

There are two possible outcomes: If the result confirms the hypothesis, then you have made a discovery. If the result is contrary to the hypothesis, then you have made a discovery.

Enrico Fermi

13.1 Quality Assurance

The requirements of quality assurance (QA) for any drug product include quality control test procedures, acceptance criteria, and testing schedule. In the United States, the U.S. Pharmacopoeia (USP) is recognized as an official compendium and the QA standards described in USP-published monographs for each drug product have served to standardize QA procedures. Other countries, similarly, also have their own Pharmacopoeias, such as the European Pharmacopoeia (EP) and the British Pharmacopoeia (BP). In the United States, the Food and Drug Administration (FDA) formally regulates the drug manufacturing process and develops the necessary requirements for good manufacturing practices (GMP) involved in manufacturing drugs.

The FDA Modernization Act of 1997 (the Modernization Act or FDAMA) directs the FDA to establish current good manufacturing practice (cGMP) requirements for PET drugs. Until the FDA develops and formalizes the GMP requirements for PET drugs, the FDAMA requires that the manufacturers of PET drugs must use the standards in USP General Chapter <823> *Radiopharmaceuticals for Positron Emission Tomography-Compounding*. The FDA has also issued a draft Chemistry, Manufacturing, and Controls (CMC) section concerning three PET drug products; Flu-deoxyglucose F18 injection, Ammonia N13 Injection, and Sodium Fluoride F18 Injection.

All radiopharmaceuticals (for both, PET and SPECT) must be tested to assure acceptable quality prior to administration to human subjects. The USP describes the general guidelines for the safety, quality assurance, and quality control of radiopharmaceuticals.

Quality control tests are also performed at various steps in the manufacturing process of every single batch (or lot) of a drug. Because of the relatively short half-lives of positron-emitting radionuclides, each batch of the PET radiopharmaceutical may be released for administration into human subjects, based on quality control tests performed only on the final batch preparation. This special requirement for PET radiopharmaceuticals is based on the following facts:

- PET drugs are produced within strict time limits
- The mass of PET drug (especially, molecular imaging probe) produced in each batch is very small (0.01–0.1 mg)
- Each batch of a PET drug is typically prepared in a small volume (10–50 mL)
- There is insufficient time between production (manufacturing) of a PET drug and administration into patients to comply with all the official QC requirement guidelines for drugs

It is important, however, to emphasize that the safety of a patient should never be compromised under any circumstances; therefore, the quality of the final drug product must meet all the criteria required to assure safety and efficacy.

13.1.1 What are CGMPs?

Manufacturing practices are the methods, facilities, and controls used in the preparation, processing, packaging, or storing of a drug. A current good manufacturing practice (cGMP) is a minimum standard which ensures that the drug meets the requirements of safety and has the identity strength, quality, and purity characteristics it is represented to possess. cGMPs are demonstrated through written documentation of procedures and practices by the manufacturer. The documents and practices may be similar or identical to those requested by other oversight bodies (e.g., NRC, and state and local agencies). Documents produced for other oversight bodies, where appropriate, can be used to provide the documentation of compliance with CGMPs. However, because of institutional, local, or state differences, some of these documents may not have sufficient overlap to address the issues in the guidance provided by FDA. Therefore, to ensure uniformity, supplemental documentation should be developed.

A more detailed description of cGMPs and regulatory controls of radiopharmaceuticals will be discussed in greater detail in Chap. 20.

13.2 Quality Control

There are two types of quality control tests; *physicochemical tests* and *biological tests*. Physicochemical tests define the quality of the PET drug as a radiochemical, while biological tests are performed to assure the *pharmaceutical quality* of the PET radiopharmaceutical.

The *criteria for quality* of the final drug product must be carefully defined for every radiopharmaceutical and appropriate tests must be designed and validated prior to implementation. As specified in the cGMPs and USP, and as required by the FDA, various quality control tests must be performed on every single batch of PET drug, manufactured for human use.

13.2.1 Physicochemical Tests

13.2.1.1 Appearance

The radiopharmaceutical drug product is inspected visually to check for the color and presence of any particulate matter. The following procedure may be used:

- Standing behind a nontinted leaded glass shield, hold the final drug product vial against the light source and gently shake it to check for the presence of any particulate matter.
- Hold the product vial against the colorless background of taped white paper and quickly look at the color of the product solution.
- Record the results.

13.2.1.2 Radionuclidic Identity and Purity

Radionuclidic purity may be defined as the fraction of total radioactivity that is present as the specified radionuclide in the final drug product formulation. Radionuclidic impurities may also be other radioisotopes of the same element as the radiolabel or some other radionuclides of different elements. The nature and levels of radionuclidic impurities depend on the type of nuclear reaction, in which the radionuclidic impurities may arise because of other undesirable nuclear reactions in the target. For example, with ^{123}I radiopharmaceuticals, the presence of ^{125}I and ^{124}I are considered as radionuclidic impurities.

With PET radionuclides, the radionuclidic identity is determined by measuring the half-life of the radioactivity (using a dose calibrator) in a sample of final drug preparation. Positron-emitting radionuclides show a major peak at 511 KeV due to annihilation photons. The levels of contaminants can be easily detected and measured by γ -ray spectrometry based on NaI(Tl) detector or Ge(Li) detector coupled to a multichannel analyzer. Radionuclidic contaminants that decay β^- emission can be measured by β spectrometry using a liquid scintillation counter.

13.2.1.3 Radioassay or Concentration

The concentration of radioactivity in every batch of the final drug preparation must be determined and expressed as mCi/mL or GBq/mL at the end of synthesis (EOS). Higher concentration of a radiopharmaceutical may generate undesirable radiochemical impurities because of radiolysis.

The total amount of radioactivity in the vial containing the final drug preparation is usually measured using a *radionuclide dose calibrator*. In order to determine the concentration, a known volume (0.5 or 1.0 mL) of the final drug preparation is withdrawn into a syringe

and then the activity in the syringe is measured. To ensure that the dose calibrator performs properly, daily quality control tests must be performed using ^{137}Cs (γ energy 611 KeV) and/or other radionuclide standards according to the NRC guidelines.

13.2.1.4 Radiochemical Purity and Identity

Radiochemical purity (RCP) of a radiopharmaceutical may be defined as the fraction of total radioactivity that is present in the desired radiochemical form of the final drug formulation. The RCP should not be confused with the labeling efficiency or radiolabeling yield, which is a measure of percent incorporation of the radionuclide during the synthesis procedure. Also, it is important that the radiolabel is in the specified molecular position. If the radiolabel is in any other position in the molecule or in any other chemical form it is regarded as radiochemical impurity. Several radiochemical impurities may originate from the radionuclide production during the manufacturing process (because of incomplete reactions, side reactions, or incomplete removal of protective groups in the precursor molecule) or even because of radiolysis.

Since the radiochemical impurities may contain the desired radionuclide, the amount of the total radiochemical impurities in the final drug formulation must be minimal, compared to the RCP of the final drug product. The presence of unacceptable levels of radiochemical impurities in the final drug formulation will produce poor quality images because of the undesirable distribution of the radiochemical impurities, *in vivo*.

During the synthesis of 2- ^{18}F fluoro-2-deoxy-D-glucose (FDG), the ^{18}F atom is incorporated into the desired molecular position, 50–80%, which implies that the labeling efficiency is 50–80%. The remaining ^{18}F radiolabel may be present in the form of undesirable radiochemical species, such as ^{18}F fluoride ion, 2- ^{18}F fluoro-2-deoxy-mannose (FDM), and partially acetylated ^{18}F fluoro-deoxy-glucose derivatives. One of the important steps in the manufacturing process is to separate (purify) the desired radiochemical form (FDG) from all other radiochemical impurities. The final drug formulation must have >90% RCP (^{18}F activity in the desired radiochemical form) and a minimal amount (<10%) of radiochemical impurities (such as ^{18}F as fluoride or in any other radiochemical form).

The radiopharmaceutical identity (that is, the desired radiochemical form) must be established by demonstrat-

ing that the chromatographic behavior of the radiolabeled drug product is similar to that of a nonradiolabeled drug product (or the “Standard”). For example, in the case of ^{18}F FDG, the standard is an ultrapure ^{19}F FDG chemical. The comparison of the radiolabeled drug product with a known standard may be performed using either a thin-layer chromatography (TLC) or a high-performance (or pressure) liquid chromatography (HPLC) method. The relative front or the retention factor (R_f) value of the radiopharmaceutical must correspond to or be within the acceptable range of the R_f value of the USP standard for that radiopharmaceutical.

In order to determine both the RCP and identity, it is very important to develop testing procedures that are practical and sensitive for the estimation of relatively small amounts of radiochemical impurities. A number of analytical methods are available depending on the chemical nature of the radiopharmaceutical and undesirable radiochemical impurities. Because of time limitations with PET radiopharmaceuticals, the routine quality control tests to determine RCP must be relatively fast, but must also be extremely reliable and reproducible.

The most applicable analytical method for radiopharmaceuticals is HPLC with an appropriate column, eluting buffer (or solvent), and detectors to identify both the chemical (such as UV detector, refractive index detector) and the radiation signals (high-sensitivity γ or β^+ radiation detectors). TLC also provides a rapid and reliable method to determine the RCP and the radiochemical impurities. However, the HPLC method provides separation of different chemical species with much greater resolution than does TLC. Figures 13.1 and 13.2 show the typical RCP testing of ^{18}F FDG and ^{11}C PIB.

13.2.1.5 Chemical Purity

The chemical purity of a radiopharmaceutical may be defined as the absence of any undesirable chemical species in the final drug product formulation. Chemical purity testing involves an estimation of the amounts (masses) of all the undesirable chemical compounds in the final drug formulation. The chemical impurities may arise from the breakdown of a precursor or other chemicals in the reaction mixture before, after or during the reaction. The mass of chemical impurities may be significantly more than the mass of the desired chemical form and may interfere with the specific interaction of the radiopharmaceutical or may cause undesirable pharmacological side effects.

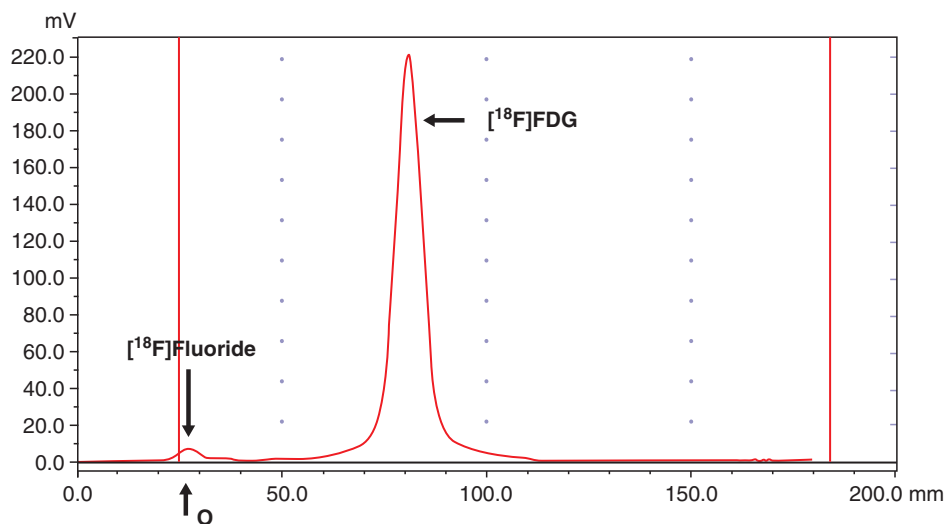


Fig. 13.1 Radiochemical purity testing of $[^{18}\text{F}]\text{FDG}$ based on TLC using SG, and acetonitrile and water (95:5). The free $[^{18}\text{F}]$ fluoride stays at the origin (O), while the migration of $[^{18}\text{F}]\text{FDG}$ is compared to that of cold FDG standard ($R_f = 0.3\text{--}0.4$)

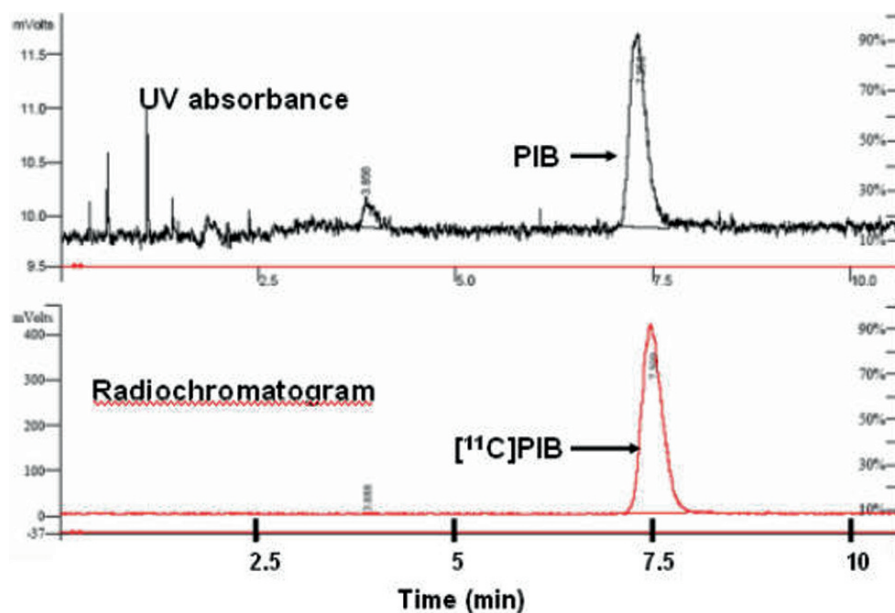


Fig. 13.2 Radiochemical purity testing of $[^{11}\text{C}]\text{PIB}$ based on HPLC using Phenomenex Prodigy $5\mu\text{ ODS}_3$ column, and 50mM of phosphate and acetonitrile (60:40) as an eluant. $[^{11}\text{C}]\text{PIB}$ is mixed with a standard PIB in order to identify PIB by UV absorbance

In the final $[^{18}\text{F}]\text{FDG}$ drug formulation, the only desirable chemical is 2-fluorodeoxy-D-glucose or FDG. However, some of the FDG will be labeled with ^{18}F while a fraction of the FDG molecules could be labeled with stable ^{19}F . Nonradioactive FDG is not a chemical impurity, but will decrease the SA of $[^{18}\text{F}]\text{FDG}$.

Some of the important chemical impurities in $[^{18}\text{F}]\text{FDG}$ drug formulation include $[^{19}\text{F}]\text{FDM}$, chloro-

deoxy-glucose (CDG), D-glucose, and aminopolyether (kryptofix 222).

To determine the chemical impurities quantitatively, HPLC is the best analytical technique. TLC with special staining techniques may also be used for the semiquantitative estimation of the levels of impurities. Kryptofix is usually determined based on a color reaction on a SG-TLC plate using an acidic iodoplatinic acid reagent. The intensity of the color depends on the kryptofix

level. The color in the test sample is compared to a known concentration of standard kryptofix.

13.2.1.6 Residual Solvents

Since most of the molecular imaging probes are organic molecules, it is necessary to use organic solvents (absolute alcohol, ether, acetonitrile, etc.) during the chemical synthesis. After the synthesis and final purification, the final drug formulation may contain small amounts of organic solvents, known as *residual solvents*, which can also be regarded as chemical impurities. Gas chromatography using a DS–PS wax column and a flash ionization (FI) detector is the most common technique for the estimation of the residual solvent concentration, as shown in Fig. 13.3.

The USP recommends acceptable levels for organic solvents in the final drug formulations, based on LD₅₀ values. The USP specified limit is no more than 0.5% dehydrated alcohol, 0.5% ether, and 0.04% acetonitrile.

13.2.1.7 Specific Activity

SA is one of the most important quality control criteria for RMIPs. It is a measure of the amount of radioactivity

per unit mass of the radiopharmaceutical and is usually expressed as mCi/μmole, GBq or TBq/μmole. For certain radiopharmaceuticals, such as [¹⁸F]FDG, radiolabeled amino acids and fatty acids, SA determination is not crucial because these radiotracers do have natural physiologically abundant levels of nonradioactive counterparts in vivo, especially in circulation. However, for receptor-binding radiotracers, radiolabeled mAbs, and peptide hormone analogs, relatively high SA is absolutely necessary. Chemical contaminants which have similar biological activity and compete with the radiotracer for binding to the specific receptors or antigen, will reduce the *apparent or effective SA*, in vivo.

The concentration of radioactivity of the radiopharmaceutical can be easily determined, as described above. The concentration of the radiopharmaceutical in terms of mass/mL (μmoles/mL) should also be determined. For most organic radiopharmaceuticals, the mass (mg or μg) can be determined based on analytical HPLC and a known concentration of an ultra pure standard. Knowing the activity/mL and mass/mL, SA can then be calculated, and expressed as mCi or MBq/μmole. When appropriate, the term, “No Carrier Added” (NCA) should be specified.

13.2.1.8 pH

All radiopharmaceutical formulations, intended for intravenous administration, should maintain relatively stable pH (a measure of hydrogen ion concentration) over an optimal range, preferably very close to the pH of blood. In general, the pH values in the range of 4.5–7.5 are quite acceptable since blood has very good buffering capacity.

Since the pH of the final drug formulation may likely vary from batch to batch, it is important that the actual pH of the final drug formulation is tested using pH paper or, if necessary, a suitably calibrated pH measuring device. The color change, following exposure to the test solution, is compared to that of known standards of the buffer solution of appropriate pH values.

13.2.1.9 Osmolality or Isotonicity

The final drug formulation of a radiopharmaceutical must be *isotonic*, meaning that the ionic strength of the drug formulation is the same or similar to that of blood. Most radiopharmaceutical preparations are generally formulated using physiological saline or a phosphate

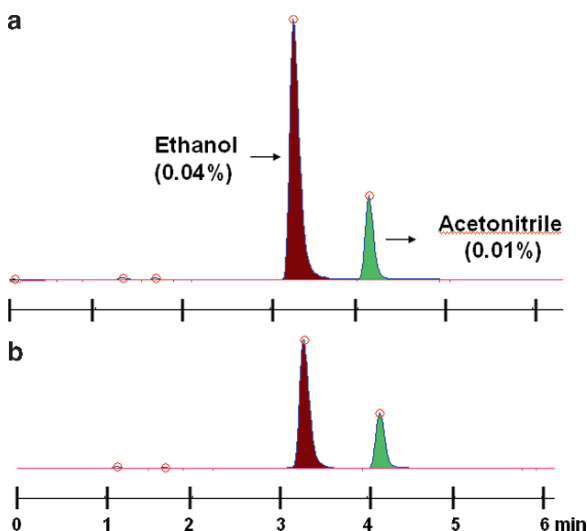


Fig. 13.3 Estimation of residual solvents using gas chromatography (GC): (a). standards; acetonitrile 0.01% and ethanol 0.04%. (b). Chromatogram of FDG solution

buffered saline (PBS) solution. If the drug formulation is prepared using some other physiologically acceptable buffer solution, such as sodium citrate and sodium acetate buffer, the ionic strength should be adjusted to maintain isotonicity of the final drug product formulation.

Routine testing of isotonicity for every batch preparation of the drug formulation may not be necessary. However, periodic testing on the decayed (nonradioactive) drug formulation may be appropriate and necessary. Osmolality (ionic strength) can be measured by the freezing point technique using an Osmometer. For any given batch, the osmolality can be in the range 285–356 mOsm Kg⁻¹.

13.2.1.10 Stability

The PET drug product should remain stable while it is being stored. Appropriate parameters should be evaluated to establish and document the stability of a PET drug product under proposed storage conditions. Examples of stability parameters include radiochemical identity and purity, appearance, pH, and SA. Appropriate stability-indicating methods that can distinguish degradation products and impurities should be used. Stability testing of the PET drug product should be performed at the highest radioactive concentration, and the whole batch volume in the intended container/closure should be stored. On the basis of the stability data, the expiration of the radiopharmaceutical can be determined.

13.2.2 Biological Tests

The USP requires that radiopharmaceuticals intended for intravenous administration must be tested to ensure that the final drug product is a sterile solution and that the *bacterial endotoxin* (*pyrogen*) concentration is within acceptable limits. The quality control tests on the finished drug product (Fludeoxyglucose F 18 injection) will be performed as described in the USP General chapter <823>, “Radiopharmaceuticals for positron-emission tomography – Compounding.”

13.2.2.1 Bacterial Endotoxin Test

The USP *Bacterial Endotoxins Test* (BET) or *pyrogenicity testing* should be performed for a sterile PET drug intended for injection. USP specifies that no more

than 175/V endotoxin units (EU)/mL of the final drug product solution, in which V is the maximum administered dose in mL, at the expiration time.

Pyrogens are fever-producing materials – that most often originate from gram-negative bacterial cell walls – referred to as bacterial endotoxin and that are readily detected by a *gel-clot technique* based on *Limulus amoebocyte lysate* (LAL). The LAL gel-clot testing is a semiquantitative method that is well suited for batch release testing of PET radiopharmaceuticals.

The BET specified in the USP<85> uses a gel-clot technique for the determination of levels of EU and requires a 60-min incubation time. The USP<283> indicates that an in-process 20-min endotoxin limit test can be used for PET drugs with a half-life >20 min; however, the standard 60-min BET must be performed and completed. If necessary, the PET drug product can be distributed, provided BET is initiated. The BET results should meet the acceptance criteria before administering the product to humans.

13.2.2.2 Membrane Filter Integrity

In order to ensure the sterility of the final drug product, most PET radiopharmaceuticals are filtered using a 0.22 μm membrane filter (such as Millipore) in the final step of the synthesis procedure. The sterility test of the final drug product must be performed using microbiological media as specified in USP. Since the sterility test is completed retrospectively, the sterility of the final drug product is indirectly assessed based on the membrane filter integrity (bubble point test) of the filter used to sterilize a single batch of radiopharmaceutical drug product.

The most widely used nondestructive integrity test is the bubble point test. The principle of this test is based on the fact that liquid is held in the pores of the filter by surface tension and capillary forces. The minimum pressure required to force liquid out of the pores is a measure of the pore diameter. The bubble point test detects minor filter defects and out-of-size pores and correlates with the bacteria passage test. Prior to using each lot of filters and performing the bubble test, it may be necessary to validate the manufacturer's specifications of filter integrity by using similar conditions and aqueous media specified by the manufacturer. These values may differ depending on the nature of the aqueous media, buffer strength and pH. For each lot of membranes, the minimum acceptable bubble point pressure (P_m) is determined first. With the membrane filter used in the synthesis

of radiopharmaceutical, the pressure at which bubbles appeared in water (P_i) is determined. The membrane filter integrity test results must show that $P_i \geq P_m$.

13.2.2.3 Sterility

A sterility test must be started within 24 h of the final manufacture of the drug product and after an appropriate decay period. The USP<71> provides information about the incubation media and incubation conditions. Sterility tests are designed to determine the presence of mesophilic bacteria and fungi in solutions. The standard USP sterility test specifies using both *soybean–casein digest broth* (SCD) and *fluid thioglycollate media* (FTM). SCD is incubated at 20–25°C and FTM at 30–35°C. SCDB normally grows aerobic bacteria and fungi, while FTM normally grows aerobic and anaerobic bacteria. Both media are normally incubated for 14 days.

The sterility testing must be conducted in a controlled area, such as a laminar airflow workbench

(LAFW) with clean-room apparel. Aseptic techniques should be used for the sterility testing. The greatest risk of false-positive results arises in the sampling and transfer of the test aliquot from the vial to the media. It may be convenient to apply direct inoculation into the commercial media. The media must be observed periodically on days 3, 7, and 14 after inoculation, but it is prudent to observe the media more often during the first week of incubation.

13.3 Quality Assurance

On the basis of the USP and FDA CMC guidelines, the regulatory specifications, procedures, and testing schedules for [¹⁸F]Fluorodeoxyglucose (FDG) final drug formulation is summarized in Table 13.1.

Similar QA and regulatory specifications have been developed for every PET and SPECT radiopharmaceutical intended for human use.

Table 13.1 FDG: Quality assurance

Test	Acceptance criteria	Procedures	Testing schedule
Appearance	Colorless and free from particles	Visual inspection	Completed prior to the release of drug
Radionuclidic identity	$T_{1/2} = 105\text{--}115.0\text{ m}$	Measure $T_{1/2}$	Same as above
Radionuclidic purity	$511 \pm 5\%$ KeV 99.5% of photons	Gamma spectroscopy	Same as above
Radioassay	10 – 200 mCi/mL at EOS	Measure using dose calibrator	Same as above
Radiochemical identity	The R_f of [¹⁸ F]FDG = R_f of Std FDG ($\pm 10\%$)	TLC-SG using acetonitrile/water (95:5)	Same as above
Radiochemical purity	NLT ^a 90.0% [¹⁸ F]FDG	TLC-SG and solvent (95:5, Acetonitrile: water)	Same as above
Radiochemical impurities	NMT ^b 5.0% [¹⁸ F]fluoride	Same as above	Same as above
pH	Range: 4.5–7.5	pH paper	Same as above
Kryptofix 222	Color intensity of drug product < the color of kryptofix 222 (50 µg/ml)	TLC-SG	Same as above
Residual solvents ^d		Gas chromatography, with flame-ionization detector	Same as above
Acetonitrile	1. NMT ^b 0.04% (w/v)		
Diethyl ether	2. NMT ^b 0.5% (w/v)		
Ethanol	3. NMT ^b 0.5% (w/v)		
Bacterial endotoxins	NMT 175/V EU/V ^c	USP<>	Same as above
Membrane Filter Integrity	$P_i \geq P_m$	Bubble point test	Same as above
Sterility testing	Sterile	USP<>	Start test within 24 h
Osmolality	Isotonic (specify range) Range: 250–350 mOsm	USP	Calculate/Validate
CIDG	NMT ^b 1.0 mg/V ^c	HPLC method	Initial validation/annual testing

^aNLT No less than; ^bNMT No more than; ^cV = Total volume of the batch of fludeoxyglucose F 18 injection produced; ^dAcceptance criteria should assure that the amount of each residual solvent impurity administered to a human subject is within the limits provided in the ICH Guidance on Impurities: residual solvents (Federal Register 1997)

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