9. TESTS

9.1 Radionuclidic purity

In the context of radiopharmaceuticals, radionuclidic purity is an important quality parameter and it is mandatory that the radionuclidic impurities are within the stipulated limits. Such radionuclidic impurities arise during the production of the radionuclide and are, hence, dependent on the production route.

In most of the cases, to state the radionuclidic purity of a radiopharmaceutical preparation, the identity of every radionuclide present and their radioactivity must be known. The most generally useful method for the examination of radionuclidic purity is by gamma spectrometry using semiconductors such as germanium or cadmium-zinc-telluride (CZT). It is not a completely reliable method because alpha- and beta-emitting impurities are not usually easily detectable. When using sodium iodide detectors the peaks due to gamma emitting impurities are often obscured by the gamma spectrum of the principal radionuclide. Also, many radionuclides used in radiopharmaceuticals emit positrons and have the characteristic 511 keV gamma emissions, hence half-life determination is important.

The individual monographs prescribe the radionuclidic purity requirements (for example, the γ-ray spectrum does not significantly differ from that of a standardized preparation) and may set limits for specific radionuclidic impurities (e.g. cobalt-60 in cobalt-57). While these requirements are necessary, they are not in themselves sufficient to ensure that the radionuclidic purity of a preparation is sufficient for human use. The manufacturer must examine the product and production route in detail and, especially, must examine preparations of radionuclides of short half-life for impurities of 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 may be obtained. In cases where two or more β^+ emitting radionuclides need to be identified and/or differentiated, as ^{18}F-impurities in ^{13}N-preparations, half-life determinations need to be carried out in addition to gamma spectrometry using a HPGe detector.

Due to differences in the half-lives of the different radionuclides present in a radiopharmaceutical preparation, the radionuclidic purity changes with time. The radionuclidic purity requirement must be fulfilled throughout the period of validity of the radiopharmaceutical. It is sometimes difficult to carry out these tests before authorizing the release for use of the batch when the half-life of the radionuclide in the preparation is short. The test then constitutes a control of the quality of production, if the production is performed under controlled circumstances.

9.2 Radiochemical purity

In the context of radiopharmaceuticals, radiochemical purity is an important quality parameter which needs to be within the stipulated limits. The relevant radiochemical impurities are listed with their limits in the individual monographs for each radiopharmaceutical.

The determination of radiochemical purity requires separation of the different chemical substances containing the radionuclide and estimating the percentage of radioactivity associated with the declared chemical substance. Radiochemical impurities may originate from radionuclide production; subsequent chemical processing; incomplete preparative separation and chemical changes during storage.

The requirement of the radiochemical purity must be fulfilled throughout the period of validity of the radiopharmaceutical.

In principle, any method of analytical separation may be used in the determination of radiochemical purity. For example, the monographs for radiopharmaceutical products may include paper chromatography (*1.14.2*), thin-layer chromatography (*1.14.1*), instant thin-layer chromatography (ITLC), electrophoresis (*1.15*), size-exclusion chromatography, gas chromatography (*1.14.1*) and liquid chromatography (*1.14.1*). The technical description of these analytical methods is set out in the monographs. Moreover, certain precautions special to radioactivity must also be taken for radiation protection.

ITLC is a rapid, miniaturized thin layer assay method developed to determine the radiochemical purity of radiopharmaceuticals. The assay uses specific binderless microfiber chromatography strips impregnated with silica gel as the stationary phase. An ITLC method offers advantages such as easy to use, rapid and can be incorporated easily in a routine quality control program.

In paper and thin-layer chromatography, a volume equal to that described in the monograph is deposited on the starting-line as prescribed in the general methods for chromatography. It is preferable not to dilute the preparation to be examined but it is important to avoid depositing such a quantity of radioactivity that dead time losses occur during measurement of the radioactivity. On account of the very small quantities of the radioactive material applied, a carrier may be added when specified in a particular monograph. After development of the chromatogram, the support is dried and the positions of the radioactive areas are detected by autoradiography or by measurement of radioactivity over the length of the chromatogram using suitable collimated counters or by cutting the strips and counting each portion. The positions of the spots or areas permit chemical identification by comparison with solutions of the same chemical substances (non-radioactive) using a suitable detection method.

Radioactivity may be measured by integration using an automatic-plotting instrument or a digital counter. The ratios of the areas under the peaks give the ratios of the radioactive concentration of the chemical substances. When the strips are cut into portions, the ratios of the quantities of radioactivity measured give the ratio of concentrations of the radioactive chemical species.

Liquid chromatography (*1.14.1*) may also be used to separate the principal radioactive substance of a radiopharmaceutical preparation from radiochemical impurities or degradation products. Some radiochemical impurities, such as colloidal impurities, may be retained on the column. In such cases, a separate method is required to determine the content of the retained radiochemical impurities. The calculation formula for the expression of the total radiochemical purity has to take into account the relative amount of the retained radiochemical impurities.

9.3 Specific radioactivity

Specific radioactivity is the radioactivity of the radionuclide concerned in the stated chemical form per unit mass of the element or of the chemical form concerned, Bq/g (GBq/µg).

Specific radioactivity changes with time. The statement of the specific radioactivity therefore includes reference to a date and if necessary, time. The requirement of the specific radioactivity must be fulfilled throughout the period of validity.

9.4 Chemical purity

The determination of chemical purity requires quantification of the individual chemical impurities specified in the monograph.

9.5 Enantiomeric purity

Where appropriate, the stereoisomeric purity has to be verified.

9.6. Biodistribution (*R3.1*)

Tests involving animals should be avoided wherever possible. Where the tests for identity and for radiochemical purity are not adequate to completely define and control the radiochemical species in a radiopharmaceutical preparation, a biological distribution test may be required.

The distribution pattern of radioactivity observed in specified organs, tissues or other body compartments of an appropriate animal species (usually rats or mice) can be a reliable indication of the expected distribution in humans and thus of the suitability for 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.

In general, the test is performed as follows.

A minimum of three animals are used per test and each animal is injected intravenously with the preparation to be tested. If relevant, the species, sex, strain and weight and/or age of the animals are specified in the monograph. The test injection is the radiopharmaceutical preparation as it is intended for human use. Where applicable, products are reconstituted according to the manufacturer's instructions. In some cases, dilution immediately before injection may be necessary.

The administration will normally be made via the intravenous route for which purpose the caudal vein is used. Other veins such as saphenous, femoral, jugular or penile veins may be used in special cases. Animals showing evidence of extravasation of the injection (observed at the time of injection or revealed by subsequent assay of tissue radioactivity) are rejected from the test.

Immediately after injection each animal is placed in a separate cage which will allow collection of excreta and prevent contamination of the body surface of the animal.

At the specified time after injection, the animals are euthanized by an appropriate method and dissected. Selected organs and tissues are assayed for their radioactivity using a suitable instrument as described elsewhere in this monograph. The physiological distribution is then calculated and expressed in terms of percentage of the radioactivity which is found in each of the selected organs or tissues, taking into account corrections for radioactive decay. For this purpose, the radioactivity in an organ may be related to the injected radioactivity calculated from the radioactive content of the syringe measured before and after injection. For some radiopharmaceutical preparations, it may be appropriate to determine the ratio of the radioactivity in weighed samples of selected tissues (radioactivity/mass).

For a preparation to meet the requirements of the test, the distribution of radioactivity in at least two of the three animals must comply with all the specified criteria.

9.7 Sterility

Radiopharmaceutical preparations for parenteral administration comply with the test for sterility.

Radiopharmaceutical preparations for parenteral administration must be prepared using precautions designed to exclude microbial contamination and to ensure sterility. The test for sterility is carried out as described in the general method for sterility (*3.2*). Special difficulties arise with radiopharmaceutical preparations because of the short half-life of some radionuclides, small

size batches and the radiation hazards. It is not always possible to wait for the results of the test for sterility before authorization of the release of the radiopharmaceutical product for patients' use. 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 results. Parametric release of the product manufactured by a fully validated process is the method of choice in such cases. When aseptic manufacturing is used, the test for sterility has to be executed as a control of the quality of production.

Radionuclide precursors comply with the test for sterility if intended for use in the preparation or production of a radiopharmaceutical for parenteral administration without further appropriate procedure for the removal of microbial contamination and to ensure sterility of the radiopharmaceutical preparation.

When the size of a batch of the radiopharmaceutical preparation is limited to one or a few samples (e.g. therapeutic or very short-lived radiopharmaceutical preparation), sampling the batch for sterility testing may not be applicable. If the radiopharmaceutical preparation is sterilized by filtration and/or aseptically processed, process validation is critical.

When the half-life of the radionuclide is very short (e.g. less than 5 minutes), the administration of the radiopharmaceutical preparation to the patient is generally on-line with a validated production system.

For safety reasons (high level of radioactivity), it is not possible to use the quantity of the radiopharmaceutical preparations as required in the test for sterility (*3.2*). The method by membrane filtration is to be preferred to limit radiation exposure to personnel. Where justified and authorized on the basis of a risk assessment, reliance of aseptic preparation of sterile radiopharmaceutical preparations may be placed on a post-filtration integrity test, omitting the pre-filtration integrity test.

Notwithstanding the requirements concerning the use of antimicrobial preservatives in parenteral preparations, their addition to radiopharmaceutical preparations in multidose containers is not obligatory, unless prescribed in the monograph.

9.8 Bacterial endotoxins - pyrogens

For certain radiopharmaceutical preparations, a test for bacterial endotoxins is prescribed. The test is carried out as described in the general method (*3.4*), taking the necessary precautions to limit radiation exposure to the personnel carrying out the test. The limit for bacterial endotoxins is indicated in the individual monograph or calculated following relevant guidelines.

For radiopharmaceuticals made with short-lived radioisotopes, endotoxins testing after product release is permitted. However, with the introduction of the kinetic (photometric) LAL test, which can be completed in 20 minutes, it is possible to complete the test for bacterial endotoxins before releasing the radiopharmaceuticals with $T_{1/2}$ of > 30 min.

In the case of radionuclide precursors (solutions for radiolabeling) the limits on the bacterial endotoxin level should allow for a contribution from other sources in the radiolabeling process if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

When the nature of the radiopharmaceutical preparation results in an interference by inhibition or activation and it is not possible to eliminate the interfering factor(s), the test for pyrogens (*3.5*) may be specifically prescribed. It is sometimes difficult to carry out these tests before releasing the batch for use when the half-life of the radionuclide in the preparation is short. The test then constitutes a control of the quality of production.

9.9 pH

The pH of radiopharmaceuticals is important and is determined as a part of quality control (QC) testing (*1.13*). Due to the small volumes involved and the radioactivity present, the conventional use of a glass pH electrode may not practical or necessary. The sample available for all the QC testing (excluding sterility) is very small and the pH range permitted is sufficiently large. Hence, the use of narrow range pH strips is adequate and is preferred as the pH can be tested with a few microliters of the sample. Further, as they are radioactive after use, they can be easily and safely disposed.