( \frac{\text{C}}{\text{Ws}} \right) \times 0.07 \times \left( \frac{\text{Wr}}{\text{a}} \right) \right]
\]

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

**Method B**

\((\text{ID/g})\) Injected dose per gram of tissue

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

Where 
- \(C_t\) = tissue concentration = activity / volume
- \(V_t\) = tissue volume
- \(W_t\) = tissue weight
- \(D_{\text{inj}}\) = 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 point of organ harvesting (e.g. 1-hour, 3-hour, 6-hour or 24-hour post-injection) with similar number of animals from same cohort group.

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