

Radiopharmacy quality control

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1. INTRODUCTION

The overwhelming majority of radiopharmaceuticals used for diagnosis and therapy in Nuclear Medicine are administered to patients as intravenous injections. The European Pharmacopoeia mandates that all injections must be sterile, apyrogenic, free from extraneous particles and be of a suitable pH. In addition, radioactive injections must be of the correct radiochemical and radionuclidic purity and have the correct radioactivity present at the stated time of injection.

The EC Guide to Good Manufacturing Practice Vol. IV [1] lays down the requirements for the minimum standards for facilities personnel and quality systems that must be in place for commercial manufacturers to be able to market such products, and similarly the PIC/S have defined standards for hospital preparation of radiopharmaceuticals [2]. In the UK, guidance for quality assurance of radiopharmaceuticals has also been issued [3].

Quality control of radiopharmaceuticals is therefore necessary for 2 distinctly separate but linked reasons as they relate to pharmaceutical parameters and radioactive parameters.

1. Pharmaceutical parameters designed to ensure that no microbiological, pyrogenic or particulate contamination can harm patients.
2. Radioactive parameter designed to ensure that the intended radiation exposure of patients is kept to a minimum by confirming that the radioactivity, radiochemical and radionuclidic purity are assured. These additional factors have an effect on the overall radiation dose to the patient, as impurities of the radionuclide and/or its chemical composition may affect the biodistribution of the injected radiopharmaceutical and consequently the radiation dose to any one particular organ or the whole body dose (ED – Effective Dose).

2. METHODS FOR ASSESSING RADIOACTIVE PARAMETERS

2.1 Activity check

There are very strict limits on the amount of radioactivity that can be administered for each type of investigation to be performed. These limits are set by the legislative bodies in individual countries. Therefore, all radioactive preparations must be checked for activity before administration [3]. Most radionuclide calibrators used in radiopharmacy are ionisation chambers. Commercial calibrators have built in scaling factors for individual radionuclides that take into account the ionising ability of the isotope and give a read out in the appropriate units (kBq or MBq, or mCi). However, this type of calibrator is not ideal for all radionuclides. Low energy radiation, such as that produced by Iodine [^{125}I], may be attenuated before reaching the gas and the measurement may be inaccurate. Also, high energy beta particles interact with the chamber wall and the measurement of activity is based on the Bremsstrahlung radiation produced. The activities dispensed in a radiopharmacy need to be measured to a precision of 2-5%. It is very important that the radionuclide calibrator used is working correctly and a strict QA regime should be followed to ensure this. Daily, the calibrator is checked for accuracy against a long-lived reference sealed source (e.g. Cobalt [^{57}Co] or Americium [^{241}Am]), there should also be an annual linearity check. The accurate determination of low activities of X-ray and gamma-emitting radionuclides can be carried out using a well scintillation counter, in which a scintillant crystal produces a flash of light when struck by an ionising particle or photon. The light produced is converted to an electrical signal by a photomultiplier, this can then be amplified and counted. Gamma and X-rays are best detected with crystals of Thallium-activated Sodium Iodide (NaI(Tl)). In a well counter the source is placed inside a well drilled in the centre of a cylindrical crystal. Liquid scintillation counting is used in the measurement of beta emitters, such as Hydrogen [^3H] and Carbon [^{14}C].

2.2 Radionuclide identity

A radionuclide can be identified by measuring the half-life of the radionuclide and/or determining the energies of the emitted radiation.

- a) The half-life is measured with a suitable detector (see above). There must be sufficient activity in the sample so that it can be measured for several half-lives, but limited to minimise count rate defects and effects such as dead time losses. Multiple measurements are made in the same geometrical conditions, for a time at least equal to three expected half-lives. A graph is drawn with the logarithm of the instrument response against time. The half-life is calculated from the graph

and should not differ by more than 5% from the half-life stated in the Pharmacopoeiae.

- b) The nature and energy of the radiation emitted may be determined by several procedures depending on the type of radiation emitted. Radionuclides which emit gamma rays or detectable X-rays can be analysed using gamma spectrometry. The spectrum can be used to identify which nuclides are present in a source and in what quantities.

2.3 Radionuclidic purity

This is the amount of radioactivity due to the radionuclide concerned compared to the measured activity of the radiopharmaceutical preparation. It is usually expressed as a percentage. The relevant monographs in the Pharmacopoeiae give limits to the radionuclidic impurities allowed in each preparation. If significant levels of other radionuclides are present then biological distribution may be altered. Radionuclide impurities can occur as a result of the manufacturing process, for example, for nuclides produced by cyclotron there can be contaminants due to impurities in the target or by the energy of the reaction. For example Yttrium [^{86}Y], for use in PET, is produced by the reaction (p,n) on a target of Strontium [^{86}Sr] using protons with energy of 16 MeV. If an energy of 30 MeV is used then Yttrium [^{85}Y] is also produced which decays to Strontium [^{85}Sr]. This radionuclide has a long half-life and targets bone, having serious implications for patients. Impurities can also arise due to the presence of the parent nuclide of the stated nuclide when the stated nuclide is obtained by a separation technique such as a generator elution, for example, the presence of Molybdenum [^{99}Mo] in a solution of Technetium [$^{99}\text{Tc}^m$]. The presence of Molybdenum [^{99}Mo] in a Technetium [$^{99}\text{Tc}^m$] radiopharmaceutical would be detrimental for patients due to the beta emission of this radionuclide and the long half-life (66 hours) giving an increased radiation dose.

The radionuclidic purity can be determined by gamma spectrometry or by the determination of the half-life. The half-life method is useful for very short-lived isotopes for use in PET, such as Oxygen [^{15}O] (half-life 2 min).

A version of the attenuation method can be used to determine the amount of Molybdenum [^{99}Mo] in a solution of Technetium [$^{99}\text{Tc}^m$] as these two radionuclides emit gamma radiation of very different energies. The activity of the sample is determined in an ionisation chamber on the technetium setting. The activity measured will be due to the Technetium [$^{99}\text{Tc}^m$] and the Molybdenum [^{99}Mo]. The sample is then placed in a lead canister with wall thickness of 6 mm and measured again on the molybdenum setting. The radiation emitted by the Technetium [$^{99}\text{Tc}^m$] has an energy of 140 keV which is absorbed by the lead. The radiation emitted by the Molybdenum [^{99}Mo] has an energy of 740 keV and is only attenuated by a third. Any measured activity is therefore due to the Molybdenum [^{99}Mo] present. The proportion of molybdenum in the sample can then be calculated. The limit allowed is 0.1% contaminating Molybdenum [^{99}Mo]. This test should be performed daily on the first elution from a generator.

Due to differences in the half-lives of the different radionuclides that can be present in a preparation, the radionuclidic purity changes with time. The requirement of the radionuclidic purity must be fulfilled throughout the period of validity.

2.4 Radiochemical purity

It is important to know that the majority of the radioactive isotope is attached to the ligand and is not free or attached to another chemical entity as these forms may have a different biodistribution. This is termed the radiochemical purity. In diagnostic scanning the different biodistribution of contaminating radioactive chemicals could interfere with the clinical diagnosis by obscuring the region of interest and interfering with the interpretation of the scan. This could result in the patient returning to the department for a repeat scan with all the cost implications for the department and a repeat radiation dose for the patient, or, more seriously, to a misinterpretation of the images. Abnormal biodistribution can also be due to other causes such as patient medication, therefore determining the RCP could help to clarify an abnormal scan by ruling out defective product. In radiopharmaceuticals used for therapy a low RCP could mean an unacceptable radiation dose to healthy organs and tissues.

For most radiopharmaceuticals the lower limit of RCP is 95%, that is, at least 95% of the radioactive isotope must be attached to the ligand. RCP determination can be carried out by a variety of chromatographic methods:

2.4.1 Paper and thin layer chromatography

In these methods a small drop of the radiopharmaceutical is put onto the bottom of a strip of support medium (e.g. paper, silica gel coated sheets). The strip is put into a tank containing a small amount of solvent. The solvent migrates up the strip. The components of the radiopharmaceutical are separated

according to the solubility in the solvent and adsorption to the support medium. Detection of the radioactivity in the strip can be carried out in a number of ways.

- a) The simplest method is to cut the strip and count the activity in the sections in a radionuclide calibrator or a well scintillation counter. The percentage of activity in each section can then be determined. For example, if a strip is cut in two sections 'A' and 'B' then the percentage activity in section A is given by:

$$\%A = \frac{A}{A+B} \times 100$$

A+B

This method does have several limitations however. Radionuclide calibrators are inaccurate for samples of low activity due to the lower level of detectability and the accuracy of the calibrator at the lower range setting, and well scintillation counters should be avoided for samples of high activity, as these can exceed the count rate capabilities due to the resolving time of the detection system.

- b) The strip can be imaged under a gamma camera. Regions of interest can be drawn around the areas of radioactivity and the percentage of counts in each region can be determined. This method has the advantage of imaging the whole chromatography strip enabling artefacts to be seen, however, it is not practicable for most hospital departments due to the cost of camera time.
- c) The strip can be imaged using a radiochromatogram scanner. A radiochromatogram scanner uses a sodium iodide detector to detect the radioactive emission. If the scanner is linked to an integrator then quantification of the peaks can be carried out. This equipment is not suitable for counting emissions from some isotopes (for example Chromium [⁵¹Cr] or Iodine [¹²⁵I]) due to statistics associated with counting low activities.
- d) Analysis of the strip can be performed using storage phosphor imaging. A phosphor screen accumulates a latent image of the distribution of radioactivity on the strip. Scanning the screen with a laser allows the location and intensity of radioactivity to be analysed and stored for future reference. Results are shown as an image of the strip. Regions of interest can be drawn and integration carried out to determine activity in each region. By varying the time of exposure of the strip to the phosphor screen, radiopharmaceuticals containing many different isotopes, or of different activities, can be analysed.

One of the biggest drawbacks to paper and thin layer chromatography methods of determining RCP is the resolving power of the methods. Most methods commonly used will only resolve one component and so two or three methods may be needed to identify all the major contaminants in a product. Time can also be a limiting factor with some methods taking 20-30 minutes to develop, or even longer.

2.4.2 Solid Phase Extraction (SPE)

A variety of bonded silica sorbents are available packed into disposable cartridges or columns. The sorbent will selectively retain specific types of chemical compounds from the sample loaded on the column. There are many different sorbents available and many suppliers and manufacturers of SPE columns and cartridges. Components can be selectively eluted by careful choice of solvents. Eluates are collected and have sufficient activity that they can be counted in a radionuclide calibrator. The procedure takes about 5 min ensuring that RCP determination can be carried out before administration of the patient dose.

2.4.3 High Performance Liquid Chromatography (HPLC)

TLC RCP methods may not be sufficient to identify all the compounds which are present in a product. HPLC has a higher sensitivity and resolving power than simple TLC methods. HPLC separation operates on the hydrophilic/lipophilic properties of the components of a sample applied. Gamma emitters are detected using a well scintillation counter connected to a rate meter. Other detectors (UV or refractive index) can be connected in series allowing simultaneous identification of compounds.

It should not be necessary to perform HPLC on radiopharmaceuticals reconstituted from licensed cold kits. It is useful to have techniques available for the purpose of eliminating a cause of any abnormal patient scan. For radiopharmaceuticals prepared 'in-house' or novel compounds for research purposes, an HPLC method for estimating radiochemical purity is essential. It should be noted that HPLC does not detect colloidal contaminants and that this should be estimated using TLC methods.

2.4.4 Electrophoresis

Electrophoresis separates components in a sample according to the charge and size of the molecules. The most frequently used electrophoresis method in radiopharmacy is in the RCP determination of radiolabelled albumin where the sample is run on a support of filter paper in a barbitone buffer.

2.5 Chemical purity

Chemical purity requires the identification and quantification of individual chemical constituents or impurities in a radiopharmaceutical preparation. A routine test for chemical purity is performed on the Technetium [$^{99}\text{Tc}^m$] Sodium Pertechnetate solution eluted from a generator. Technetium [$^{99}\text{Tc}^m$] Sodium Pertechnetate solution may be contaminated with aluminium, which originates from the alumina bed of the generator column. The presence of aluminium can interfere with the preparation of some Technetium [$^{99}\text{Tc}^m$] -colloidal preparations and also with the labelling of red blood cells with Technetium [$^{99}\text{Tc}^m$], causing their agglutination. The aluminium in the eluate can be detected using a simple paper test which is commercially available. Filter paper is impregnated with a colour complexing agent. A standard solution of aluminium (10mgml^{-1}) is supplied. A spot of the standard solution causes a colour change in the paper. A spot of generator eluate is compared to the standard spot. If the colour is more intense in the eluate spot then the eluate contains more than 10mgml^{-1} aluminium and implies a lack of stability in the column, consequently the eluate should be discarded.

It should not be necessary to perform other chemical tests on licensed radiopharmaceuticals. Unlicensed radiopharmaceuticals should be checked for chemical purity to ensure the quality of the product. This would include the quantification of the normal constituents of a labelling kit i.e. the ligand and reductant. Synthesis precursors or catalysts used in the preparation should be tested for. Commonly, this can be carried out using HPLC. Gas chromatography methods are used to test PET tracers for residual solvents used in the synthesis of these agents.

3. METHODS FOR ASSESSING PHARMACEUTICAL PARAMETERS

3.1 pH

The pH of the preparation should be in the physiologically acceptable range (5.5 - 8) or at the optimum pH for the stability of the preparation. It is especially important to check the pH of PET radiopharmaceuticals as preparative operating procedures may include extreme pH conditions which need neutralization. pH can be checked by placing a drop of solution on pH paper. Reading the paper is done by referring to a colorimetric pH scale. Micro pH meters are available which can read a drop of liquid (10 μl) placed on the electrodes.

3.2 Apyrogenicity

Bacterial endotoxins (pyrogens) are polysaccharides from bacterial membranes. They are water soluble, heat stable, and filterable. If they are present in a preparation and administered to a patient they can cause fever and also leukopenia in immunosuppressed patients. To minimise the chances that pyrogens are present it is important that preparations are manufactured and dispensed under aseptic conditions and that all consumables and equipment used have been heat treated and known to be pyrogen free. Most licensed products are guaranteed pyrogen free. PET products, whether manufactured commercially or in Hospitals, must be tested for pyrogens prior to or immediately following use. The most popular method is the limulus amoebocyte lysate test. However data exists confirming that some radiopharmaceuticals may directly cause precipitation or interfere with the forming gel.

3.3 Visual examination

As with all pharmaceutical preparations, radiopharmaceuticals should undergo a visual examination. The examination should take into account radiation protection issues for the operator and be conducted as quickly as possible with the preparation behind suitable shielding. Vials should be examined for insecure closures, cracks, glass particles in the liquid, and particulate contamination. Syringes should also be examined for particulate contamination.

3.4 Sterility

In the UK it is recommended that retrospective sterility testing of products is undertaken three times a week. A sample (0.3-0.5ml) of the first elution (used) of each new generator is aseptically transferred to two different sterile culture media contained in 100ml rubber stoppered vials. These are then incubated within the Radiopharmacy department at the correct temperature for at least one week to ensure total radioactive decay. They are then transported for further culturing as necessary by a Pharmacy Microbiological Testing Laboratory. In a similar fashion, the last elution from each generator (unused) is tested, as is a sample taken from the remnants of a product which has been prepared during the week and from which patient doses have been dispensed. If a positive growth is found, an investigation must be held to determine possible cause(s).

References

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Further Reading

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