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Theranostic Radiopharmaceutical Development and Manufacturing

From early radiochemistry and drug development to cGMP manufacturing and clinical supply.

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Theranostic Radiopharmaceutical Development and Manufacturing

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Radiopharmaceuticals have brought a new perspective to cancer care. Covering diagnostics and therapy, this theranostic portfolio enables multi-level care that a single drug would not otherwise provide. The diagnostic radiopharmaceutical is often called a radiotracer, a gamma-emitting radiolabeled precursor specifically accumulated in tumor tissues, leading to an immediate cancer assessment prior to treatment. The same chemical precursor radiolabeled with an alpha- or beta-emitting isotopes allows for a cellular-specific delivery of lethal radiation directly at the tumor site. This therapeutic strategy is particularly attractive to limit healthy tissue irradiation, often occurring with radiation therapy.

Radiopharmaceuticals may be applicable to several cancers depending on the nature of the targeting precursor. Ideally, a radiopharmaceutical consists of a high-affinity precursor specifically binding to receptors over-expressed on the surface of the tumor or metastatic sites. While a preference is given to internalizing agent for therapeutic pairs, cell

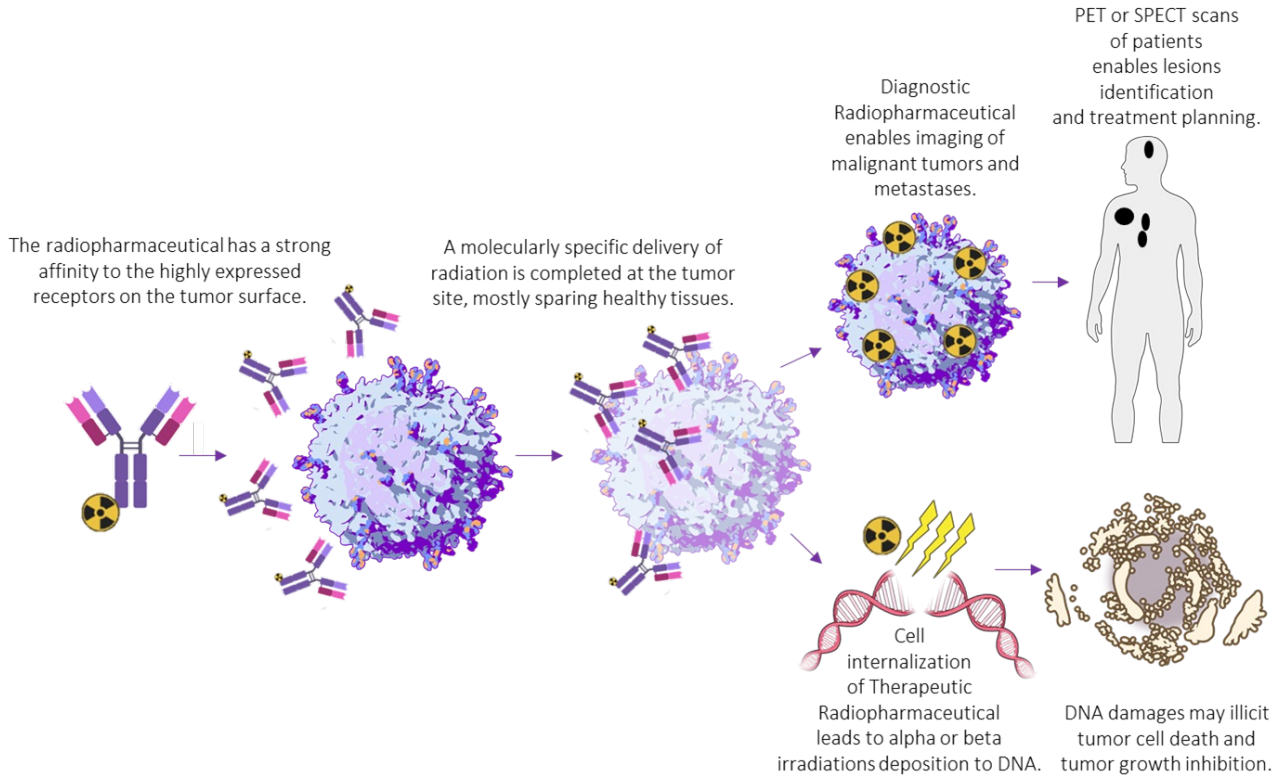

Figure 1: Tumor-specific recognition of diagnostic or therapeutic radiopharmaceuticals

internalization is not always necessary for the diagnostic purpose, (3, 4).

Radiopharmaceuticals come in all sizes and shapes, spanning from particles such as 90Y-microspheres, monoclonal antibody or protein fragments, peptides, or as small as the atom itself such as 223RaCl2 or 89SrCl2 (Figure 2). The launching of a radiopharmaceutical to first-in-human clinical trials is primarily pathed using diagnostic radiotracers . Gamma-emitting radiometals such as 111In (for SPECT), or 64Cu and 89Zr (for PET) and others are strongly bound by the chelator-conjugated precursor, forming the radiotracer. Conveniently, alpha- and beta-emitting isotopes such as 225Ac, 177Lu, 67Cu, and others classified as lanthanides or transition metal, can usually be complexed by the same chelator. Utilizing the same chemical precursor associated with a diagnostic or therapeutic radionuclide leads to a theranostic platform. Radiotracer and therapeutic analogs are more likely to result in similar organ distribution in a patient, supporting easier treatment cohort selection and dosimetric planning for clinical trials.

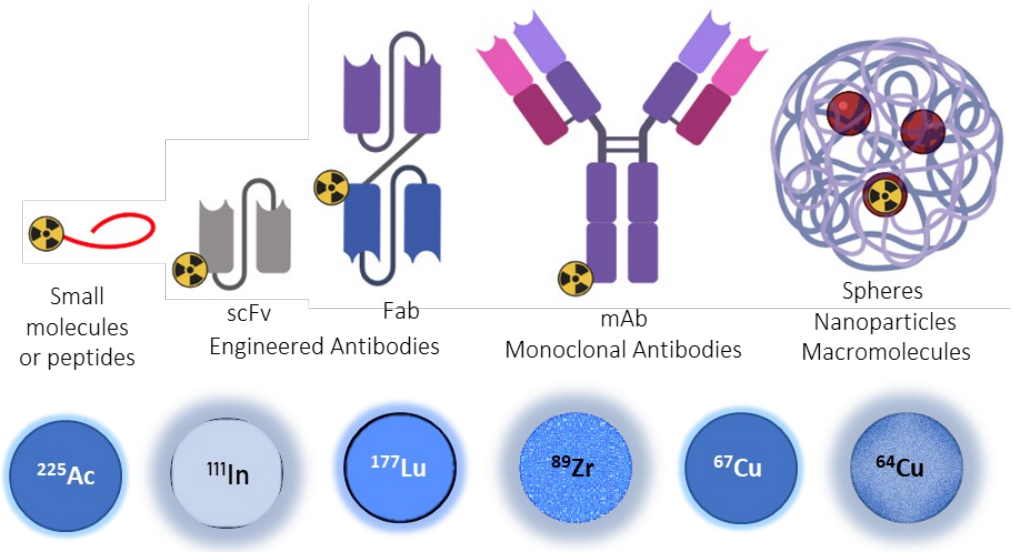

Figure 2: Examples of diagnostic and therapeutic radiopharmaceuticals

[1] Scheinberg, D. A., Lovett, D., Divgi, C. R., Graham, M. C., Berman, E., Pentlow, K., ... & Gee, T. S.. A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: specific bone marrow targeting and internalization of radionuclide. Journal of Clinical Oncology, 9(3), 478-490 (1991).

[2] Sgouros, G., Bodei, L., McDevitt, M.R. et al. Radiopharmaceutical therapy in cancer: clinical advances and challenges. Nat Rev Drug Discov 19, 589–608 (2020).

[3] Carter LM, Poty S, Sharma SK, Lewis JS. Preclinical optimization of antibody-based radiopharmaceuticals for cancer imaging and radionuclide therapy-Model, vector, and radionuclide selection. J Labelled Comp Radiopharm.;61(9):611-635 (2018).

[4] Kleynhans J, Ebenhan T, Cleeren F, Sathekge MM. Can current preclinical strategies for radiopharmaceutical development meet the needs of targeted alpha therapy? Eur J Nucl Med Mol Imaging.51(7):1965-1980 (2024).

[5] Oncology Therapeutic Radiopharmaceuticals: Dosage Optimization During Clinical Development, Draft Guidance for Industry, August 2025, FDA-2025-D-1757

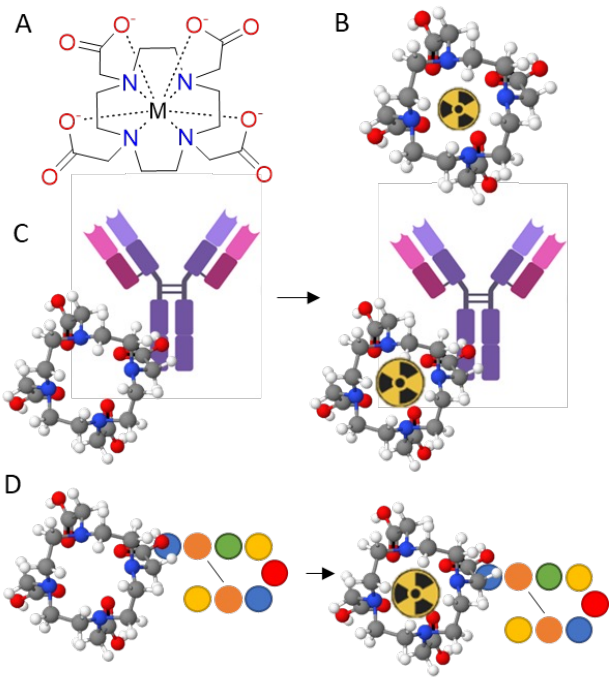

Figure 3: (A-B) Chelator-radiometal complex and 3D modeling of DOTA; (C) radiolabeling of a monoclonal antibody; (D) radiolabeling of a peptide.

Successful radiopharmaceuticals are governed by critical chemical and radiochemical criteria which must be met throughout the drug development. Radiolabeling, formulation and stability are evaluated via radiochemical and chemical purity and identity of the radiotracer or drug product, along with consistent controlled pH, appearance, and excipient contents throughout the shelf life. Similarly, at scale-up and manufacturing, those defined attributes are determined as specifications, controlled under Current Good Manufacturing Practice (cGMP) procedures. In addition, endotoxin controls and aseptic process are required for patient dose manufacturing. Instrument qualifications and method validations are performed in accordance to regulatory guidance in order to establish high quality and reliable batch release data.

Process development for both diagnostic and therapeutic radiopharmaceuticals generally follows the same steps, including three stages: early phase, development and optimization, and, finally, manufacturing. The following discussion outlines each stage from the benchtop to manufacturing, leading to the finalization of a patient dose deliverable to clinics.

Early Phase	Drug Development	Manufacturing
Chelator bioconjugation	Radiolabeling & purification optimization	Scale-up & automation
Diagnostic and/or therapy radiolabeling	Formulation stability and expiry	cGMP validations and aseptic process
Chemical / Radiochemical QC method development	Immunoreactivity & biological testings	Support for regulatory submission

Figure 4: Radiopharmaceutical development from early stage to manufacturing

I- Early Phase

Choice of radiometals and chelator

The choice of the isotope or radiometal is governed by the biological application of the radiopharmaceutical. Diagnostic isotopes may be chosen among the medically utilized gamma-emitting radionuclides. For instance, ^{111}In is a SPECT isotope, with a half-life of 2.8 days. ^{111}In is well-suited for mAb radiolabeling; however, ^{111}In often results in challenging, low-resolution patient scans due to the physical nature of the isotope. ^{64}Cu is a PET isotope with a short half-life of 12.7 hours and, consequently, a short expiry. This isotope is well-established in clinical PET diagnostic. ^{89}Zr is also a PET isotope and defined with a longer half-life of 78.4 hours, which is better suited for long-circulating radiotracer.

Similarly, alpha- and beta-emitting isotopes may be chosen in function of their half-lives and the irradiation type for treating the intended cancer. Alpha-emitting isotopes, such as ^{225}Ac, ^{212}Pb, and ^{211}At are characterized by high linear energy transfer (80-100 keV/µm) and very short emission pathlengths equivalent to a cell diameter. When specifically targeted to tumors, the alpha particle deposits highly potent radiation, often resulting in potentially lethal outcomes for tumor cells, while largely sparing neighboring healthy tissue. In contrast, beta-emitting isotopes such as ^{177}Lu, ^{67}Cu, or ^{90}Y emit lower linear energy transfer (0.1-1 keV/µm) with longer pathlengths (up to cm) than alpha-emitters. These isotopes are generally utilized to target aggressive primary solid tumors.

Conversion of a bioactive ligand into a radiopharmaceutical most often begins with the chemical attachment of a chelator to the ligand. DOTA or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid is the most versatile chelator [6]. While DOTA adequately complexes many radiometals, other chelators may bind specific radionuclides longer. The radiometal to be chelated needs to be chosen according to diagnostic or therapeutic intent, in alinement with the ligand body residency time and the treated cancer.

Bioconjugation of the chelator with the original ligand

The chelator conjugation to the ligand may be achieved via amino-, thiol- or a click-chemistry approach. The amino-bioconjugation is completed by nucleophilic substitution of a deprotonated amine held by the ligand to a highly reactive isothiocyanate or succinimide bi-functionalized chelator, forming a stable amide functional group.

The thiol-bioconjugation is proceeded by connecting the chelator to the ligand protein via a reactive maleimide prosthetic group with a reduced cysteine carried by the original ligand. This Michael addition has been reported less stable in *vivo* than the amino-conjugation [7].

Finally, the click-chemistry approach requires prior chemical modifications of both the ligand and the chelator bearing orthogonally reactive prosthetic groups. This reaction is based on a

[6] Dai L, Jones CM, Chan WTK, Pham TA, Ling X, Gale EM, Rotile NJ, Tai WC, Anderson CJ, Caravan P, Law GL. Chiral DOTA chelators as an improved platform for biomedical imaging and therapy applications. Nat Commun. 2018 Feb 27;9(1):857. doi: 10.1038/s41467-018-03315-8. PMID: 29487362; PMCID: PMC5829242.

Diels-Alders reaction. This conjugation strategy has demonstrated to be highly selective, stable and fast [8].

Chelator conjugation and purification

Once the chelator is covalently bound to the ligand, the precursor is ready for radiolabeling. Purifying excess chelator after conjugation ensures stable radiolabeling, reduces off-target radiation, and ensures regulatory compliance — all critical for safety, efficacy, and consistency of the radiopharmaceutical preparation.

Pilot radiolabeling and characterization

The newly synthesized precursor is then chemically identified and radiolabeled to confirm efficiency and radiochemical purity. High-Performance Liquid Chromatography (HPLC) and mass-spectrometry may be undertaken to evaluate the number of chelators per precursor.

Chemical and radiochemical initial QC methods

Pilot radiolabeling assays may be completed using the desired radionuclide, providing a first assessment of successful chelator conjugation. Upon completion, chemical and radiochemical characterization are initiated, evaluating methods using thin layer chromatography (TLC), HPLC equipped with UV and radiometric detection.

II- Drug Development

The next phase, defined as drug development, is initiated at the completion of pilot radiolabeling (RCP% ≥ 95%) supported by successful TLC and HPLC initial methods. In this phase, the radiolabeled drug substance is converted into a drug product (Figure 5).

Radiopharmaceutical development includes repeatability (at least n=3), yield improvements, and accuracy and specificity verifications of Quality Control (QC) methods. Once the obtained drug substance is formulated into a drug product, shelf life and expiry are evaluated.

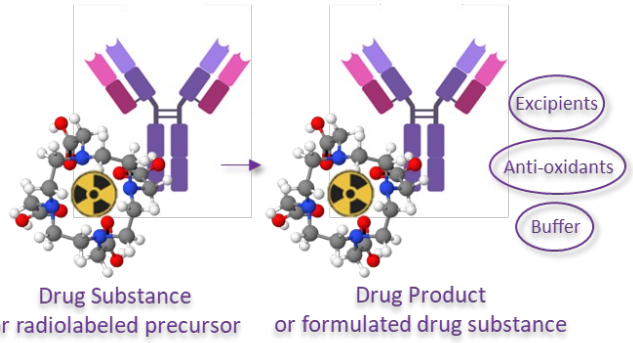

Figure 5: Conversion of a drug substance into a drug product radiopharmaceutical

Radiolabeling optimization: repeatability, yields and early stability

Reaction parameters are evaluated, including: temperature, buffer, pH, volume, activity amounts, and specific activity aiming for highest yields and reaction efficacy. Specific activity

[7] Fontaine SD, Reid R, Robinson L, Ashley GW, Santi DV. Long-term stabilization of maleimide-thiol conjugates. Bioconjug Chem. 2015 Jan 21;26(1):145-52. doi: 10.1021/bc5005262. Epub 2014 Dec 26. PMID: 25494821.

[8] Brian M. Zeglis, Kuntal K. Sevak, Thomas Reiner, Priya Mohindra, Sean D. Carlin, Pat Zanzonico, Ralph Weissleder, Jason S. Lewis; A Pretargeted PET Imaging Strategy Based on Bioorthogonal Diels–Alder Click Chemistry; Journal of Nuclear Medicine Aug 2013, 54 (8) 1389-1396; DOI: 10.2967/jnumed.112.115840

is defined as the activity amount per mass of precursor. Depending on the radiolabeling strategy, diagnostic radioimmune-agents may be prepared with a 100 to 1000-fold higher specific activity to ^{225}Ac- analogs[9,10], (7, 8). Drug substance early stability is evaluated over 48 hours following the end of synthesis and beyond when possible.

Drug product formulation, expiry and analytical methods

The conversion of the drug substance into a drug product is achieved in the formulation step. Stabilizing buffers, excipients, and antioxidants are added to the drug substance, enabling shelf life extension. Both diagnostic and therapeutic radiopharmaceuticals are subject to radiolysis and degradation over time. Drug product degradation is mitigated with the addition of appropriate excipients and radiolytic stabilizers in order to achieve the desired shelf life. Stability is evaluated using the pre-defined QC methods, examining each attribute of the drug from end of synthesis to expiry date under different storage conditions. Radiochemical identity and purity (>95%), chemical identity and purity, appearance, pH, radioactive concentration, specific activity, excipients content, and residual solvents are listed in the form of a specification sheet (Figure 6).

REQUIRED TESTS FOR ROUTINELY PRODUCED [^{11}C] MET BATCH

TESTS	METHODS	SPECIFICATIONS
Appearance of the solution	Visual examination	Clear, colourless solution
pH	pH paper or potentiometry	4.5 to 8.5
Radiochemical identification	Cation exchange HPLC with UV/radioactivity detectors[a]	Rt ± 10% (comparison with standard)
Radiochemical purity	Cation exchange HPLC with UV/radioactivity detectors [a]	Not less than 95.0%
Enantiomeric purity	Chiral HPLC with UV/radioactivity detectors [b]	Not less than 90.0%
Activity per volume determination	Radiometry–isotopic calibrator	185 to 740 MBq/mL
Chemical impurity: L-homocysteine thiolactone hydrochloride	Cation exchange HPLC with UV detector	Not more than 0.06 mg/mL
Chemical impurity: L-homocysteine	Cation exchange HPLC with UV detector	Not more than 0.2 mg/mL
Chemical impurity: L-methionine	Cation exchange HPLC with UV detector	Not more than 0.2 mg/mL
Residual solvents: ethanol	Gas Chromatography (Varian 3400)	Not more than 8 mg/mL
Osmolality	Osmometry	250-300 mOsmol/kg
Bacterial endotoxin content	LAL test	Less than 20 IU/mL
Sterility	Direct inoculation	Sterile
Filter integrity test	Bubble point	> 50 psi

Figure 6: Example of specification sheet for a ^{11}C-radiotracer reported by the IAEA TECDOC-1856 report: Quality Control in the Production of Radiopharmaceuticals[11].

[9] Luurtsema G, Pichler V, Bongarzone S, Seimbille Y, Elsinga P, Gee A, Vercouillie J. EANM guideline for harmonisation on molar activity or specific activity of radiopharmaceuticals: impact on safety and imaging quality. EJNMMI Radiopharm Chem. 2021 Oct 9;6(1):34

[10] Maguire WF, McDevitt MR, Smith-Jones PM, Scheinberg DA. Efficient 1-step radiolabeling of monoclonal antibodies to high specific activity with 225Ac for α-particle radioimmunotherapy of cancer. J Nucl Med. 2014 Sep;55(9):1492-8.

[11] Quality Control in the Production of Radiopharmaceuticals, IAEA-TECDOC-1856, ISBN 978-92-0-107918-3

The specification sheet may provide the initial support for regulatory submission of the Investigational New Drug (IND) application.

Preliminary Biological Testing

In vivo and *in vitro* testing may be initiated at this stage or earlier to evaluate whether the biological properties of the original ligands are maintained upon chelator modification and radiolabeling. Immunoreactive assay, cell binding affinity, and animal organ distribution of the newly developed radiopharmaceutical may support regulatory submission for first-in-human trials.

III- cGMP Manufacturing

Once the formulated drug product has demonstrated stability through expiry and the analytical methods have been verified robust for the process (Figure 7), the development continues to cGMP manufacturing (Figure 8).

cGMP manufacturing is conducted in a dedicated compliant facility hosting qualified equipment, controlled verified processes, robust quality management systems, and trained personnel. The expertise spans from mastering the art of handling high levels of radiation to controlled standardized radioactive procedures in an aseptic environment. Due to the high technicality and complexity of the operation, it is not uncommon to set equipment, personnel, and power redundancies to ensure uninterrupted operations and supply chain resilience.

Technical & Manufacturing Capabilities

At this stage, projects may be incoming through tech transfer. Smooth transfer from sponsor laboratories to cGMP suites is essential to enable fast and efficient project initiations. Often, tech transfer is supported by dedicated teams verifying documentation alignment, process adaptation, and validation support.

cGMP project initialization usually undergoes radiolabeling scale-up matching the clinical phase needs. Per 21 CFR Part 210 and 211, sterility

Analytical Procedure	Radioactivity assay	Radionuclidic Identity (1/2 life)	Radionuclidic Identity	Radiodionuclidic purity (Limit Test)	Radiodionuclidic purity (after decay)	Radiochemical Identity (HPLC/TLC)	Radiochemical Purity* (HPLC/TLC)
Characteristics							
Accuracy	+	-	+	+	+	-	+
Precision (Repeatability)	+	+	-	-	(+)	-	(+)
Intermediate Precision	-	-	-	-	(+)	-	(+)
Specificity	+	+	+	+	+	+	+
Detection Limit	-	-	-	+	-	-	-
Quantification Limit	-	-	-	-	+	-	+
Linearity	+	+	-	-	+	-	+
Range	+	+	-	-	+	-	+
*RadioEnatiomeric purity shoud be validated analougously (+) may not be possible with short-lived isotopes							

Figure 7: Criteria for robustness of radiopharmaceutical analytical testing (REF: Gillings N, Todde S, Behe M, Decristoforo C, Elsinga P, Ferrari V, Hjelstuen O, Peitl PK, Koziorowski J, Laverman P, Mindt TL, Ocak M, Patt M. EANM guideline on the validation of analytical methods for radiopharmaceuticals. EJNMMI Radiopharm Chem. 2020 Feb 12;5(1):7. doi: 10.1186/s41181-019-0086-z. PMID: 32052212; PMCID: PMC7016057).

and aseptic process are mandated in order to achieve cGMP compliance. Aseptic cGMP may include preparations in classified cleanrooms and ISO 5 aseptic suites, maintaining controlled particle and microbiological levels to complete sterile drug product vial fill, ready for human administration.

Once the vial is ready for distribution, logistics for proper packaging, labeling, shipping, and distribution to the clinical site are then engaged. Container closure integrity test, excursion studies, and other logistical steps are verified

Documentation traceability, written procedures, and robust quality management system (QMS) are supported by standard operating procedures, document control, batch records, defined quality control release specifications, change management, and deviation/CAPA programs aligned with International Council for Harmonization (ICH) Q10 principles.

Appropriate facilities and equipment for radiochemistry, microbiology, and scalable manufacturing process are outfitted with lead-shielded hot cells equipped with manipulators,

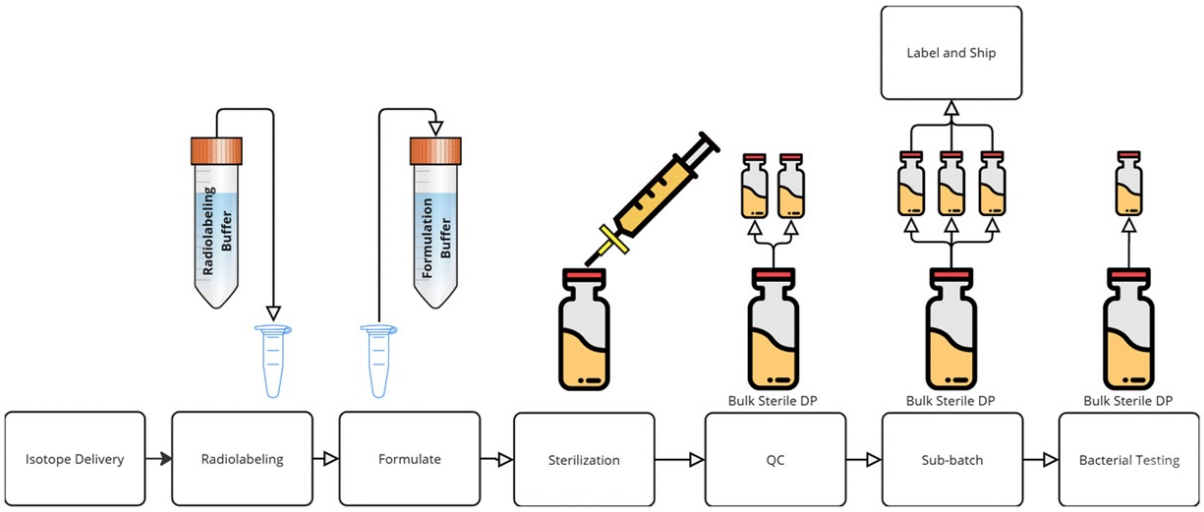

Figure 8: Flow chart depicting steps of a Drug Product Manufacturing process

in preparation of the finished vial shipping. Information tracking and rapid communication to sponsors and sites of delivery are essential.

isotope-specific dedicated fume hoods, automated synthesizing units, air filtration systems, cleanrooms, gowning procedures.

Regulatory & Quality Assurance

Quality assurance is responsible for compliance to radiation safety and health authority regulatory requirements during the radiopharmaceutical manufacturing.

Method and process validations are the foundations of GMP manufacturing. Repeatability, precision, accuracy, specificity, and robustness are the essential criteria to scientifically justify and qualify each analytical

method involved in the operation. Validations are driven by a data-supported process in accordance to ICH, U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) recommendations for drug and radiopharmaceutical development. Data collected throughout studies completed in development and manufacturing are compiled into research reports feeding the chemistry, manufacturing and control (CMC) writing. These reports are referenced within the U.S. FDA regulatory submissions such as IND, New Drug Application (NDA), or others.

Regulatory and Quality resources are critical for maintaining audit readiness. Inspections by health authorities for cGMP compliance, customer audits, and evaluations by radiation regulatory agencies can significantly impact or even halt drug development. These teams ensure ongoing compliance and keep the organization in a constant state of audit preparedness.

Project Management

A dedicated project manager for sponsor interface facilitates clear timelines and milestone expectations with regular reporting using industry-standard project management tools. The management approach is based on proactive capacity planning ensuring availability of materials, personnel, and equipment to meet project needs.

Strategic Value to Sponsors

Radiopharmaceutical manufacturing is a time-sensitive operation. The rollout of manufacturing steps needs to be well-exercised and executed with high precision and coordination. As such, dedicated teams of experts are identified for each level of the process. Trained and experienced personnel are essential to complete rapid and reliable manufacturing, release testing, and distribution from sponsor to clinical sites. In this service, the ability to scale batches, add new suites, or pivot to different processes or chemistry without compromising compliance or timelines requires flexibility and versatility of the manufacturing teams.

Sponsors may not have the financial capabilities to support the specific facilities, equipment, staff, and licenses necessary for a radiopharmaceutical development. Specialized radiopharmaceutical Contract Development and Manufacturing Organizations (CDMO), such as NorthStar Medical Radioisotopes, can provide a cost-effective approach as all elements of drug development through manufacturing supply are provided from a single site.

Conclusion

Radiopharmaceutical development comprises three main process components: (1) Early stage: bringing a molecule never radiolabeled into a radiopharmaceutical preparation; (2) Drug development: optimizing analytical methods, radiolabeling, formulation and establishing stability of the radiopharmaceutical; (3) cGMP manufacturing or the art of radiolabeling scale up to support aseptic, large-scale radiopharmaceutical distribution. These three crucial steps are meant to support the sponsors endeavor for clinical trial supply.

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