UT Southwestern
Medical Center
Radiology

Radiopharmacy Essentials

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Radiopharmaceutical Preparation – Compounding – Manufacturing

- Radiopharmaceutical preparation, preparation with minor deviations and compounding are performed or supervised by a pharmacist licensed by a state board of pharmacy (or under practice of medicine).
- Manufacturing is the mass production of drug products that have been approved by the Food and Drug Administration (FDA).

Radiopharmaceutical Preparation – Compounding – Manufacturing

- Preparations follow manufacturer preparation instructions, taking into account appropriate radiation safety considerations, appropriate environmental controls, and aseptic handling practices

- Compounding radiopharmaceuticals is the combining, mixing, diluting, pooling, reconstituting or otherwise altering a drug or bulk drug substance other than as provided by the manufacturer's package insert

Radiopharmacy Design

Manufacturing Facility vs Compounding Facility

Design of a Cyclotron Manufacturing Facility

The 4000 sq ft facility houses:

- A CGMP Clinical Production Lab
- Research Radiochemistry Lab
- Solid Target Research Radiochemistry Lab
- Quality Control Lab
- Cyclotron Electronics and Work Room
- Cyclotron Vault

CHARACTERISTICS OF THE VAULT

- 24 feet below ground
- 6 (or 3) feet concrete walls
- 6 feet concrete ceiling with an additional 5 feet dirt

CHARACTERISTICS OF THE PLUG-DOOR

- Framed with steel and filled with concrete
- 6 feet thick
- 15 tons weight (30,000 lb)

Flow of Radiopharmaceutical Production

Clinical Production Lab

Cyclotron

^{15}O, ^{13}N
^{11}C, ^{18}F

SOPs

Research Radiochemistry Lab

^{11}C, ^{18}F

^{64}Cu, ^{55}Co
^{89}Zr, ^{86}Y

Solid Target Lab

Quality Control (QC) Lab

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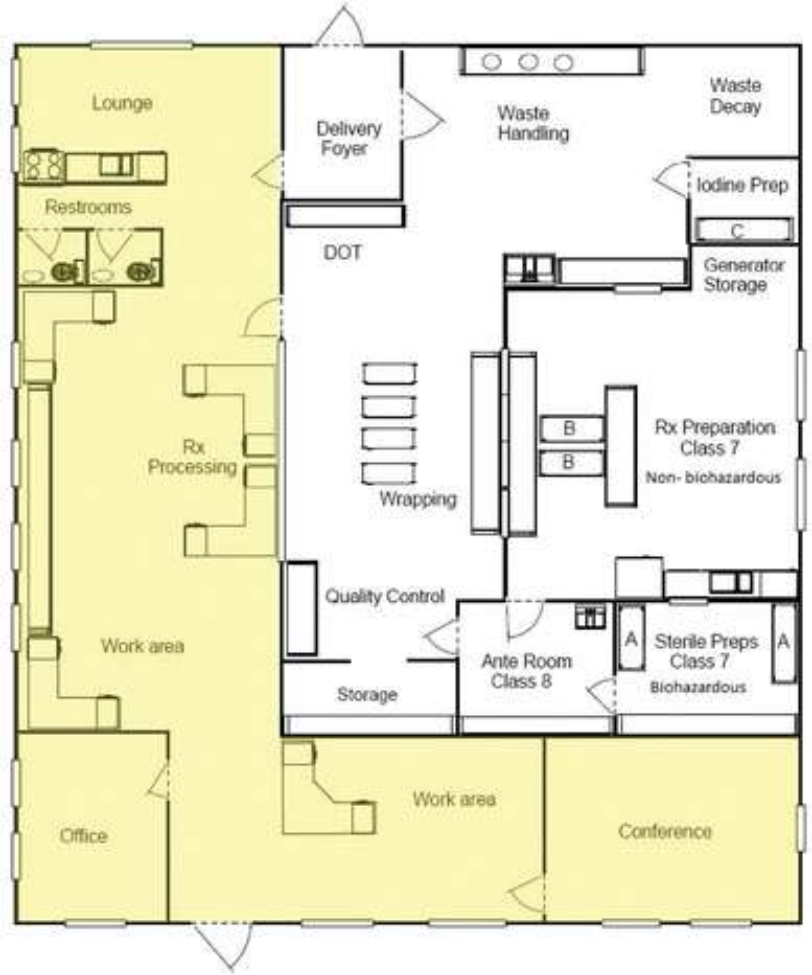
Design of a Compounding Radiopharmacy



- Facilities designed to minimize airborne contamination
- The PEC must be certified to meet ISO Class 5 or better conditions
- The PEC must be located in a SEC, which may be either an ISO-classified buffer room with ante-room or an SRPA
- Restricted area for the preparation of volatile or airborne radiopharmaceuticals

https://pharmacylibrary.com/doi/10.21019/9781582122830.ch13

PEC : Primary Engineering Control
SEC: Secondary Engineering Control
SRPA: Segregated Radiopharmaceutical Preparation Area

Design of a Compounding Radiopharmacy

Pictures provided by Huy Chu

RLS Radiopharmacies

Business Unit Manager - Dallas

First Attendance Verification Code:

1651

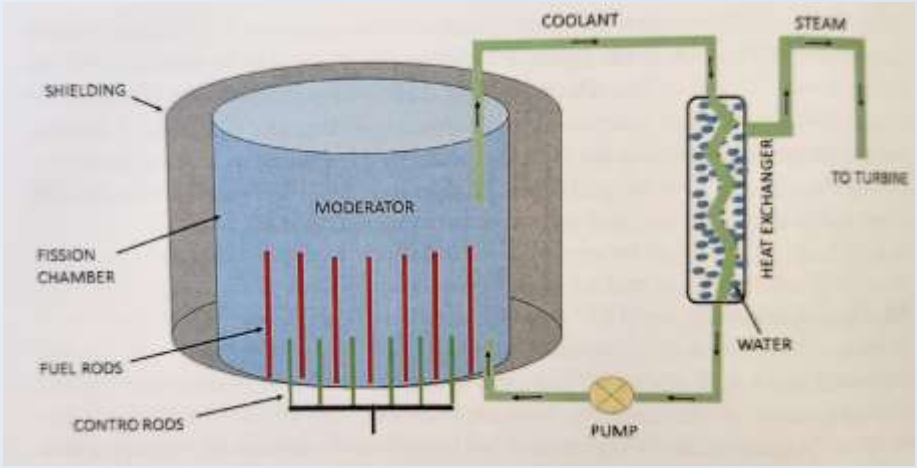
Radionuclide Production

Nuclear Reactors – Cyclotrons - Generators

Nuclear Reactor Radionuclide Production

- Fission is a process in which smaller radionuclides are created from larger radionuclides

Fission in an uncontrolled environment would release so much energy the result would be the explosion of an atomic bomb

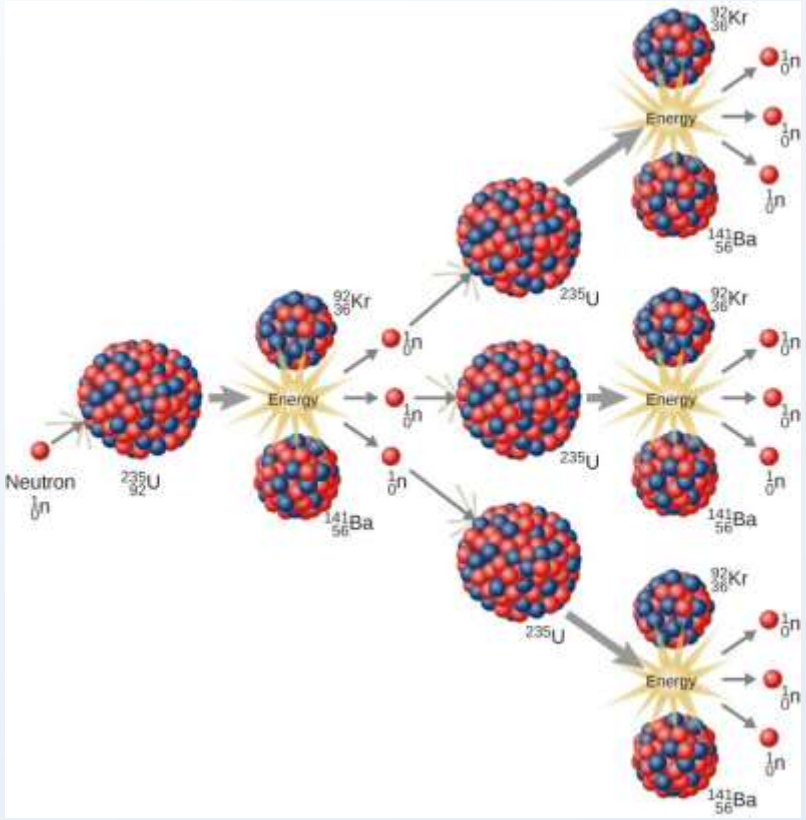


- Fuel rods contain the fuel (fissile) material (e.g. ^{235}U, ^{239}Pu) that undergo spontaneous fission
- Excess neutrons are removed by the control rods (cadmium)
- The reactor is either surrounded by water and/or graphite reflector and/or concrete to shield the radiation being produced
- The moderator is a low molecular weight material (water, beryllium, graphite) used to slow down high energy neutrons making them more useful
- Heat produced is removed by heat exchangers to produce electricity

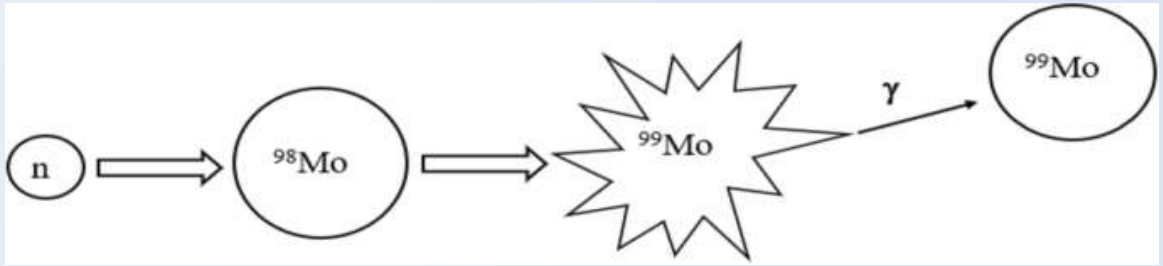
Saha, G.B. (2018) Fundamentals of Nuclear Pharmacy. Seventh Edition, Springer, New York, Heidelberg, Dordrecht and London.

Fission and Neutron Capture Reactions

- Fission is a breakup of a heavy nucleus into two fragments of approximately equal mass



- In neutron capture the produced excited nucleus releases γ energy to produce an isotope of the same element

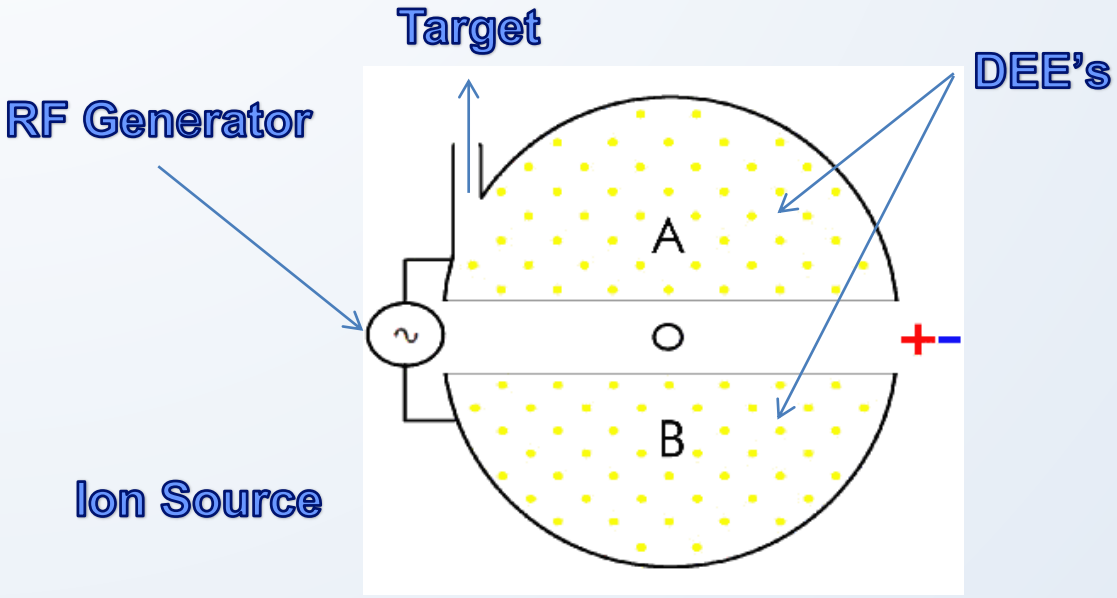


https://phys.libretexts.org/Bookshelves/University_Physics/University_Physics_(OpenStax)/University_Physics_III_-_Optics_and_Modern_Physics_(OpenStax)/10%3A__Nuclear_Physics/10.06%3A_Fission

https://link.springer.com/article/10.1007/s42452-020-03524-1

Cyclotron Produced Radionuclides

- Cyclotron accelerates charged particles (protons, deuterons, or alpha particles) to bombard targets for radioisotope production.



Cyclotron Produced Radionuclides

- Accelerated particles can be protons, deuterons, or alpha particles

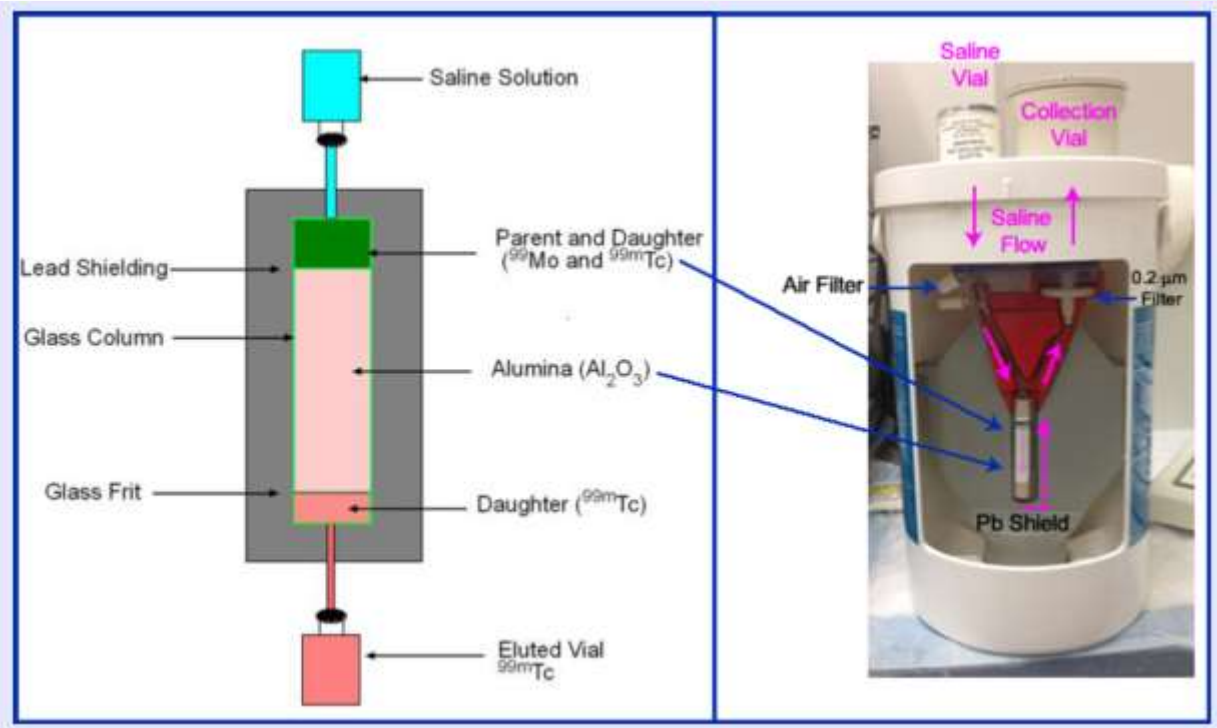
Isotope	Half-life	Nuclear Reaction	Target Media
^{15}O	2 min	$^{14}N(d,n)^{15}O$	N_2 + 1 %O_2
^{13}N	10 min	$^{16}O(p,\alpha)^{13}N$	[^{16}O]H_2O
^{11}C	20.3 min	$^{14}N(p,\alpha)^{11}C$	[^{11}C]CO_2: N_2 + 0.5 %O_2 [^{11}C]CH_4: N_2 + 10 %H_2
^{18}F	110 min	$^{18}O(p,n)^{18}F$	[^{18}F]F^-: ^{18}O-enriched H_2O [^{18}F]F_2: ^{18}O-enriched O_2 + O_2/Ar
^{68}Ga	67.7 min	$^{68}Zn(p,n)^{68}Ga$	^{68}Zn-enriched ZnO

- Radionuclidic impurities can originate from side reactions or impurities in the targe material

Radionuclide Generators

- Radionuclide generators are devices that produce a short-lived medical radionuclide (known as “daughter”) from the radioactive transformation of a long-lived radionuclide (called a “parent”).

Radionuclide Generators



- Radionuclidic impurities due to parent isotope breakthrough

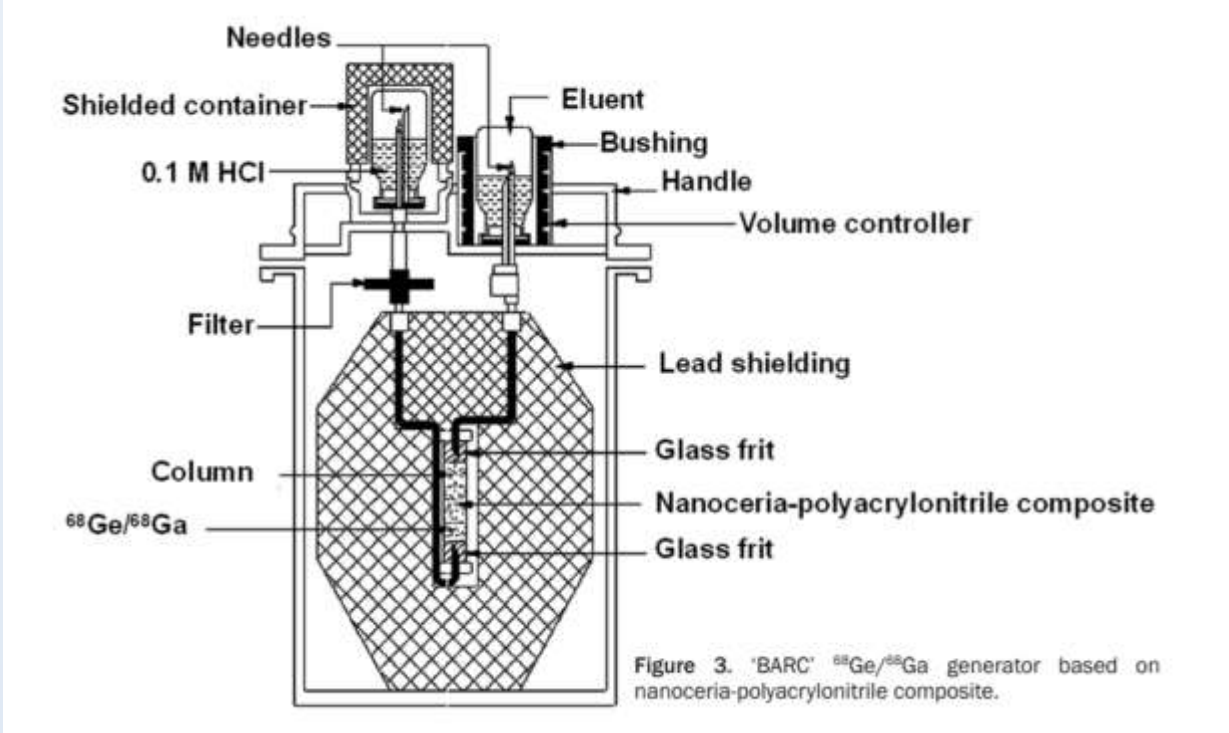
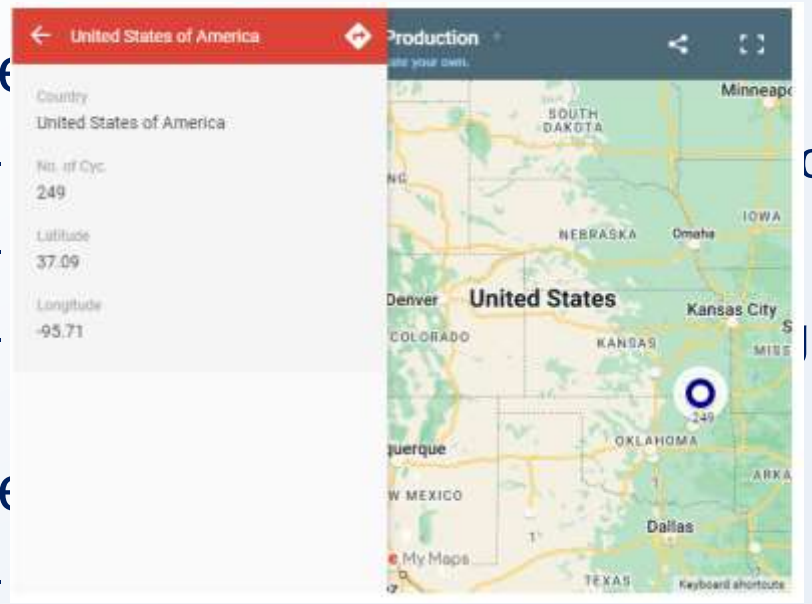
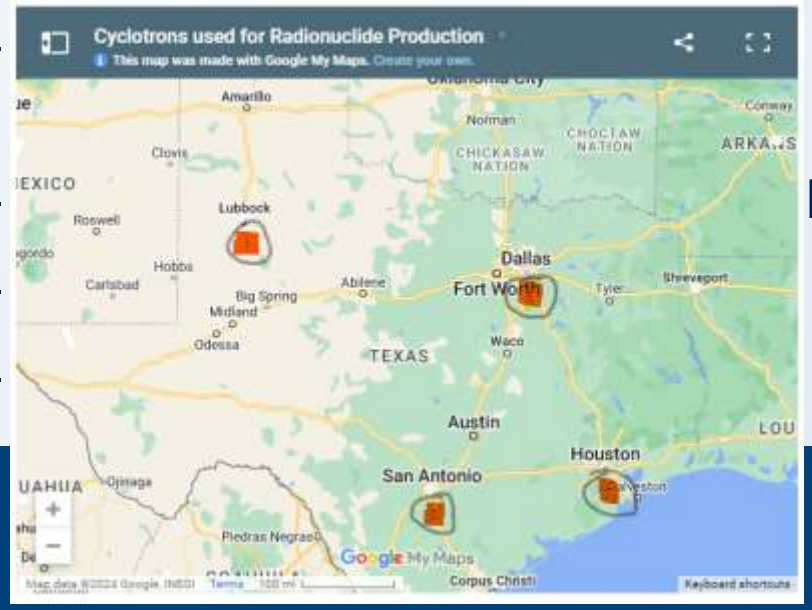


Figure 3. 'BARC' ^{68}Ge/^{68}Ga generator based on nanoceria-polyacrylonitrile composite.

Cyclotron vs Generator Produced ^{68}Ga

- Ge
  - onths
  -
  - gh
- Ge
  -
  - h)
  -
  -





- Cyclotron pros
  - Larger activities obtained
  - Consecutive productions with no down time
  - Independence from ^{68}Ge production sites can avoid shortages
- Cyclotron cons
  - Cyclotron facility needed
  - Larger number of employees
  - Cost of instrument/cyclotron maintenance

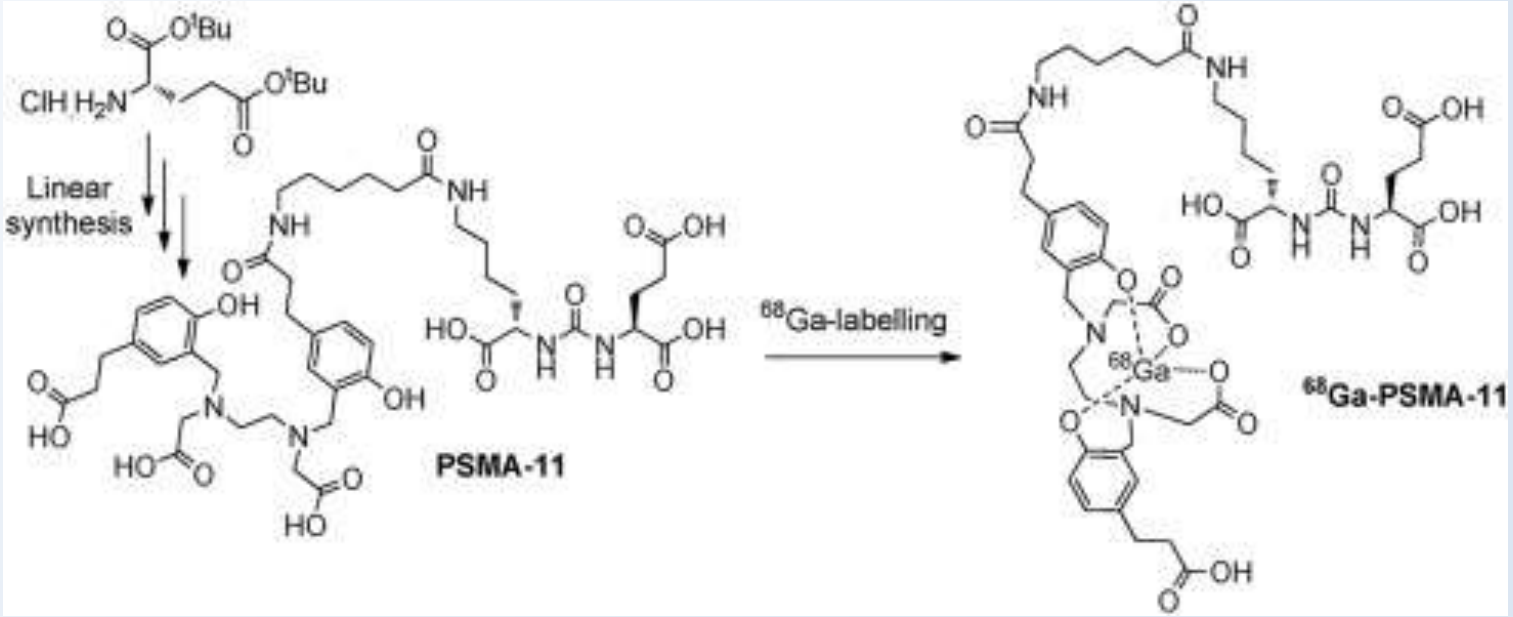
https://nucleus.iaea.org/sites/accelerators/Pages/Cyclotron.aspx

Radiopharmaceutical Production

Kit Preparation vs Manufacturing

Radiopharmaceutical Kit Preparation

- Kits include all reagents needed for the preparation of the drug
- Precursor and materials are checked for identity and purity
- Reagents, containers and closures are sterile and checked for pyrogens



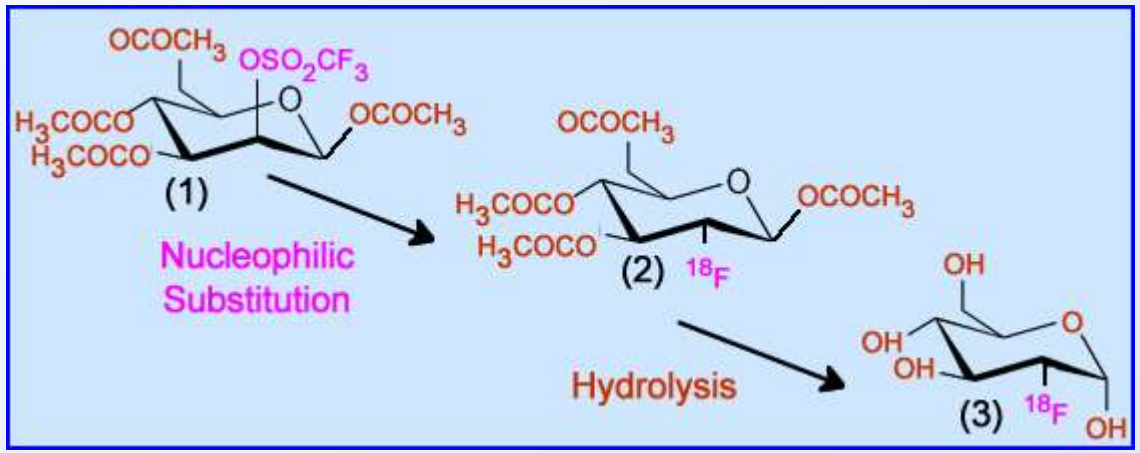
- Labelling with radiometals (e.g. ^{68}Ga, ^{111}In, ^{99m}Tc)

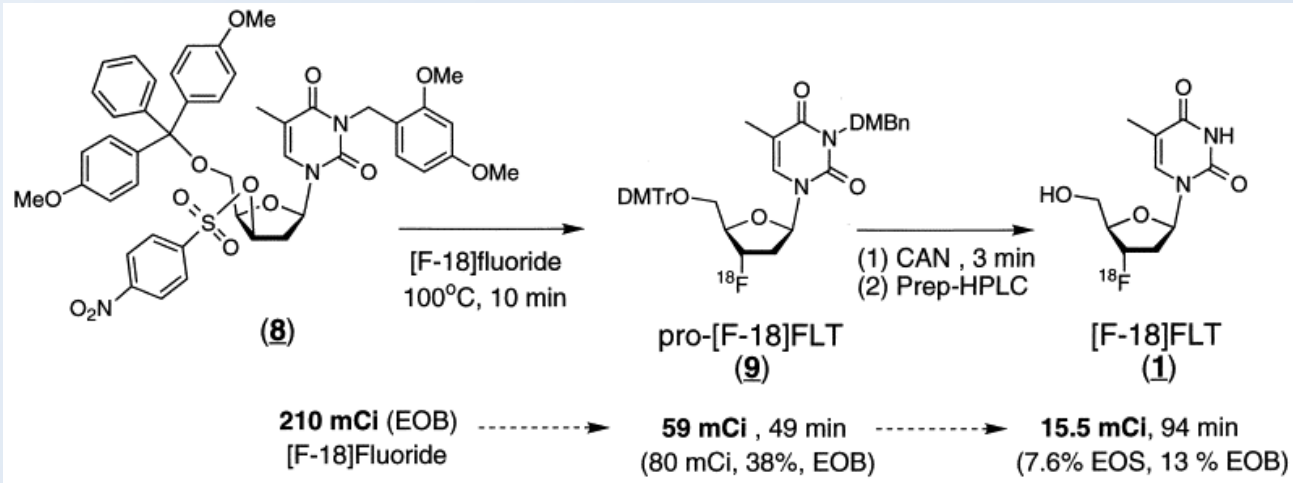
^{68}Ga-Gozetotide (Illuccix) Kit Preparation

- Green: normal saline
- White: empty evacuated vial
- Red: acetate buffer
- Blue: precursor

Radiopharmaceutical Manufacturing

- Manufacturing of radiopharmaceuticals can involve more that one steps and purification of intermediates
- Molecules are built from smaller starting compounds
- Reagents and starting compounds are not sterile – sterilization is required





- Labelling with non-radiometals (e.g. ^{18}F, ^{11}C, ^{15}O)

Grierson, J.R.; Shields, A. F. Nuc. Med. Biol. (2020), 27, 143 - 156

^{18}F-FLT Production

Cyclotron

1. Load target with [^{18}O]water
2. [^{18}F]Fluoride from cyclotron by $^{18}O(p,n)^{18}F$ reaction

[^{18}F]Fluoride -vial

3. [^{18}F]Fluoride in [^{18}O]water was sent to V-vial in FX-FN module

[^{18}F]Fluoride separation cartridge

4. Trap [^{18}F]Fluoride in a QMA cartridge; [^{18}O]water was sent to waste
5. Elute [^{18}F]Fluoride to reaction vessel using 1 mL of K_{222} and 0.1 mL of K_2CO_3 solution

Reaction vessel

6. Evaporate water and acetonitrile at 60°C for 2 min, followed by 7 min at 105°C
7. Add 15 mg of FLT precursor in 1 mL of acetonitrile to reactor; React at 125 °C for 6 min
8. Cool to 80°C; evaporate acetonitrile at 80°C for 20 seconds
9. Cool to 70°C; add 0.5 mL of 1 N HCl to reactor; react at 105°C for 5 min; then cool to 50°C

HPLC sample transfer vial

10. Add 3.5 mL of 20% sodium acetate solution and 0.1 mL ethanol to reactor; transfer reaction mixture through a light alumina N cartridge to HPLC transfer vial

HPLC column

11. Inject the reaction mixture onto the HPLC column; run HPLC for purification

HPLC collection flask

12. Collect the product in the HPLC collection flask

2-neck formulation vial

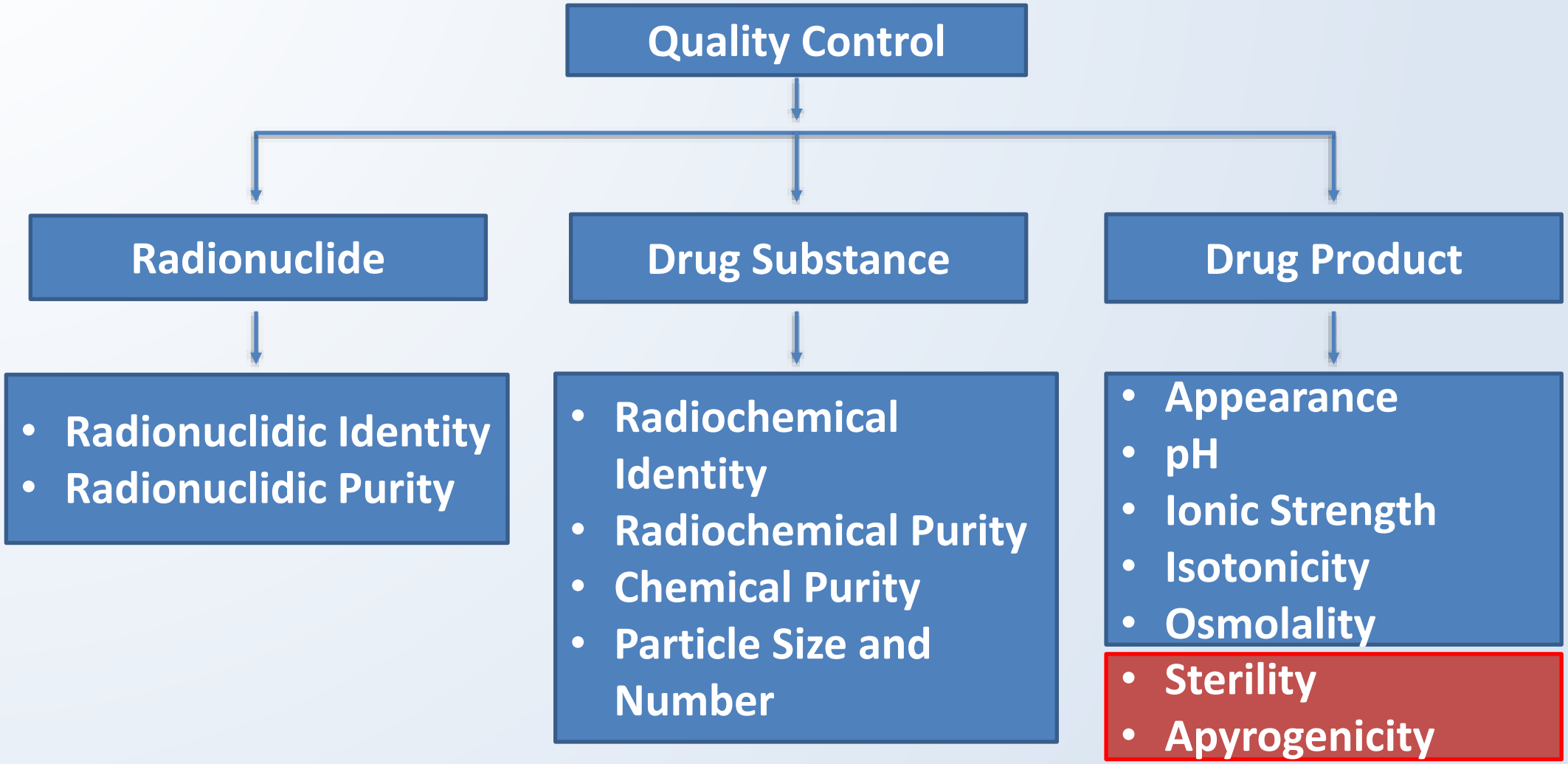
13. Transfer the collected fraction to the 2-neck formulation vial pre-filled with 1.2 mL of 23.4% saline solution

Sterilizing filter and final product vial

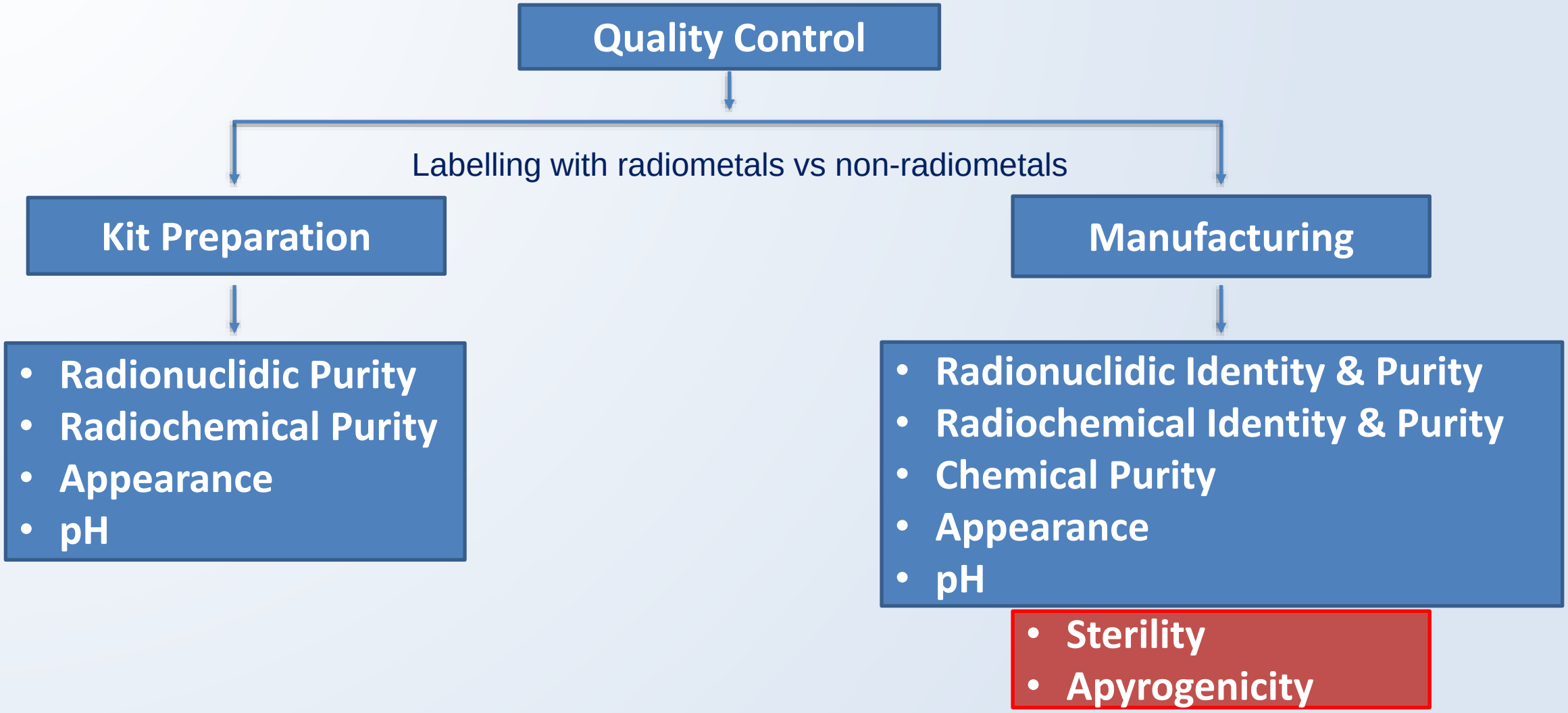
14. Filter the final drug product through a sterilizing filter into the final product vial inside the dispensing hot cell
15. Assay the vial and remove samples for QC testing

Radiopharmaceutical Quality Control

Quality Control (QC) of Radiopharmaceuticals



QC of Prepared vs Manufactured Radiopharmaceuticals



Quality Control of Radionuclide

Radionuclidic Purity

- Radionuclidic Purity (RNP) is the ratio of the stated radionuclide activity to the total radioactivity given as a percentage

$$RNP = \frac{\text{Activity of desired Radionuclide}}{\text{Total Activity}} 100\%$$

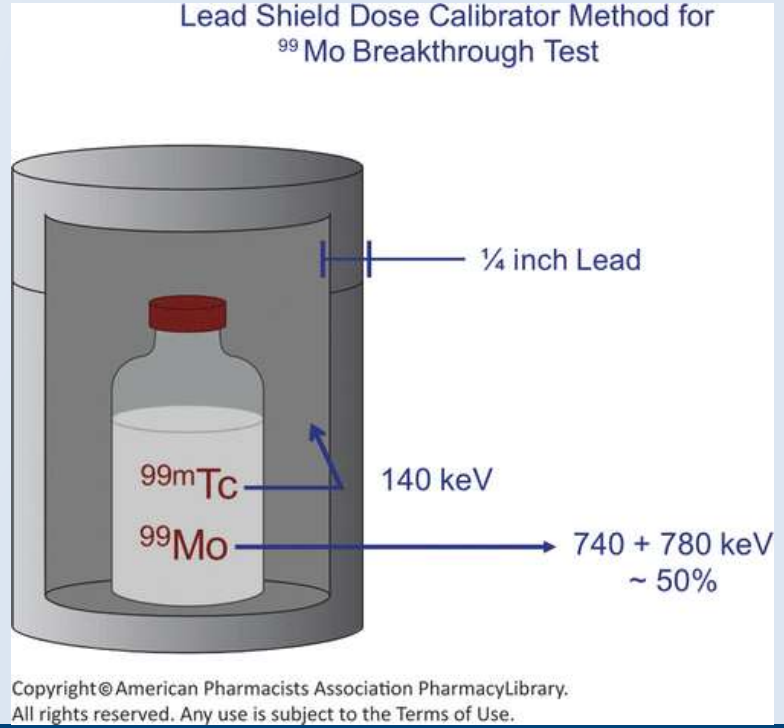
Radionuclidic impurities can originate from:

- extraneous nuclear reactions
  - isotopic impurities in target material
  - fission process occurring in a reactor
- generator breakthrough
  - $^{99}Mo \leq 0.15$ mCi per mCi of ^{99m}Tc at time of patient administration (66 h vs 6 h)
  - $^{68}Ge \leq 0.005\%$

Determination of ^{99}Mo Breakthrough

- **Molybdenum** breakthrough is measured using a dose calibrator.

The amount of ^{99}Mo breakthrough, during elution is normally determined by placing the eluate from the generator in a lead shield and measuring the penetration of any ^{99}Mo (740- and 780-keV) photons.



https://pharmacylibrary.com/doi/10.21019/9781582122830.ch4
https://www.sciencedirect.com/topics/medicine-and-dentistry/molybdenum-99#:~:text=The%20amount%20of%2099Mo%20contamination,and%20780%2DkeV)%20photons.

Determination of Radionuclidic Identity & Purity

- **Half-life** is used for radionucliding identity testing

of radiopharmaceuticals with a short half-life

Performed using a dose calibrator and linear regression analysis

$$T_{½} = (\ln 2)t \div \ln(A_0/A)$$

A_0 = initial assay, A = assay after t minutes (t ≥ 10 min), t = elapsed time between the two assays

Determination of Radionuclidic Identity & Purity

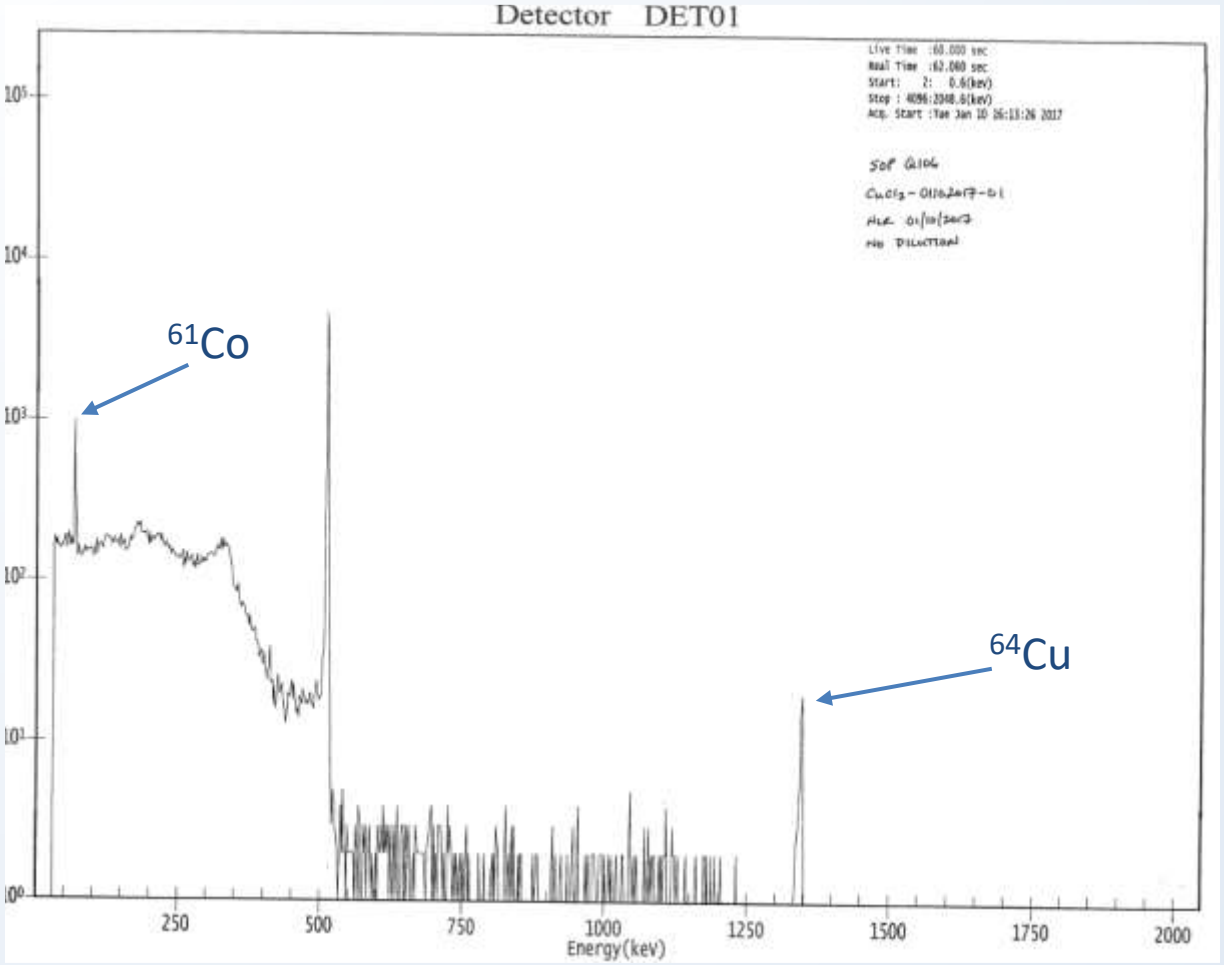
- Radiometal analysis (longer half-life) require a high-resolution multichannel analyzer (germanium detector)

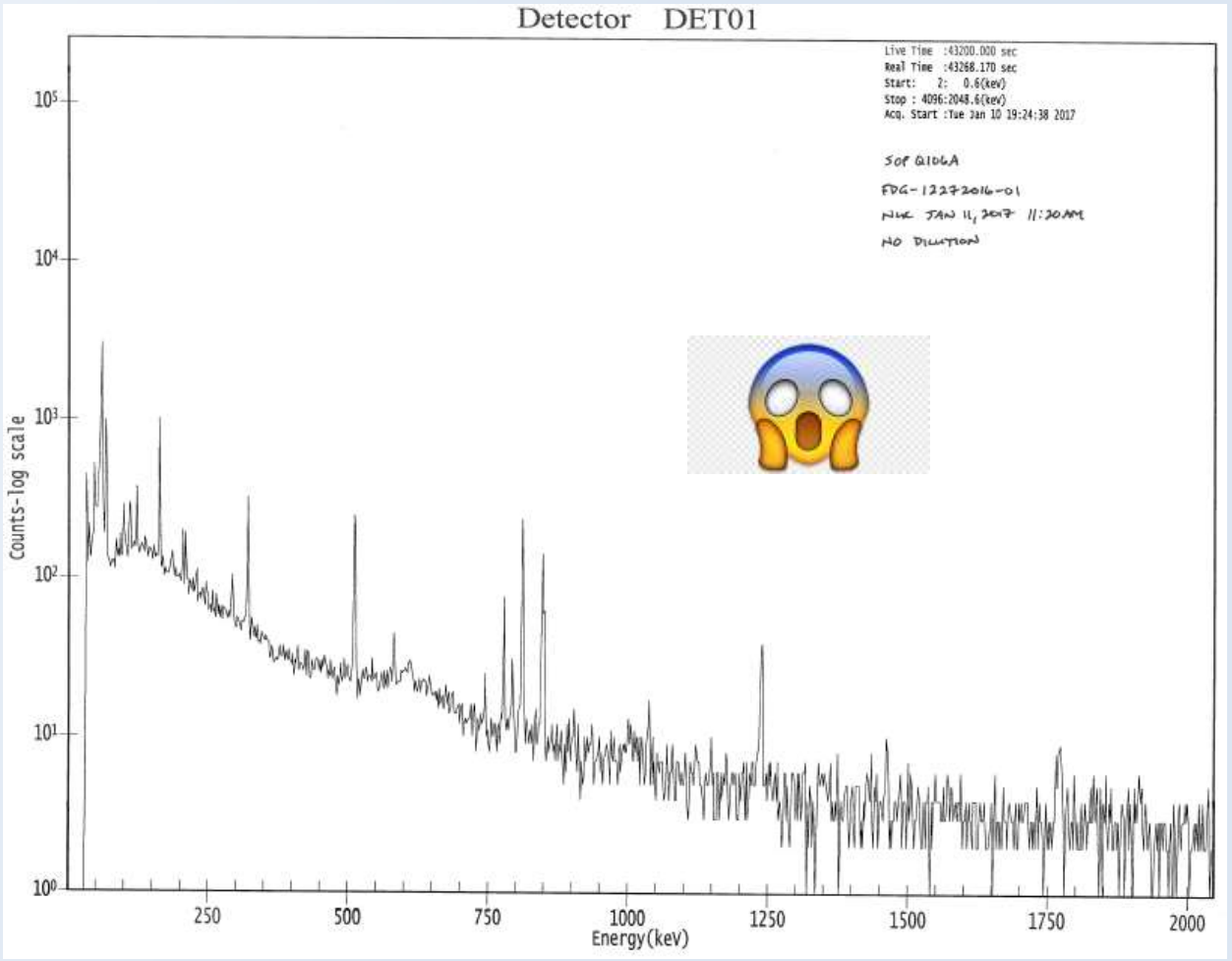
  example: $^{64}Ni(p,n)^{64}Cu$ vs $^{64}Ni(p,a)^{61}Co$

- Long-lived impurities determined periodically using a high-resolution multichannel analyzer

  example: produced during target body irradiation

Determination of Radionuclidic Identity & Purity



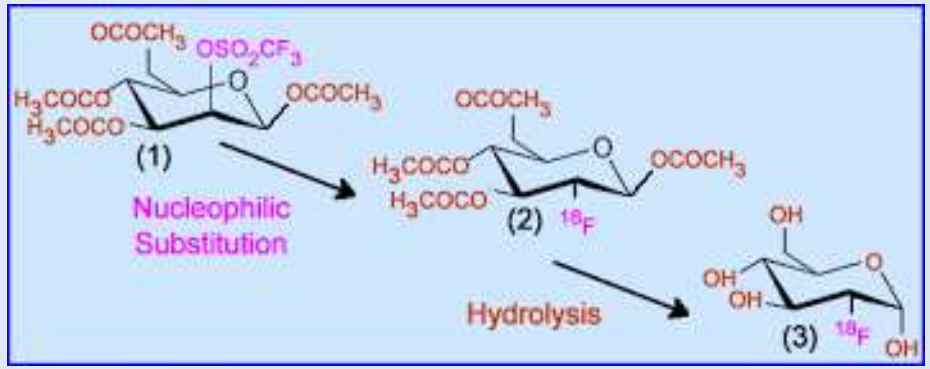


Quality Control of Drug Substance

Radiochemical Identity and Purity

- Radiochemical Purity (RCP) of a radiopharmaceutical is defined as the percent of the total radioactivity present in the desired chemical form in a radioactive pharmaceutical

$$RCP = \frac{\text{Activity in desired form}}{\text{Total Activity}} 100\%$$

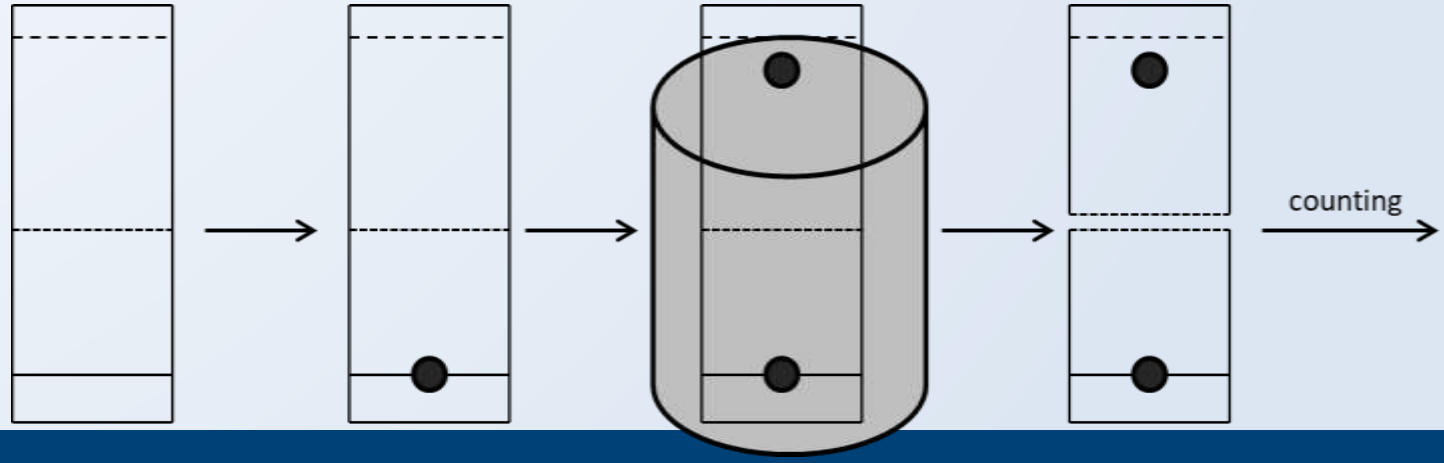


- Radiochemical Identity: the retention of the sample must agree with that of the reference standard within ±10%

Thin Layer Chromatography

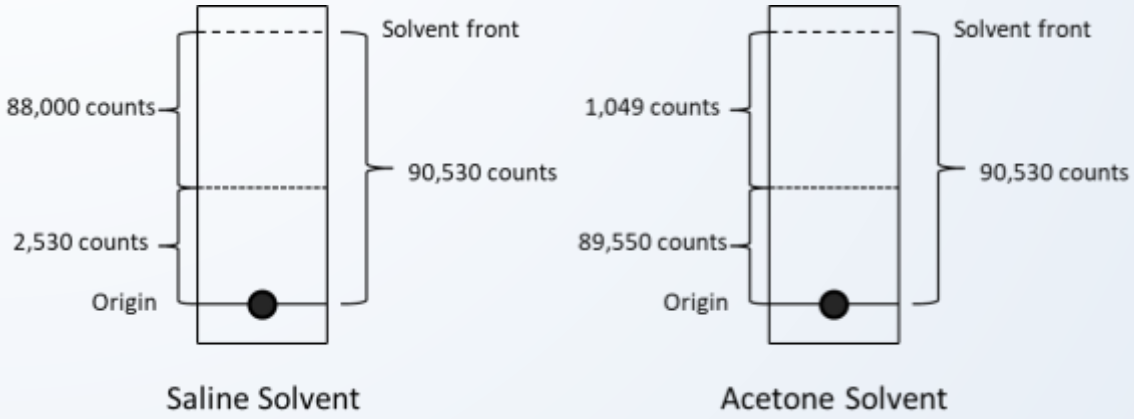
- Small size sample (1 uL) loaded on TLC strip
- TLC strip placed in a chamber with appropriate solvent
- TLC strip developed and dried
- TLC strip cut according to known R_f values
- Number of counts determined for each region

- Paper or Instant thin layer chromatography (iTLC)
- iTLC strips made of glass fiber impregnated with silica gel (SG) or polysilicic acid (SA) etc.
- iTLC-SG



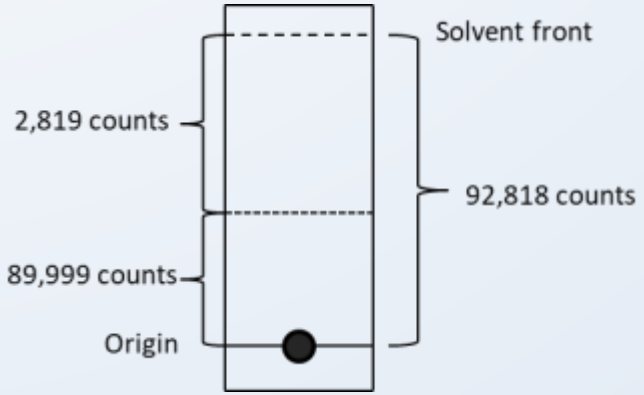
Radiochemical Purity – ^{99m}Tc radiopharmaceuticals

- Soluble ^{99m}Tc radiopharmaceuticals



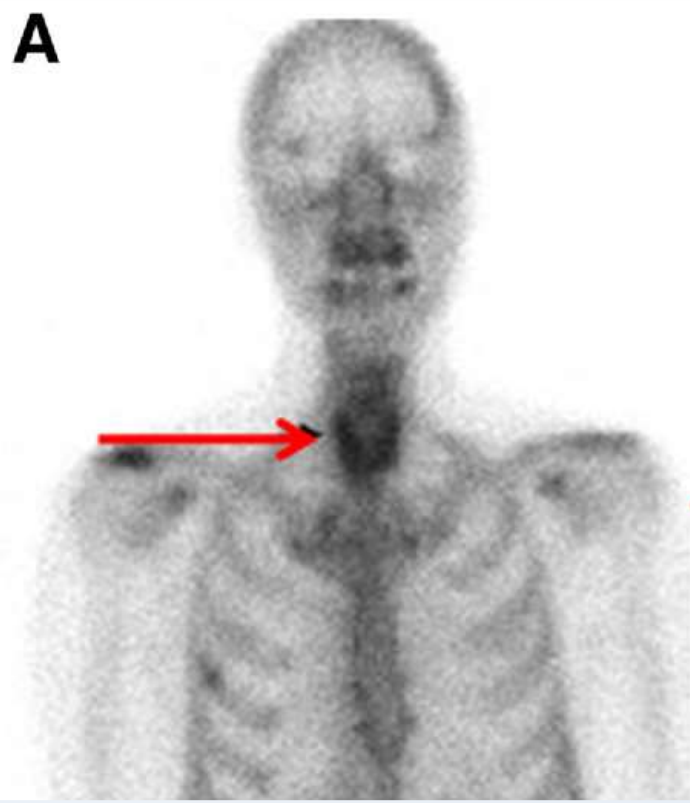
R_f values for a soluble technetium ^{99m}Tc radiopharmaceutical		
	R_f values for Saline	R_f values for Acetone
^{99m}Tc-labeled complex	1.0	0.0
Hydrolyzed ^{99m}Tc	0.0	0.0
$^{99m}TcO_4^-$	1.0	1.0

- Particulate ^{99m}Tc radiopharmaceuticals



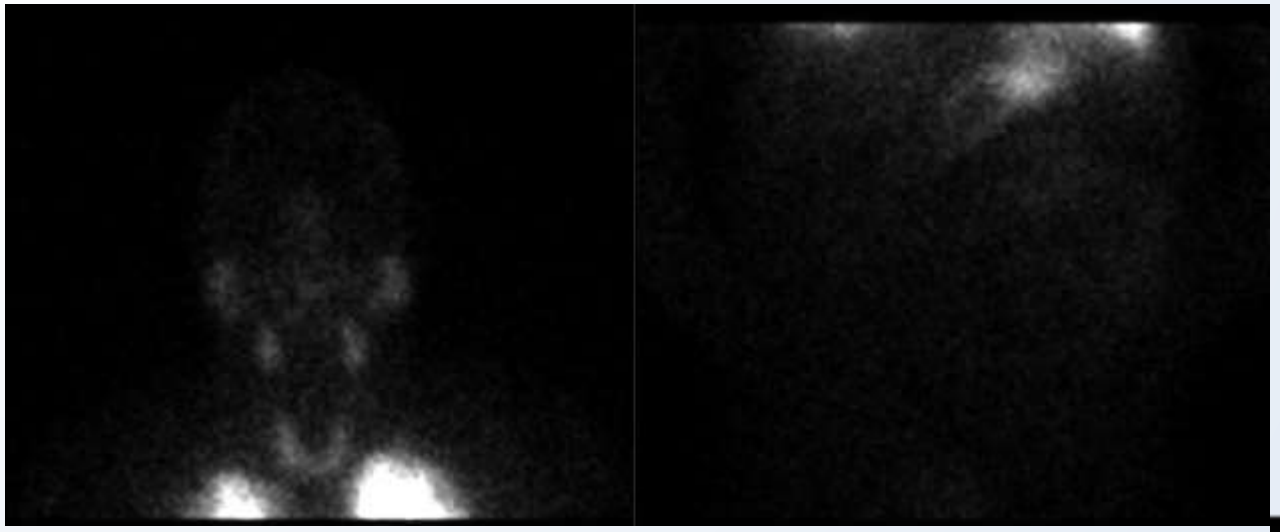
R_f values for a soluble technetium ^{99m}Tc radiopharmaceutical	
	R_f values for Saline
^{99m}Tc-labeled complex	0.0
Hydrolyzed ^{99m}Tc	0.0
$^{99m}TcO_4^-$	1.0

Biodistribution of Free ^{99m}Tc

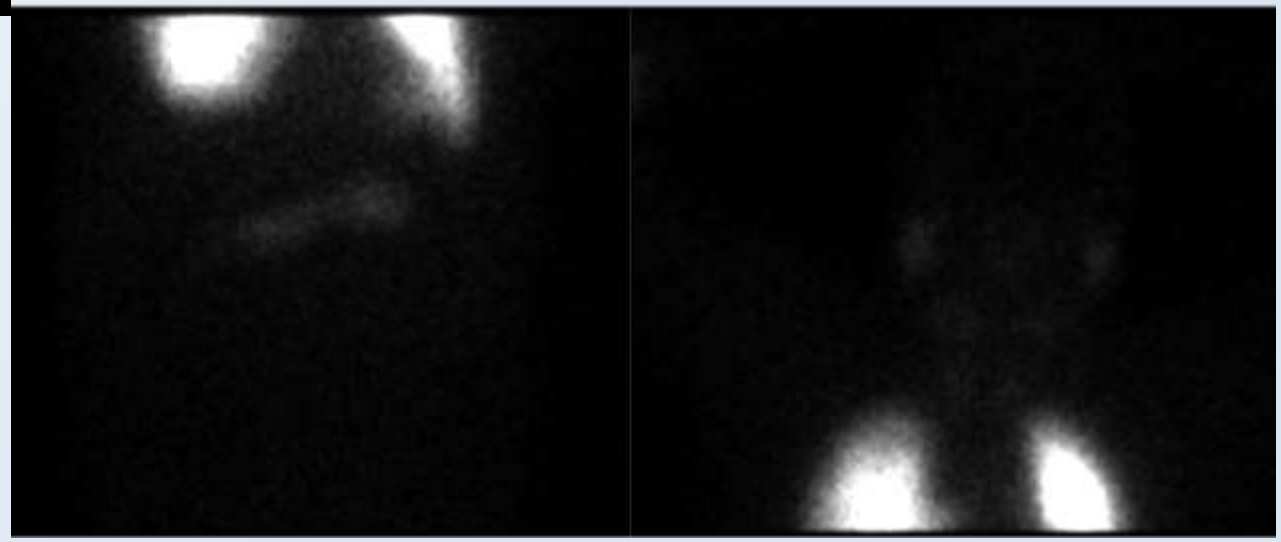


- Free ^{99m}Tc ($^{99m}TcO_4^-$ pertechnetate) localizes in thyroid and gastric mucosa

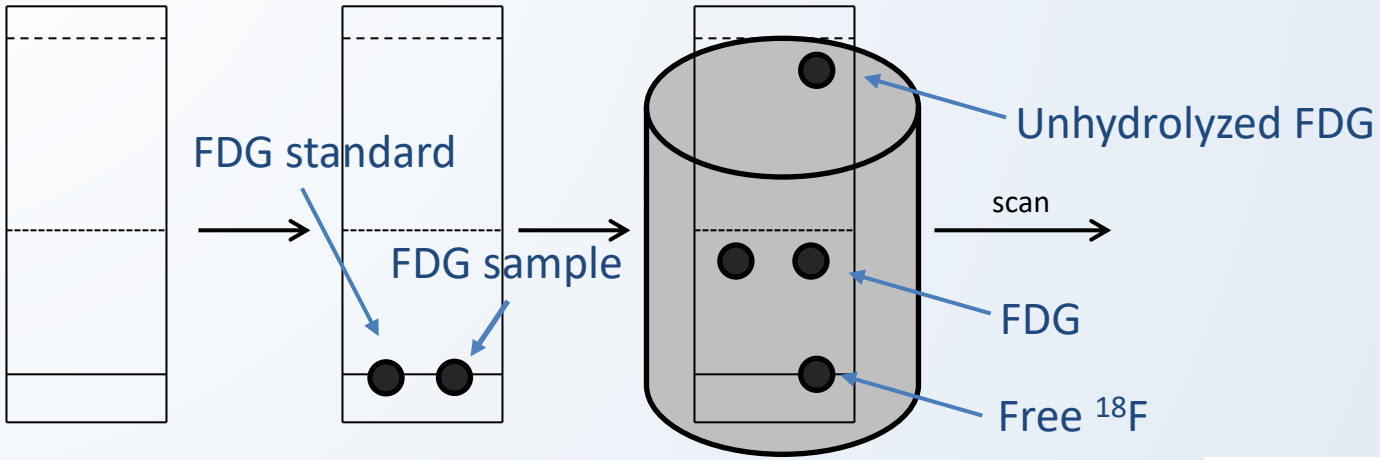
Biodistribution of Free ^{99m}Tc

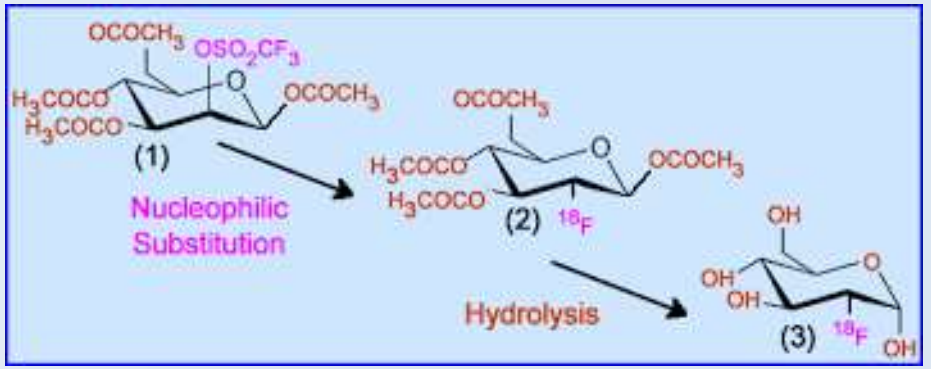


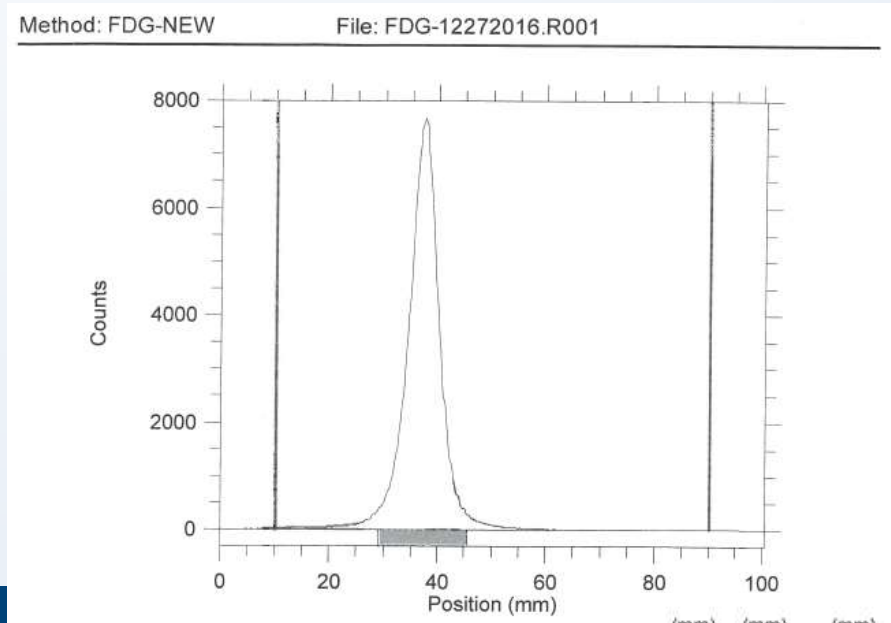
- 2 consecutive VQ scans on November 10, 2023
- Elevated uptake in thyroid and stomach



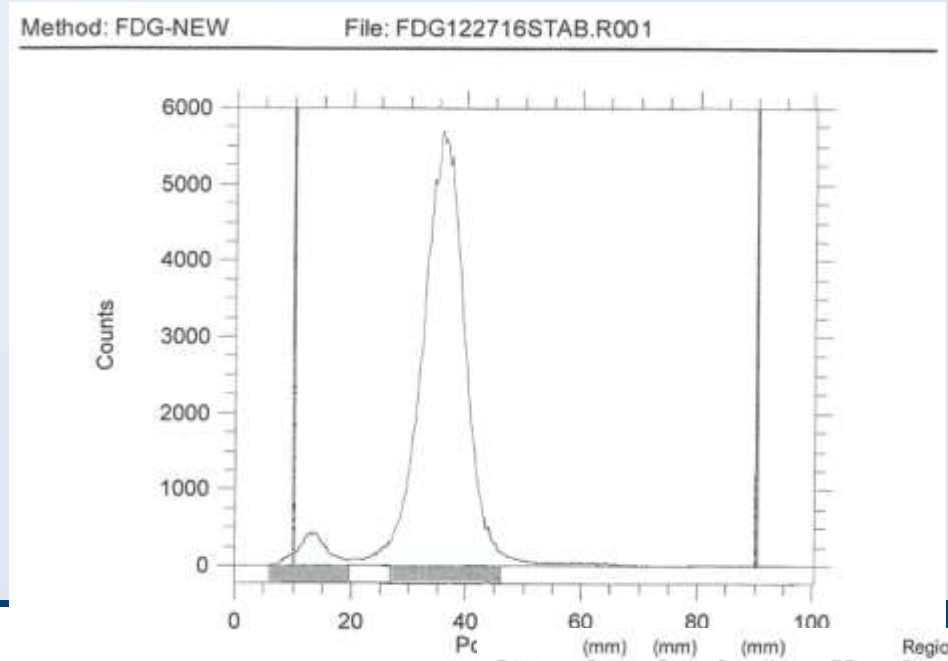
Radiochemical Identity and Purity by TLC





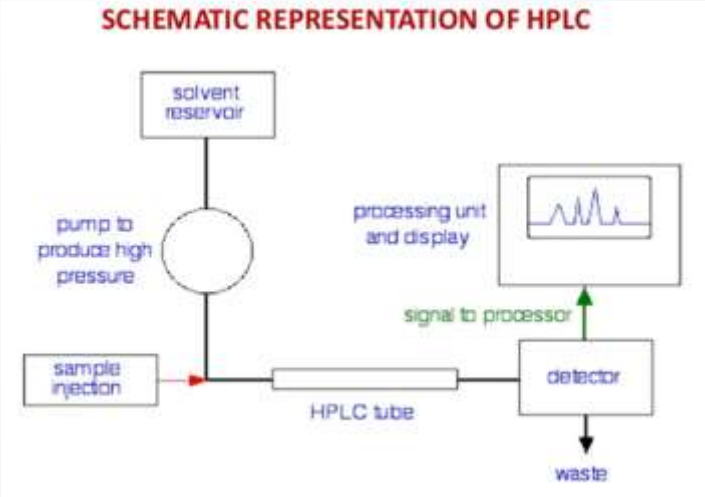


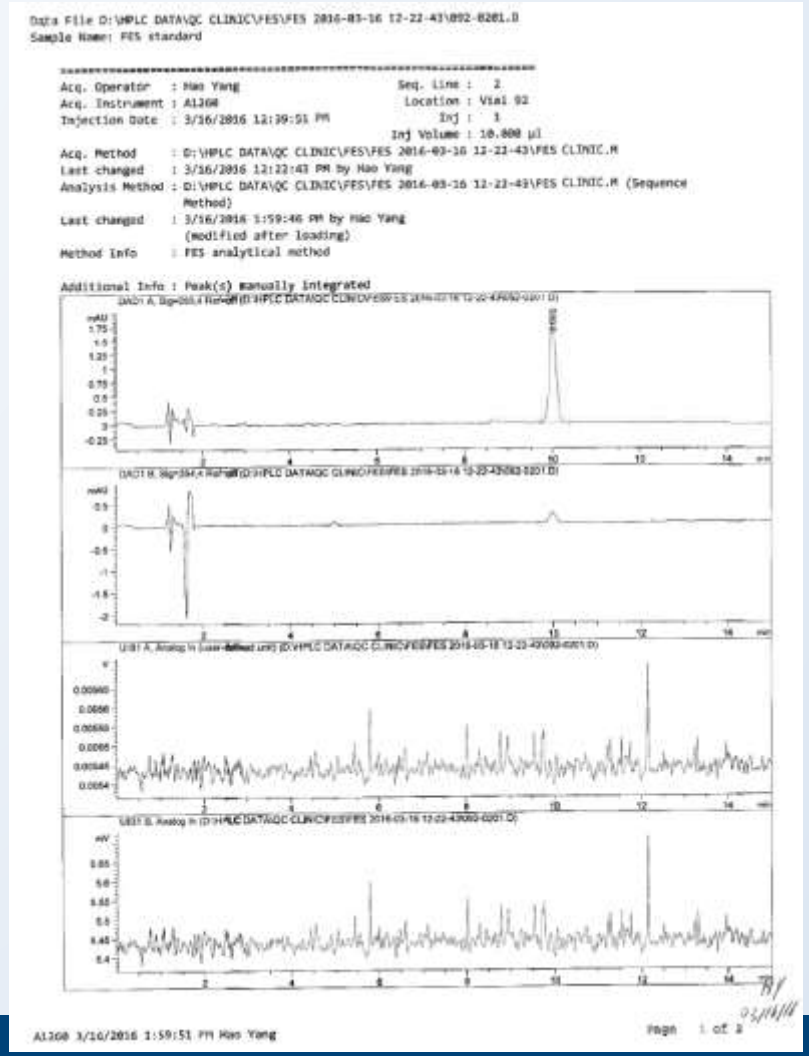
Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	29.1	45.5	37.2	0.339	194101.0	647003.3	94.54	100.00
1 Peaks					194101.0	647003.3	94.54	100.00

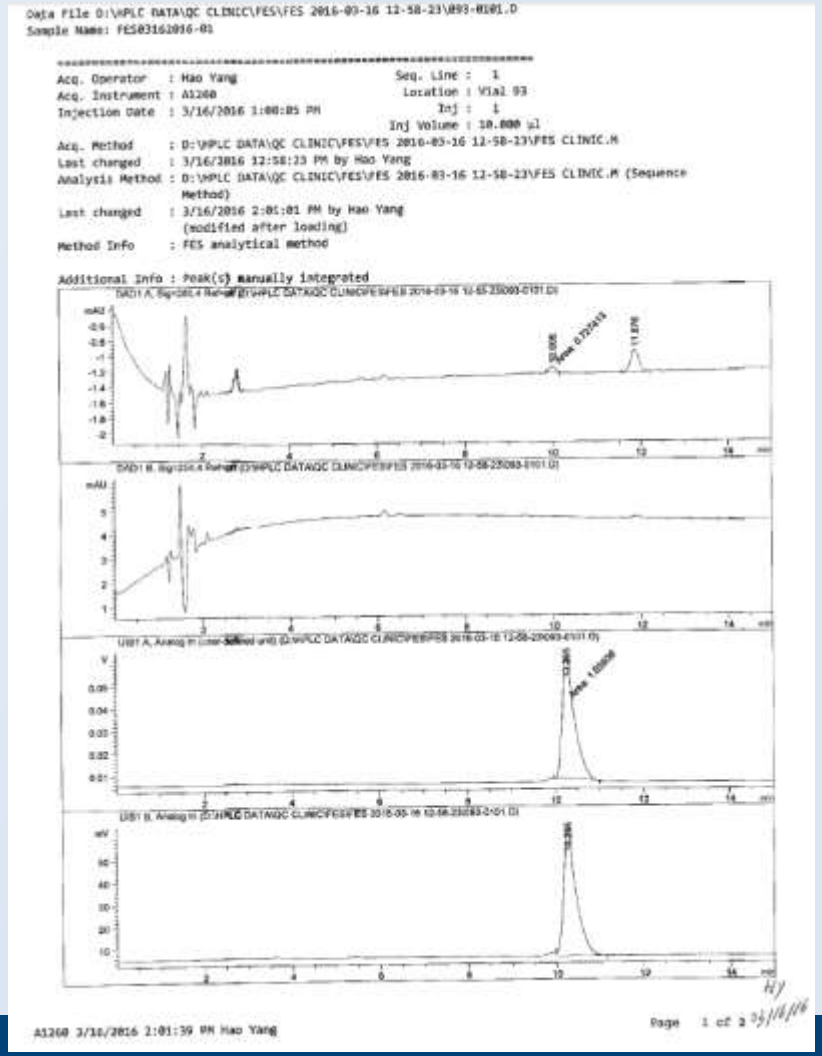


Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	5.9	19.5	13.3	0.041	9546.0	9875.2	4.68	4.89
Rgn 2	26.7	46.1	35.9	0.323	185867.0	192276.2	91.11	95.11
2 Peaks					195413.0	202151.4	95.79	100.00

Radiochemical Identity and Purity by HPLC







HPLC: High Performance Liquid Chromatography

Chemical Purity of Radiopharmaceuticals

- **Chemical Purity** is the proportion of the total mass present in the stated chemical form
- **Chemical Impurities** are all the **nonradioactive** substances that can either affect radiolabeling or directly produce adverse biological effects
- Chemical impurities are analyzed using chromatography or colorimetric methods
- Chemical impurities must be identified, when possible, and their acceptable limit must be determined

Common Chemical Impurities

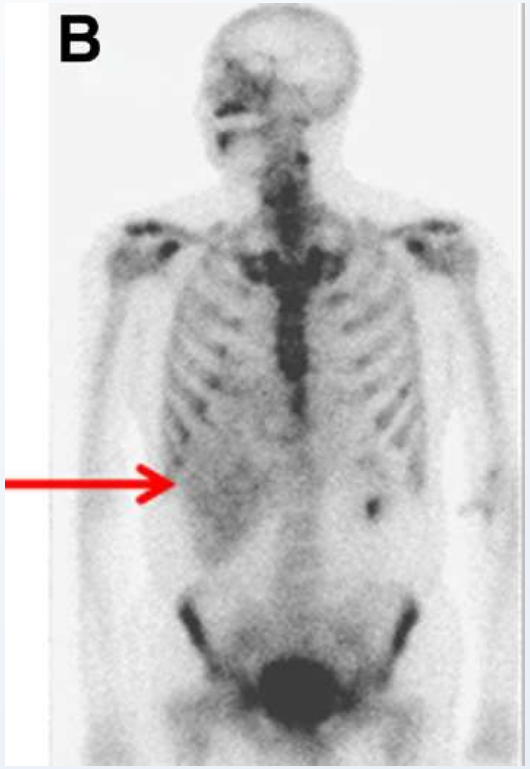
- Alumina breakthrough

  Limit 10 μg Al^{3+} per mL of ^{99m}Tc eluate

- Stannous ion present in commercial kits
- Since synthetic methods are utilized in the preparation of PET drugs analysis of chemical purity is necessary
  - Kryptofix 2.2.2 (for 18F labeled drugs)
  - By-products
  - Residual solvents
- "Cold" Compound

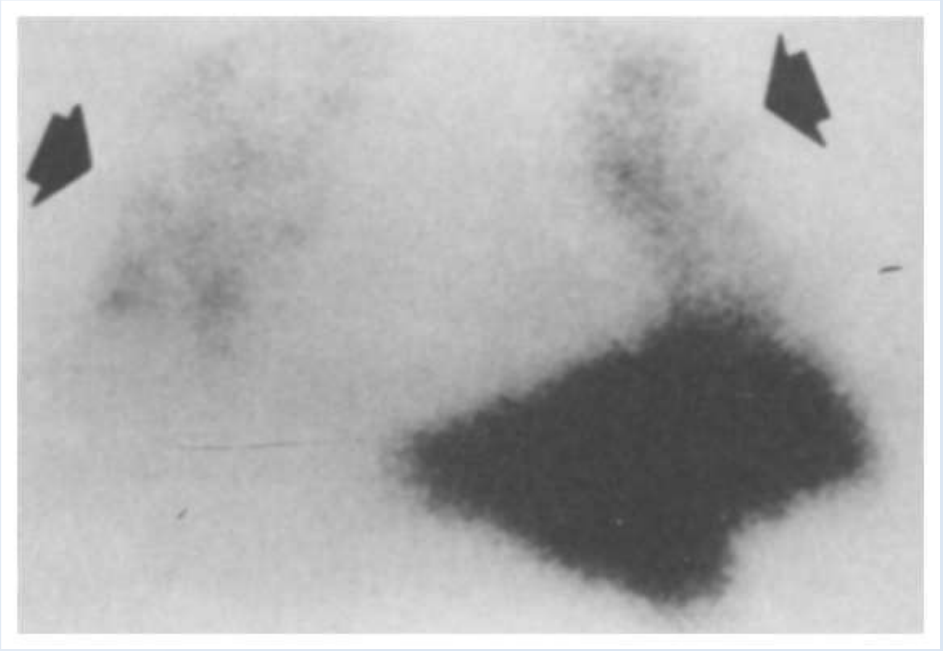
Effect of Chemical Impurities in Biodistribution of ^{99m}Tc Radiopharmaceuticals

^{99m}Tc methylene diphosphonate (MDP) bone scan



- Excess of stannous ions leads to the formation of insoluble particles that localize in the liver

^{99m}Tc sulfur colloid liver scan



- Presence of alumina leads to the increased size of particles that are trapped in the lungs

Vallabhajosula S. et al. Semin Nucl Med 2010 Jul; 40:220-241

Hung, J. C. et al. Semin Nucl Med 1996 Oct; 26: 208-255

Second Attendance Verification Code:

1668

Quality Control of Drug Product

pH – Colorimetric Methods

pH is a measure of the hydrogen ion concentration of a solution:

$$pH = -\log[H^+] \quad (0 < pH < 14)$$

- Appropriate pH to maintain stability and integrity
- Ideally the pH of the radiopharmaceutical should be that of blood (pH = 7.4)
- Due to blood's high buffer capacity 2 < pH < 9

Method: Colorimetric, Narrow-band pH paper validated against standard buffer

- **Colorimetric Method**

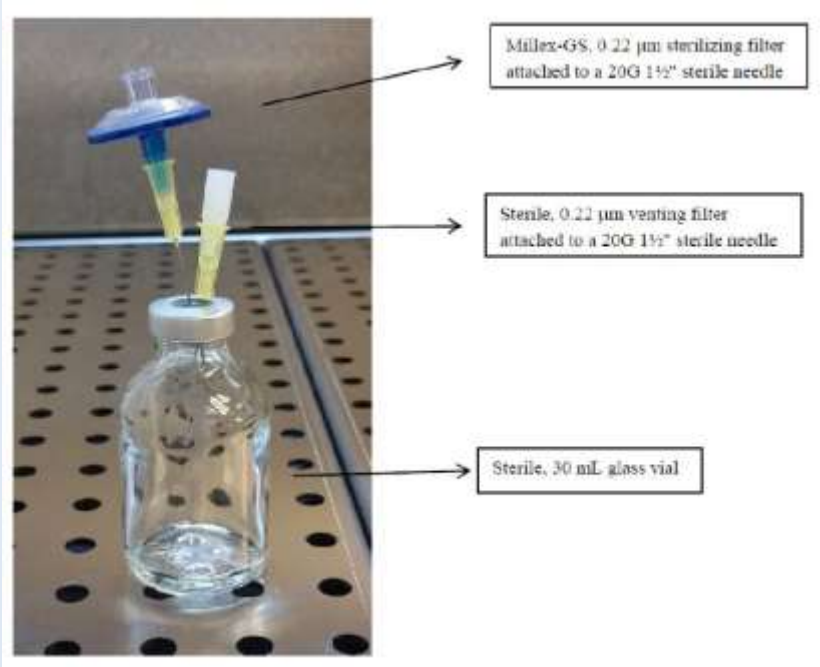
A method of determining the concentration of a chemical element or chemical compound in a solution with the aid of a color reagent

Microbiology Considerations - Sterility

Sterility

- Due to the short half-life of PET drugs sterility testing is performed **post-release**
- Sterility testing must be initiated within 30 hours post End Of Synthesis
- Testing in two different media
- No growth must occur in either media

PET radiopharmaceuticals are sterilized through a 0.22 µm membrane filter and the integrity of the filter is performed using the bubble point measurement immediately after production



Microbiology Considerations – Apyrogenicity

- Bacterial Endotoxin Test is performed **before** releasing the PET drug to determine the presence of bacterial endotoxins in the drug solution
- The test uses limulus amebocyte lysate (LAL) which reacts with bacterial endotoxins

Atlantic horseshoe crab (Limulus polyphemus)

?h?X?
PTS V712F 3/26/2015 01/12/17 11:32 S/N:8
668 AS/N:Endosafe

******** ENDOSAFE Test Record ********
V712F 3/26/2015

DateTime: ..... 01/12/17 @ 01:37:59PM
Device: 8668
OperatorID: ..... NLR
Cartridge: ..... Endotoxin
Temperature: .. Start: 37.0C End: 37.0C
Method: KX-122
Cartridge Lot#: 5238145
Cartridge Cal Code: 512141566750
Range: 5-0.05
Range Time: ..... Sec: 121-815
Onset Times: ..... >815 290 >815 210
Slope: -0.414 ..... Intercept: +2.372
Dilution: 50
Sample Lot: FDG-01122017-01
Sample ID: FDG-01122017-01
Sample Rxn Time CV: ..... 0.0% Pass
Spike Value: ..... 0.817 EU/mL
Spike Rxn Time CV: ..... 22.6% Pass
Spike Recovery: ..... 94% Pass
Test Suitability: ..... Pass
Sample Value: ..... <2.50 EU/mL
:

NLR
JAN 13, 2017

Thank you