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New PET Radiopharmaceuticals: Challenges in the Development of Analytical Methods

Continuing Education for Nuclear Pharmacists
and
Nuclear Medicine Professionals

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NEW PET RADIOPHARMACEUTICALS: CHALLENGES IN THE DEVELOPMENT OF ANALYTICAL METHODS

STATEMENT OF LEARNING OBJECTIVES:

1. Definition of validation of analytical methods used in the routine quality control of PET radiopharmaceuticals.

2. Understanding the seven performance characteristics that comprise analytical methods validation in PET.

3. Understanding the use of system suitability in QC testing of PET radiopharmaceuticals.

4. Identification of the impurities in the production of [^{18}F]FDG and the analytical methods used to determine them.

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QUALITY ASSURANCE

A systematic approach to quality in the production of PET radiopharmaceuticals consists of several critical elements. These elements may be summarized in a "quality system" that ensures the suitability of PET radiopharmaceuticals for human use.[1] A quality assurance (QA) program is one of the most important elements of a quality system. A common theme in QA is "building quality into the product" through documentation, specifications, operator training, validation and quality control (QC). Quality control (QC) testing is the part of QA concerned with routine sampling, testing and release of materials and finished products. QC testing is sometimes erroneously referred to as "QA testing," but QC testing is actually a sub-set of a QA program. A good example of QC testing in PET is the determination of the radiochemical purity of a PET radiopharmaceutical prior to release for human administration. Another example is the analysis of a PET precursor to ensure its proper identity and purity prior to release for use in the production of a PET radiopharmaceutical.

Perhaps no element of a QA program embodies "building quality" more than validation. Validation applies to production processes used to make a product, as well as the analytical methods used to test the product. Validation studies address fundamental questions about the process or method, such as reproducibility, reliability and stability.

Numerous methods are used for the routine QC testing of PET radiopharmaceuticals, including pH determinations, radiation measurements, chromatographic methods and biological assays, such as bacterial endotoxin and sterility tests. This paper will focus exclusively on the challenges associated with the validation of chromatographic methods used for these purposes, but it is important to note that many of the validation principles discussed here also apply to non-chromatographic methods.

METHODS DEVELOPMENT VS. METHODS VALIDATION

The development and validation of analytical methods for QC testing are different, but closely related, processes. Before discussing methods validation, it is important to differentiate it from methods development. Generally speaking, the development of an analytical method occurs before validation, but the two processes often overlap each other and may even undergo several iterations before the entire process is complete.

The development process addresses fundamental issues, such as the type of analysis used in the QC test (chromatographic, spectroscopic, biologic, etc.). For chromatographic analyses, key questions include the type of chromatography, the stationary phase, the mobile phase and the type of detector. The development process also addresses acceptance criteria for the QC test and user requirements like the time and equipment involved in the testing process. Once a QC test has been developed, validation studies are performed to ensure that the method is reproducible, reliable and stable in routine usage.

REGULATIONS AND REGULATORY GUIDELINES

The key regulatory driver behind the validation of analytical methods in the United States is the FDA's Good Manufacturing Practice regulations, or GMP. For PET radiopharmaceuticals, GMP regulations have been under revision by the FDA since the late 1990's and, as of this writing, have yet to be finalized.[2,3] Similar to GMP regulations for non-PET products, the final PET GMP regulations will likely contain requirements that "analytical methods must be suitable for their intended use and must be sensitive, specific, accurate and reproducible.[4,5] Thus, producers of PET radiopharmaceuticals for human use are legally required to perform validation studies on the analytical methods used to test their products.

In addition to regulations, regulatory guidelines and guidance documents describe analytical methods validation. Unlike regulations, regulatory guidelines do not carry the force of law, but regulatory agencies frequently use guidelines to delineate more extensive details than is appropriate for regulations. Still, guidelines tend to be rather vague, which often leads to non-standard practices in the industry. In the United States, the most important guidelines that apply to analytical methods validation are written by the United States Pharmacopeia (USP),[6] the International Conference on Harmonization (ICH)[7] and the FDA.[8] None of these guidelines address specific issues associated with the validation of analytical methods for PET.

Numerous publications in the world of "big pharma" describe validation of the QC tests for drug substances, drug products, biologics, impurities, etc. These publications typically contain detailed descriptions of validation schemes used for analytical methods. Although some publications describe quality assurance programs for PET,[9,10] there is currently a lack of universally accepted standards for the validation of analytical methods in PET. This may lead to unexpected consequences if the PET community and regulatory authorities erroneously assume that methods validation requirements for PET are same as those for big pharma. The unique nature of PET, with its short half life, highly distributed production environment and unique staffing model, will likely have important ramifications regarding the appropriate standards for analytical methods validation that evolve for PET. Therefore, the goal of this paper is to support the development of industry-wide standards for PET.

THE USE OF ANALYTICAL METHODS IN PET

High quality PET imaging studies demand high quality radiopharmaceuticals. Analytical methods and QC testing play a critical role in this equation by assuring the identity, strength and purity of PET radiopharmaceuticals.

The most predominant chromatographic methods used in PET are high pressure liquid chromatography (HPLC), thin layer chromatography (TLC) and gas chromatography (GC). The application of these methods that will be discussed in this paper include the determination of:

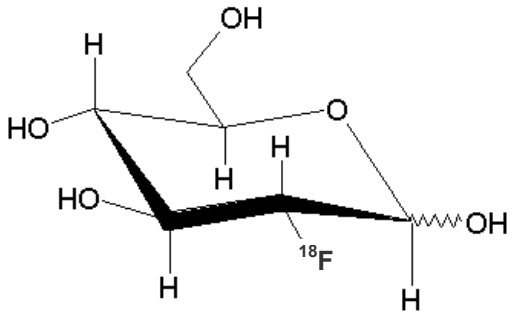
- Radiochemical identity and purity
- Chemical identity and purity
- Specific activity.

Radiochemical Identity

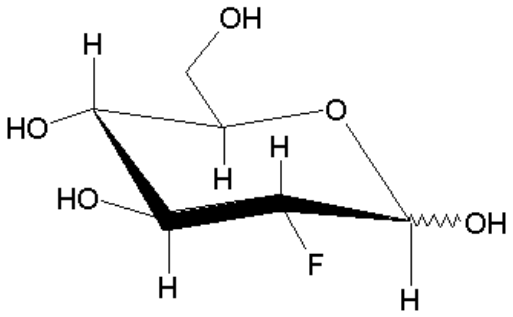
Methods validation studies play an important role in radiochemical identity determinations because the clinical outcome of a PET scan is fundamentally based on the identity of the radiolabeled molecule that comprises the PET radiopharmaceutical.

Radiochemical identity may be defined as the molecular structure of the compound that contains the positron-emitting radionuclide. Since it is nearly impossible to analyze the structure of radiolabeled compounds with the traditional tools used for organic structure determination, the radiochemical identity of a positron-emitting compound must be determined indirectly. This process begins with the preparation and characterization of a non-radioactive analog, which is commonly referred to as the

"cold compound." The radiolabeled compound is then chromatographically analyzed simultaneously with the cold compound. The identical response of the two compounds demonstrates the structural identity of the radiolabeled compound. To illustrate this process, consider the most widely used positron-emitting compound, [^{18}F]fluorodeoxyglucose ([^{18}F]FDG). [^{18}F]FDG was first synthesized by Brookhaven National Lab in 1978.[11] These workers prepared and characterized a non-radioactive analog of [^{18}F]FDG, then showed that the two analogs identically responded to HPLC analysis. This comparison provided strong evidence for the structural identity of [^{18}F]FDG.



[^{18}F]Fluorodeoxyglucose
[^{18}F]FDG



[^{19}F]Fluorodeoxyglucose
[^{19}F]FDG

In routine QC testing, the inclusion of a "cold" compound corrects for the normal variation in chromatographic conditions that could lead to erroneous results. For example, the TLC radiochemical identity test for [^{18}F]FDG includes the analysis of [^{19}F]FDG. The identical response of [^{18}F]FDG and [^{19}F]FDG during the TLC analysis confirms the identity of the [^{18}F]FDG.

Radiochemical Purity

Methods validation studies play an important role in radiochemical purity determinations because radiolabeled impurities may affect the clinical outcome of PET imaging studies due to non-specific uptake.

Radiochemical purity may be defined as the proportion of a radionuclide that is present in the desired chemical form.[9] In the case of [^{18}F]FDG, radiochemical impurities include other species labeled with ^{18}F, such as [^{18}F]fluoride ion or radiolabeled intermediates and by-products. The chromatographic method must effectively separate these species in order to assess radiochemical purity.

Chemical Identity and Purity

Methods validation studies play an important role in chemical identity and purity determinations because the relevant impurities may present a safety hazard if they are present in sufficient quantities. Chemical identity and purity address non-radioactive materials in the PET radiopharmaceutical, including by-products, solvents and other residual components used in the production process. Non-radioactive materials (e.g., stabilizers, additives, etc.) that are intentionally added to the PET radiopharmaceutical may also be included in this category.

Some examples of non-radioactive components that require chemical identity and purity determinations in PET are:

- Chlorodeoxyglucose by-product in [^{18}F]FDG
- L-DOPA by-product in [^{18}F]FDOPA
- Acetonitrile residual solvent in [^{18}F]FDG
- Kryptofix 222 in [^{18}F]FDG.

Specific Activity

The specific activity of a PET radiopharmaceutical is a measure of the amount of the radiolabeled species (measured in radioactivity units) per unit mass of the non-radioactive analog. The non-radioactive analog is frequently referred to as "carrier." In the case of [^{18}F]FDG, the "carrier" is [^{19}F]FDG (see structures above). The units of specific activity are commonly stated in Ci/mmol, mCi/µmol, or MBq/µmol.

Specific activity determinations require the measurement of carrier. These determinations are important when the amount of carrier in the PET radiopharmaceutical is high enough to be pharmacologically significant in the biological system of interest. Since the carrier and PET analogs compete equally well for the desired target, the presence of high quantities of carrier may profoundly impact the uptake of the radiolabeled tracer. In some cases, high quantities of carrier may raise concerns about toxicity. Of course, these concerns do not apply to all PET radiopharmaceuticals. This is the case for [^{18}F]FDG where the amount of carrier [^{19}F]FDG is so low that routine specific activity measurements are not necessary.[12]

METHODS VALIDATION PERFORMANCE CHARACTERISTICS

The validation of a chromatographic method encompasses seven performance characteristics:

- Specificity
- Precision
- Accuracy
- Linearity and range
- Detection limit
- Quantitation limit
- Ruggedness and robustness.

Specificity

Specificity is the ability to assess the desired component in its intended formulation, including impurities, by-products, additives, etc. A chromatographic method is specific if it can separate the component of interest from other components in the formulation. A common example of specificity in PET is the TLC method for the determination of [^{18}F]FDG radiochemical purity. In this case, the method must separate [^{18}F]FDG, [^{18}F]fluoride ion and other ^{18}F labeled products. In order to demonstrate specificity, it is necessary to prepare each ^{18}F labeled component and subject a mixture of the components to the TLC analysis. Separation of the individual components demonstrates the specificity of the method.

Precision

The precision of a method is the degree of agreement between individual test results. In its simplest form, precision is a quantitative measure of the repeatability of the method under normal operating conditions. Precision is measured by multiple repetitions of the test and determination of the standard deviation in the results. In addition to the standard deviation, another common measure of precision is the relative standard deviation (RSD), which is the standard deviation divided by the mean of the sample population. The higher the RSD, the less precise the method. A chromatographic method is precise if the RSD is on the order of 5 to 10%. A common example of precision as it applies to PET is the GC method for the determination of acetonitrile concentration in [^{18}F]FDG. In order to demonstrate the precision of this method, it is necessary to perform multiple analyses of acetonitrile standards. RSD values less than 10% demonstrate the precision of the method.

Accuracy

The accuracy of a method is the degree of agreement between the test result and the true value. Accuracy is the ability of the method to provide the correct answer. Accuracy may be measured by the analysis of a known standard and comparison of the measured value to the actual value. For example, GC analysis of a known solution containing 0.03% acetonitrile must provide a result that is within experimental error of 0.03%.

The accuracy of a QC method may also be measured by verifying the results with an independent method. This technique is especially useful in HPLC radiochemical purity determinations where radiochemical purity is routinely determined by integrating the areas under the radioactivity peaks. It is also possible to collect fractions of the solution as it elutes from the column. The measurement of the radioactivity in each fraction offers an independent means of determining radiochemical purity. The HPLC method is accurate if the value for the radiochemical purity is the same as that obtained by the fraction counting method.

Linearity and Range

Linearity may be defined as the proportional response of a method as a function of the amount of analyte. A linear relationship exists if the detector response is directly proportional to the amount of analyte in the sample. The range of the method defines the region where the response is linear. For example, the linearity of the GC method for acetonitrile may be determined by the analysis of standards containing different known concentrations of acetonitrile. The method is linear if the acetonitrile peak area increases proportionately with increasing acetonitrile concentration. The linear range of the method must cover the concentrations of acetonitrile found in routine QC testing.

Detection Limit

The detection limit is the lowest amount of analyte that can be detected. Frequently, the detection limit is two or three times the baseline noise level encountered in the chromatographic method. This characteristic is most important in chemical and radiochemical impurity determinations. It is also important in carrier determinations used to measure specific activity.

Quantitation Limit

The quantitation limit is closely related to the detection limit. The quantitation limit is defined as the lowest amount of analyte that can be measured. This limit is frequently taken as ten times the baseline

noise level of the chromatographic method. This characteristic is important in measurements of impurity levels in chemical and radiochemical purity determinations. This characteristic is especially critical for carrier determinations used to measure specific activity.

Ruggedness and Robustness

Ruggedness is the degree of reproducibility obtained under a variety of conditions. A method is rugged if the identity and purity determinations do not depend on the manufacturer of the analytical equipment or on the analyst performing the measurement. For commercial suppliers of PET radiopharmaceuticals, ruggedness may be thought of as the ability of all analysts at one production facility to obtain the same result and the ability of all production facilities to obtain the same result.

Robustness is the ability of a method to remain unaffected by small, deliberate changes. A method is robust if the results remain unaffected by small changes in temperature, flow rate, concentration of mobile phase, etc.

When to Perform Methods Validation

It is only necessary to perform methods validation studies at certain key stages of the development and commercialization process for PET radiopharmaceuticals. It is not necessary to perform methods validation studies for PET radiopharmaceuticals in the pre-clinical or investigational (IND) stage of development. This position was asserted by the FDA in public meetings held in 2006.[13] In these meetings, the FDA noted that IND's for PET radiopharmaceuticals typically contain some methods validation data (e.g., specificity, linearity, precision and accuracy), but acknowledged that complete methods validation is not necessary until submission of a new drug application (NDA). It is also not necessary to validate analytical methods that are described in USP monographs. The assumption here is that the analytical method was validated as part of the process of accepting the method in the monograph.

Practical Recommendations

As noted earlier, methods validation begins with successful methods development. The successful completion of the entire development and validation cycle requires a systematic approach aimed at several key milestones.

First, it is crucial to understand how the method will be used in routine QC testing. Of course, the short half-life of positron-emitting radionuclides creates significant time constraints on the execution of QC tests. In some cases, it may not even be possible to complete certain QC tests prior to release of the product. This may result in additional burdens placed on the analytical methods development and validation process. Beyond time constraints, other user requirements may also present significant obstacles. For example, the type of equipment and the experience of personnel may create requirements that drive the entire method selection process. Thus, it is important to pay special attention to user requirements at all stages of development and validation. Erroneous assumptions during this cycle can easily lead to regulatory delays and failures or delays in the implementation of the method for routine use.

Second, it is important to obtain a well-characterized and purified sample of the analogous cold compound early in the development process. Since the cold analog is necessary for most of the validation studies, a reliable supply is critical. If the cold analog is not commercially available, it will be necessary to prepare it in-house, or with a custom synthesis laboratory. In addition to its use as a standard in the validation studies, the cold analog is often subjected to degradation studies to provide insight into the stability of the PET compound. Depending on the results of degradation studies, it may even be necessary to prepare degradation products for use in the methods validation studies. Depending on the chemical complexity of the cold analog, it's synthesis, purification, characterization and degradation may require several months to complete. Therefore, plan for these studies early in the development process.

Third, it is important to prepare and maintain detailed records and notebooks to document the methods validation studies. The old adage applies here: "if it isn't documented, it didn't happen." Prepare a written validation protocol beforehand that describes the validation studies. Make sure records are complete, orderly and properly approved. Summarize the results of the validation studies in a report and maintain on file.

System Suitability

A properly executed validation study results in a QC method that may be implemented in numerous laboratories and environments. Although it is not necessary to repeat the methods validation studies in each of these settings, it is necessary to ensure that each analytical system used in QC testing functions as a whole. This is accomplished with system suitability parameters, which demonstrate that the

complete analytical system, including the instrument, reagents, columns, etc., is suitable for the intended application. System suitability parameters must be routinely measured to assess the "health" of the equipment used in the analytical system. For the chromatographic methods discussed earlier, common system suitability parameters include:

- Tailing factor (measure of peak asymmetry)
- Resolution (measure of separation between two peaks)
- Efficiency (measure of resolving power).

It is also necessary to assess the ability of the analyst to perform the test. This is accomplished with analyst qualification tests, which may be considered a fourth system suitability parameter. Analyst qualification tests typically consist of replicate analyses to ensure the analyst is capable of repeating the test and obtaining the same results.

Purity Characteristics, Impurities and QC Methods for [^{18}F]FDG

Reliable QC testing methods are built on a foundation of methods validation and system suitability. A complete list of the QC tests for [^{18}F]FDG is shown in Table 1, which also summarizes the purity characteristics and potential impurities associated with each QC test for [^{18}F]FDG.

Table 1. Purity Characteristics, Impurities and QC Methods for [^{18}F]FDG.

Purity Characteristic	Potential Impurities	QC Test Method*
Physical Characteristics Color Clarity Visible particulate matter	Coloration Turbidity Particulates	Visual inspection through leaded glass
Radiochemical purity	[^{18}F]Fluoride ion and partially hydrolyzed [^{18}F]fluorinated intermediates	Thin-layer chromatography
Radionuclidic purity	Target activation products	Half-life determination Gamma spectra obtained on decayed samples
pH	Non neutral solution	Paper pH strips
Chemical purity	Acetonitrile	Gas chromatography

Purity Characteristic	Potential Impurities	QC Test Method*
	Ethanol Kryptofix 222 Chlorodeoxyglucose	Gas chromatography Spot test HPLC
Bacterial endotoxin	Bacterial endotoxins	Limulus amebocyte lysate
Sterility	Bacteria and other microbes	Inoculation into two growth media

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ASSESSMENT QUESTIONS

1. Which of the following is a measure of the degree of agreement between individual test results?

    a. Precision
    b. Resolution
    c. Specificity
    d. Accuracy

2. Which of the following is a system suitability parameter?

    a. Specificity
    b. Resolution
    c. Radiochemical purity
    d. Accuracy

3. Radiochemical purity is a routine QC control test preformed on PET radiopharmaceuticals. Radiochemical purity is the:

    a. proportion of a radionuclide that is present as the desired radionuclide.
    b. molecular structure of the compound containing a positron-emitting radionuclide.
    c. lowest amount of the PET compound that can be detected.
    d. proportion of a radionuclide that is present in the desired chemical form.

4. The chemical purity of [^{18}F]FDG includes which of the following?

    a. the amount of [^{18}F]fluoride ion that is present in the formulation.
    b. the presence of a turbid solution.
    c. the concentration of chlorodeoxyglucose.
    d. the presence of bacterial endotoxins.

5. The following performance characteristics are included in a methods validation study except:

    a. Linearity
    b. Efficiency
    c. Detection limit
    d. Precision