Quality control of radiopharmaceuticals

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Abstract
Radiopharmaceuticals are special class of radiolabelled compounds which are mainly used as diagnostic tools and a small percentage is used as therapeutic agents. Since they are administered in humans, the Quality Control (QC) tests should be done with utmost care following the standard operating procedures as per the monograph as mentioned in Pharmacopeia.(USP,BP,IP etc.) QC tests are done to ensure safety and efficacy of the drug. In the case of radiopharmaceuticals, some special tests are done besides the tests done for the pharmaceuticals, to ensure the radiological safety. The QC tests and the instrumentation used in the case of radiopharmaceuticals are briefly given in the article.

Introduction
Radiopharmaceuticals are medicinal products having radioisotopes which are safe and effective for diagnosis or treatment in humans. Nowadays use of radioisotopes is very common for diagnosis purpose as in imaging of organ function and disease status, moreover Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and Ultrasonography are the tools which are providing us the exact metabolic and physiological functioning of tissue or organs. [1, 2] Apart from that Iodine-131(Radioisotope) is in use since 1946 for the trusted treatment of thyroid cancer and still it is a most efficacious method for the treatment of hypothyroidism and thyroid cancer [3, 4].

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Radiopharmaceuticals are classified into two types i) Diagnostic radiopharmaceuticals ii) Therapeutic radiopharmaceuticals. They are also further subdivided into a) Oral radiopharmaceuticals
b) Injectable radiopharmaceuticals. The essential difference between the diagnostic and therapeutic radiopharmaceuticals is the quantity of radioisotope used in terms of radioactivity. Since radiopharmaceuticals are intended for human administration, it is essential that they undergo strict quality control tests in order to meet the predetermined specifications as normal pharmaceuticals. In the case of radiopharmaceuticals the main concern is the radiological safety besides the other safety aspects. The radiological safety will ensure the patient does not get unnecessary radiation exposure during diagnosis or therapy. The QC tests for the most of the radiopharmaceuticals are mentioned in the Pharmacopeia (USP, BP, IP etc.)

QUALITY CONTROL TESTS
The quality control of radiopharmaceuticals includes the purity, potency, product

identity, biologic safety and efficacy of the radiopharmaceuticals.

The main tests include:

1. Physicochemical tests
2. Biological tests.

Quality Control procedures should always be performed immediately because of the short-lived nature of the radioisotope present in the radiopharmaceuticals.

In Physicochemical tests following parameters are involved

Appearance
Particle size
Radioactivity Assay
Radionuclidic Purity
Radionuclidic Identification
Radiochemical Purity

In Biological tests:

Sterility
Bacterial Endotoxin
Bio-distribution

When all the tests are complied as per the monograph, the identity, purity and safety is ensured. In the case of Radiopharmaceuticals Radioactivity assay, Radionuclidic purity and the Radiochemical purity tests ensure the radiological safety.

Physicochemical tests:

Appearance: A radiopharmaceutical's physical appearance is important, both at the time of receipt and throughout its useful shelf life.

Particle Size: Particle size and number of particles is critical to achieve desired bio-distribution of particulate radiopharmaceuticals i.e., it determines where they get localised**.** For performing liver/spleen studies, (Technetium-99m) ^{99m}Tc sulphur colloid must have a particle range of 0.1-1µm.

Radioactivity assay: Radioisotopes are frequently used either for imaging or for therapeutic purposes. Prescribed radioactivity in the form of radiopharmaceuticals should be administered to patients through the prescribed route .If the radioactivity is increased or decreased it will affect the quality of imaging in diagnostic radiopharmaceuticals whereas it will affect the therapeutic effect in the case of therapeutic radiopharmaceuticals. Radioactivity assay is routinely done using dose calibrators. The dose calibrator picture is shown below.

Radionuclidic purity (RNP): It is defined as the percentage of the total radioactivity present in the form of stated specified radionuclide.

– The radionuclide must have specific nuclear characteristics, for the gamma radiation energy to allow the acquisition of good scintigraphies;

– The radionuclide must have adequate radiation energies and half-life such that the internal irradiation dose delivered to the patient after administration of radiopharmaceutical, for the whole duration of the medical procedure, to be as low as possible;

– The radionuclide must have certain chemical properties enabling its biological behaviour to answer the desired purposes (localization only in the targeted organ) and the time until its clearance from the organism (biologic half-life) to be as short as possible. The product must have a selective affinity toward certain organs or groups of organs and in some specific cases to be metabolized, being physiologically inert and non toxic.

- Radionuclidic impurities may arise from impurities in the target material, differences in the values of various competing production cross section and excitation functions at the energy or energies of the bombarding.
- Radionuclidic impurities may be isotopic or non isotopic and shorter or longer-lived than the specified radionuclide.
- The need for Radionuclidic purity is to avoid degradation of the image quality and to limit error on measurements *in-vivo*.

Radionucidic purity is routinely checked by using HPGe detector.

Fig 1: HPGe Detector

Radionuclidic Identification: It is done using HPGe detector. They require radionuclide should be present in the prepared radiopharmaceutical. Otherwise it will affect the radiological safety of the patient.

Radiochemical purity (RCP): It is the percentage of the total radioactivity present in the desired chemical form in a radiopharmaceutical. RCP plays an important role in both diagnostic as well as in the therapeutic of radiopharmaceuticals. Generally RCP is done by any chromatographic technique such as paper, TLC, HPLC, Electrophoresis followed by radioactivity detection. In the case of paper and TLC after the development of chromatogram, they are scanned for radioactivity using radioactive scanner. In diagnostic radiopharmaceutical, the unacceptable level of RCP will lead to poor quality of the imaging and as a result accurate image interpretation will be difficult whereas in therapeutic radiopharmaceutical the unacceptable RCP will lead to unnecessary radiation dose to non- target organs.

Radiation Detection Instrumentation:
The following radiation detection instruments can be used in the determination of RCP: Gas Filled Detectors, Well Scintillation Counters and Radiochromatogram scanners. The determining factor for choosing the instrument is the amount of radioactivity used and the form of sample.

Gas Filled Detectors
The operation of a gas-filled detector is based on the ionization of gas molecules by radiations, followed by collection of the ion pairs as current with the application of a voltage between two electrodes. The two most commonly used gas-filled detectors are ionization chambers and Geiger-Muller (GM) counters.
Example of ionization chamber is *dose calibrator*, which is used for measuring the high intensity radiation sources, such as radioactivity of radiopharmaceuticals.

Well Scintillation Counter
A sodium iodide [NaI(Tl)] crystal well detector is an excellent choice for determining radioactive counts from paper and ITLC strips; however, the scintillation well counter's count rate capabilities must be known in order not to exceed its maximum counting rate.

Fig 2: Dose Calibrator

Radiochromatogram Scanner
A Radiochromatogram scanner is very useful in the identification and quantification of the radioactivity distribution on a radiochromatography strip.

Fig 3: TLC Scanner

It has the advantage of being able to quantify relative amounts of radiochemical species over a broad range of radioactivity. It is routinely used for the RCP checking.

BIOLOGICAL TESTS:

Sterility Test: Sterility can be defined as the freedom from the presence of viable microorganisms. Sterility testing of medical devices is required during the sterilization validation process as well for routine quality control. Sterilization is necessary for the complete destruction or removal of all microorganisms (including spore-forming and non-spore-forming bacteria, viruses, fungi, and protozoa) that could contaminate radiopharmaceuticals or other materials and thereby constitute a health hazard.

The various sterilization processes are as follows:

- Heating in an autoclave (steam sterilization)
- Dry-heat sterilization
- Exposure to ionizing radiation
- Gas sterilization
- Filtration

The sterilization process commonly used in radiopharmaceuticals is filtration.

Sterilization by filtration is employed mainly for thermo labile solutions. These may be sterilized by passage through sterile bacteria-retaining filters, e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. Usually, membranes of not greater than 0.22 μm nominal pore size should be used.

Bacterial Endotoxin Test: The Bacterial Endotoxin Test (BET) is a test to detect or quantify endotoxins from Gram-negative bacteria using appropriate amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are three techniques for this test: the gel-clot technique, which is based on gel formation; the turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate; and the chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromogen complex. Proceed by any of the three techniques for the test. In the event of doubt or dispute, the final decision is made based upon the gel-clot limit test unless otherwise indicated in the monograph for the product being tested. The test is carried out in a manner that avoids endotoxin contamination. Gel-clot method using LAL reagent is popularly used.

Limulus amebocyte lysate (LAL):

The LAL assay is the most sensitive method for the detection of bacterial endotoxins currently approved by the FDA. The first methodology used to determine the LAL test results was the formation of a gel-clot in the bottom of a glass reaction tube. It has also been observed that the test solution becomes turbid prior to gel formation. The time required to produce a specified level of turbidity is inversely proportional to the amount of endotoxin in a sample.

Fig 4: Sterile container

Bio-distribution Test:

Biodistribution is a method of tracking where compounds of interest travel in an experimental animal or human subject. Biodistribution method is mandatory for radiopharmaceutical injectables. Often the first indication that something might be wrong with a radiopharmaceutical is an unexpected pattern of biodistribution found during an imaging procedure. The ultimate test of the quality of a radiopharmaceutical is its biodistribution because finally the radiopharmaceutical is to be administered into a biological system mainly **into** humans.BD has to be carried out for every new batch of radiopharmaceutical injectables.In normal practice, BD is carried out in small animals (Wistar rats or Swiss mice)Biodistribution studies generally done by killing the animals at certain times after administration of the radiopharmaceutical and measuring the distribution of the radioactivity in tissues by counting techniques.The above mentioned Biological Tests are done only for Injectable radiopharmaceuticals not for Oral radiopharmaceuticals.

Radiopharmaceuticals for diagnosis:

^{99m}Tc--SULPHUR COLLOID

^{99m}Tc - Sulphur colloid is used as liver and spleen imaging agent for evaluation of functional morphology of reticulo endothelial system. Delineation of space occupying lesions in liver.

^{99m}Tc-DTPA (Diethylene Triamine Penta Acetic acid)

^{99m}Tc -DTPA is used as renal imaging agent for quantitative evaluation of renal functions (vascular, secretion, excretion) of individual kidneys.Estimation of glomerular filtration rate (GFR).

^{99m}Tc-PHYTATE

^{99m}Tc -Phytate is used as liver imaging agent for delineation of space occupying lesions in liver. Work up of patients in oncology: Assessment of metastases in liver – helpful in staging primary malignancy.

^{99m}Tc-MDP

^{99m}Tc-MDP-is used as a skeletal imaging agent. For detection and evaluation of infective bone diseases like osteomyelitis. In staging primary malignancy.

SODIUM FLUORIDE ^{18}F

Bone disease.

^{99m}Tc(V)-DMSA

^{99m}Tc (V)- DMSA is used as a tumor imaging agent, primarily in cases of medullary carcinoma of thyroid. Post surgery, to delineate metastases in lymph nodes in medullary carcinoma of thyroid.

SODIUM CHROMATE ^{51}Cr

Blood volume studies.

^{99m}Tc-MEBROFENIN

^{99m}Tc - Mebrofenin is used as hepatobiliary function imaging agent for assessment of hepatobiliary patency. Evaluation of intra as well as extra hepatic biliary obstruction.

^{99m}Tc-ECD

^{99m}Tc –ECD is used as brain imaging agent for assessment of regional cerebral blood flow using SPECT. Mapping of cerebral perfusion.

MIBI-^{99m}Tc

A MIBI scan or sestamibi scan is now a common method of cardiac imaging. Parathyroid imaging the drug is also used in the evaluation of breast nodules.

^{131}I-META-IODOBENZYLGUANIDINE (^{131}I-mIBG)

Imaging to identify the location of Pheochromocytoma (tumour of neuroendocrine origin and a cause of essential hypertension), particularly when CT scan is negative or inconclusive.

Radiopharmaceuticals for therapy:

^{153}Sm-EDTMP-

^{153}Sm-EDTMP used for Pain Relief of Bone Metastases from Prostate and Breast Cancer and Other Malignancies.

SODIUMIODIDE-Na^{131}l

For extensive therapy of thyroid cancer.

^{131}I-META-IODOBENZYLGUANIDINE (^{131}I-mIBG) :

Therapy of Pheochromocytoma and neuroblastoma.

Conclusion: Radiopharmaceuticals are unique medicinal formulations containing

radioisotopes which are used in major clinical areas for diagnosis and or therapy. QC of radiopharmaceuticals includes pH, appearance, particle size, radioactivity assay, radiochemical purity, radionuclidic purity, sterility, bacterial endotoxin test and bio-distribution test.

The above mentioned tests are carried out to ensure safety and efficacy of the product. In the case of radiopharmaceuticals, the radiological safety is also ensured by the special tests such as radioactivity assay, radiochemical purity and radionuclidic purity. In case of radiopharmaceuticals the tests should be faster and effective since radionuclides have short half life. QC is responsible for testing and release of reports in time so that the product can be released.

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Abbreviations:

CT : Computed Tomography
MRI : Magnetic Resonance Imaging
QC :Quality Control
USP : United States Pharmacopeia
BP : British Pharmacopoeia
IP : Indian Pharmacopoeia
RNP : Radionuclidic purity
HPGe detector: High purity germanium detector
RCP : Radiochemical purity
TLC :Thin Layer Chromatography
HPLC : High Performance Liquid Chromatography
GM : Geiger-Muller
BET : Bacterial Endotoxin Test
LAL : Limulus amebocyte lysate
FDA : Food and Drug Administration
BD : Bio-distribution
Tc : Technetium
DTPA : Diethylene Triamine Penta Acetic acid
GFR : Glomerular filtration rate
^{99m}Tc-MDP : 99mTc-Methylene diphosphonate
^{99m}Tc(V)-DMSA: Pentavalent 99mTc Dimercaptosuccinic acid
^{99m}Tc-ECD: 99mTc -Ethyl cysteinate dimer
MIBI-^{99m}Tc: 99mTc - Methoxyisobutylisonitrile
^{131}I-mIBG: ^{131}I-Meta-Iodobenzylguanidine
^{153}Sm-EDTMP: Samarium-153- Ethylene Diamine Tetramethylene Phosphonate

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