Production and Quality Control of [^{18}F]FDG

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Slide 1

[^{18}F]FDG Production

Objectives—

- Introduction to chemistry
  - Nomenclature, reagents, reactions
  - Potential by-products and impurities
- Quality Control (QC)
  - Relation to chemistry

Slide 2

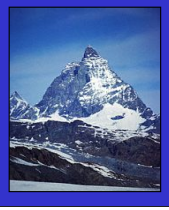
This presentation introduces you to the chemistry behind the production of [^{18}F]FDG. I will discuss nomenclature, reagents, reactions, potential by-products and impurities. I'll also relate these topics to Quality Control processes relevant to the production of [^{18}F]FDG.

First of all, what do the initials "FDG" stand for? "FDG" is an abbreviation for 2-[^{18}F]fluoro-2-deoxyglucose, or simply fluoro-deoxy-glucose for short. In the picture in the upper right corner of this slide, you see that [^{18}F]FDG has a similar structure to that of glucose. The red atoms in this graphic depict oxygen, the gray atoms carbon and the green atom the F-18. The small white atoms represent hydrogen.

[18F]FDG Production

Six steps—

- [18F]Fluoride ion production
- Trap and release the [18F]fluoride ion
- Preparation of the [18F]fluoride ion
- Radiolabeling
- Hydrolysis
- Purification

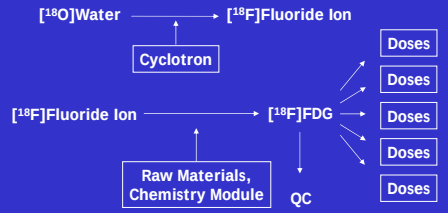


Slide 3

I'm going to divide the chemistry portion of the discussion into six steps. First, I'll discuss the production of [18F]fluoride ion. Next, I'll talk about trapping and releasing the [18F]fluoride ion, followed by it's preparation for the subsequent processing steps. Then I'll discuss these processing steps in detail, including the radio-labeling step, the hydrolysis step and the purification process.

[18F]FDG Production

Process Overview—



Slide 4

This slide provides an overview of the [18F]FDG production process. We begin with [18O]water, which is similar to ordinary water except it is enriched with the stable isotope O-18. Upon bombardment with a cyclotron, this stable isotope is transformed into F-18, which is in the chemical form of [18F]fluoride ion. Next, the [18F]fluoride ion is converted into [18F]FDG with raw materials and a chemistry module. After some purification, the [18F]FDG is collected in a single vial. We perform quality control on a small portion of this vial before sending doses out the door.

[^{18}F]FDG Production

Cyclotrons—

1931

Today

Slide 5

To make [^{18}F]FDG, you need a particle accelerator, in most cases a cyclotron. This slide shows a picture of the world's first cyclotron, which was invented in 1931 by E.O. Lawrence. For this work, Lawrence received the Nobel Prize in physics. Cyclotrons accelerate sub-atomic particles like protons or electrons to very high velocities. The picture on the left is the cyclotron invented by Lawrence. It is about the size of a grapefruit and easily fits on a tabletop. It accelerated a mere trickle of protons.

The cyclotron on the right is a slightly different machine. This machine is located at the TRIUMF Research Consortium in Canada, and is the largest in existence today. It is 18 meters across, and in case you can't determine the scale of this photograph, note the human being on the *inside* of that cyclotron. Cyclotrons have certainly come a long way in the last 70 years! The cyclotrons used today in the production of [^{18}F]FDG fall between the extremes pictured on this slide, and typically accelerate protons to 11 to 17 MeV.

[^{18}F]Fluoride Ion Production

- Proton bombardment of [^{18}O]water in specially designed target
- ^{18}O(p,n)^{18}F reaction produces [^{18}F]fluoride ion
- Only small fraction of protons undergo reaction
- Transfer solution of [^{18}F]fluoride ion/[^{18}O]water from target to chemistry module
- Pass through ion exchange cartridge
  - Recover [^{18}O]water
  - "Trap and release" [^{18}F]fluoride ion

Slide 6

So let's get back to the process. Recall that we begin with [^{18}O]water, which is placed inside a target that is specially designed to contain the [^{18}O]water during the bombardment process. The F-18 nuclei are produced via the p-n nuclear reaction wherein an O-18 nucleus captures a proton and ejects an energetic neutron. Only a very small fraction of the protons in the beam result in the production of F-18. Most of the protons are simply lost as heat in the target. For this reason, the target must be engineered to efficiently remove the immense quantity of heat produced during the bombardment process. This is challenging because the volume of [^{18}O]water is so small. The volume of the [^{18}O]water in the target varies depending on the design of the machine, but typically ranges from 1 to 3 ml. Of course, it is important to minimize the volume of [^{18}O]water due to the expense of this enriched isotope.

The chemical form of the F-18 is [^{18}F]fluoride ion, the same chemical form as the non-radioactive version used in toothpaste. Once the bombardment is complete, the radioactive solution containing the [^{18}F]fluoride ion is transferred from the target to the chemistry processing module. This is a seemingly simple, but critical step in the production of [^{18}F]FDG. The transfer must be reliable and efficient. Due to the high radiation levels resulting from the [^{18}F]fluoride ion, the transfer takes place remotely under computer control. During this step in this process, the solution passes through an ion exchange resin, where the [^{18}F]fluoride ion is removed from the solution and the [^{18}O]water recovered for reprocessing. [^{18}O]Water is a precious resource, so it's important to recover it for reuse.

The process of extracting the [^{18}F]fluoride ion from the [^{18}O]water is commonly referred to as a "trap and release process." Let's discuss this in more detail.

[^{18}F]Fluoride Ion Trap and Release

What is Ion Exchange?

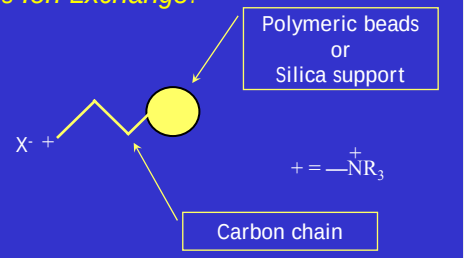
- Solid phase process based on resins
- Relies on electrical charge
- Anion exchange resins positively charged
- Cation exchange resins negatively charged

Slide 7

The "trap and release" process is based on ion exchange, which is a solid-phase process that relies on electrical charges to conduct a separation. Anion exchange resins are positively charged because they exchange negatively-charged anions, and cation exchange resins are negatively charged to exchange cations.

[^{18}F]Fluoride Ion Trap and Release

What is Ion Exchange?

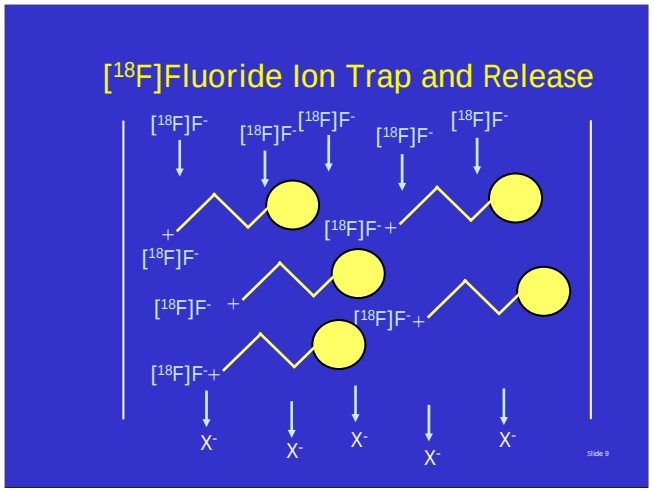


Slide 8

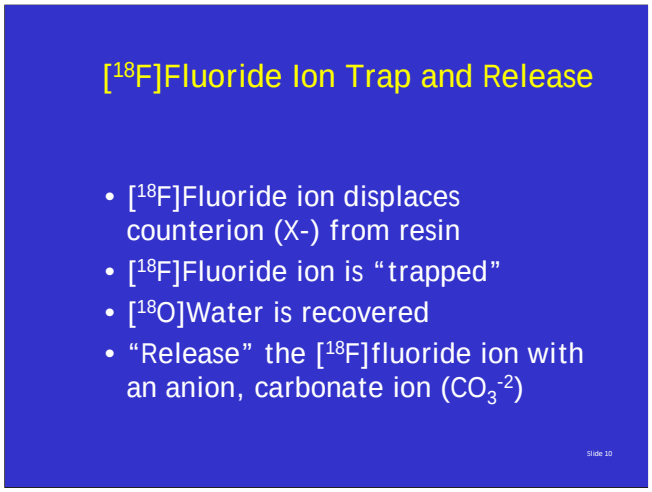
If you closely look at an ion exchange resin, you see that it consists of a carbon chain bound to beads. The beads are made from either an organic polymer or a silica support. To illustrate this concept, this slide contains a highly magnified view of a single resin bead with a single hydrocarbon chain. In reality, of course, a resin bead has a very irregular surface with many, many pores. Each bead contains numerous immobilized hydrocarbon chains.

The end of each hydrocarbon chain contains a positive or negative charge. Anion exchange resins, like that depicted on this slide, contain a positive charge at the end of the hydrocarbon chain. The moiety responsible for the positive charge is typically a quaternary ammonium salt.

To balance the electric charge on an anion exchange resin, there must be an anion, typically hydroxide, carbonate or chloride ion. An anion exchange resin with hydroxide counterions is said to be in the hydroxide form. As we shall see in a moment, the choice of counterion is very important because it can get involved in subsequent processing steps.

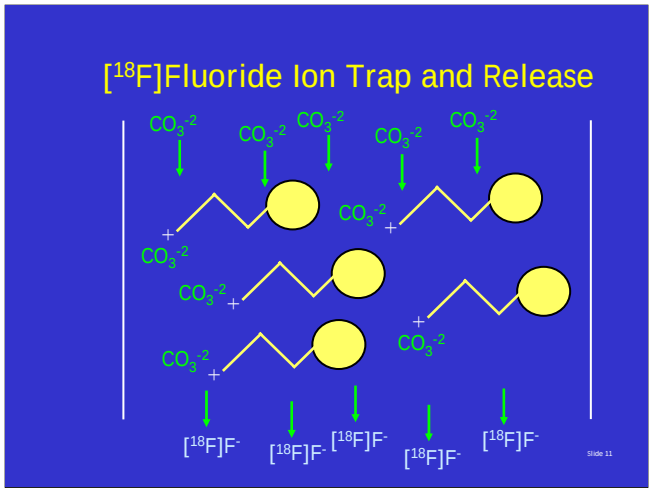


Now, imagine a column containing many, many resin beads. This slide depicts an anion exchange column with five resin beads, each with a counterion that we'll call "x." When we pass a solution of [^{18}O]water containing [^{18}F]fluoride ion through the resin bed, the solution enters the top of the column. As it passes though the column, the [^{18}F]fluoride ion displaces the counterion "x". The [^{18}F]fluoride ion is trapped by the positive charge on the resin. The [^{18}F]fluoride ion has been "exchanged" with the "x" counterion.

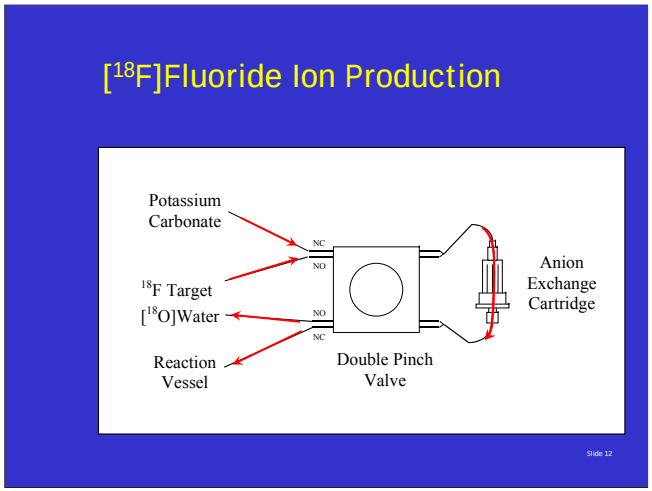


During the exchange process, the counterion "x" is displaced from the resin, and exits the bottom of the column, now in solution with [^{18}O]water. So, at the end of this process, we have achieved two things. First, we have exchanged, or "trapped," the [^{18}F]fluoride ion onto the resin, and, second, we have recovered the [^{18}O]water.

The next step in the [^{18}F]fluoride ion "trap and release" process is to release the [^{18}F]fluoride. We accomplish this with a solution of potassium carbonate.



The next slide returns us to our cartoon of the resin column where [^{18}F]fluoride ions have been trapped onto the positively charged ends of the hydrocarbon chain. We pass a solution of carbonate ions through the column to "exchange" or displace the [^{18}F]fluoride ion from the resin. Since each carbonate ion has two negative charges, it is possible for each carbonate to displace two [^{18}F]fluoride ions, so this cartoon isn't quite accurate from the standpoint of electric charge balance. As the carbonate solution passes through the column, the [^{18}F]fluoride ion exits the bottom of the column.



The hardware required to accomplish the "trap and release" process is very simple. This slide illustrates one possibility that uses a commercially available anion exchange cartridge and a double pinch valve with flexible tubing. The [^{18}O]water solution containing [^{18}F]fluoride ion takes the "normally open" or "NO" pathway through the cartridge and exits into a container to collect the [^{18}O]water. During this process, of course, the [^{18}F]fluoride ion binds to the cartridge. Next, electrical power is applied to actuate the valve. Then, an aqueous solution of potassium carbonate passes through the normally closed pathway, strips the [^{18}F]fluoride ion from the cartridge, and passes into the reaction vessel for further processing.

[^{18}F]Fluoride Ion Trap and Release

What is the [^{18}F]fluoride ion counterion?

- [^{18}F]Fluoride ion is displaced from resin with potassium carbonate
- Therefore, the counterion is potassium
- Potassium carbonate can be
  - In aqueous solution

    or…
  - Dissolved in acetonitrile/water with Kryptofix® (more on this later)

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I wanted to briefly mention the counterion for the [^{18}F]fluoride ion after it is stripped from the resin. Since the counterion of the carbonate solution is potassium, it's simple to see that the counterion of the [^{18}F]fluoride ion is potassium.

[^{18}F]Fluoride Ion Trap and Release

Summary—

[^{18}F]Fluoride Ion/[^{18}O]water

↓

K^+/[^{18}F]Fluoride Ion/[^{16}O]water

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This slide summarizes the trap and release process for the [^{18}F]fluoride ion. We begin with an [^{18}O]water solution of [^{18}F]fluoride ion and end with an [^{16}O]water solution of [^{18}F]fluoride ion with a known counter ion.

Interestingly, no one really knows the counterion of the [^{18}F]fluoride ion while it is dissolved in the [^{18}O]water. As it comes off the target, the counterion is likely H+, or some other cation that is endogenous to either the target body or the [^{18}O]water.

[^{18}F]Fluoride Ion Production

By-product of bombardment—

- [^{16}O]Water produces ^{13}N
- ^{16}O(p,α)^{13}N reaction
- [^{13}N]Nitrites, [^{13}N]nitrates, [^{13}N]N_2 gas
- 90% Enriched [^{18}O]water yields mCi's

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The next slide introduces us to some potential by-products that may be produced during the bombardment process. The first by-product is ^{13}N, which results from proton bombardment of [^{16}O]water via a (p,α) reaction. Since [^{18}O]water is not 100% enriched and contains some [^{16}O]water, ^{13}N is produced in this fashion.

The ^{13}N potentially exists in several chemical forms, including [^{13}N]nitrite and [^{13}N]nitrate, both of which are negatively charged, or [^{13}N]nitrogen gas, which is just like the gas surrounding us here today.

What happens to these by-products? First, [^{13}N]nitrogen is a gas that may escape during the target unload process unless it is trapped. That is an easy process. Next, the negatively charged nitrite and nitrate trap on the anion exchange resin just like the [^{18}F]fluoride ion that we just discussed. Unlike [^{18}F]fluoride ion, however, nitrite and nitrate have a very high affinity for the anion exchange resin, so they bind irreversibly to the cartridge.

When the enrichment of the [^{18}O]water is 90%, the proton bombardment process may produce millicurie amounts of ^{13}N. In my experience, most of this ^{13}N exists in the chemical form of [^{13}N]nitrogen gas.

[^{18}F]Fluoride Ion Production

Other by-products of bombardment—

- Target window
  - Havar® (alloy containing Co, Ni, Cr, Fe)
    - Yields ^{56}Co, ^{57}Co, ^{58}Co, ^{52}Mn, ^{54}Mn
  - Titanium
    - Yields ^{48}V
- Target body (Ag or Ti)
  - Silver produces ^{109}Cd
  - Titanium produces ^{48}V
- Removed at various steps in the production process

Slide 16

The next slide describes other potential impurities. To understand the source of these impurities, it is important to remember that the bombardment of the [^{18}O]water takes place in a solid target body that is isolated from the rest of the cyclotron with a thin foil. The target body and the thin foil produce proton activation products that must be removed form the [^{18}O]water during the production process. This slide lists the most prevalent activation products from the two most common foil materials (Havar® and titanium), as well as the most common target bodies (silver and titanium).

Each activation product is removed during the [^{18}F]FDG product process, and studies have shown that the resulting product is free of activation products.

[18F]Fluoride Ion Production

Sources of [19F]fluoride/chloride ion—

- Target walls, windows
- [18O]Water
- Potassium carbonate
- Anion exchange resin

Slide 17

The last impurities I'll discuss in the context of the bombardment process are [19F]fluoride ion and chloride ion. Each of these ions are present in very small, but measurable quantities in the target walls, target windows, [18O]water and the anion exchange resin. Of course, [18F]fluoride ion is also know as "carrier" fluoride, and chloride is a halogen that has very similar chemical properties as fluoride.

[18F]Fluoride Ion Production

Impact of [19F]fluoride/chloride—

- [19F]Fluoride ion (carrier) produces carrier FDG
- Chloride ion produces chloro-deoxyglucose (Cl-DG)

Slide 18

What happens to these impurities and why do we care? Of course, carrier fluoride ion is chemically identical to ^{18}F, so it produces carrier FDG. The more carrier fluoride ion that is present, the lower the specific activity of the [18F]FDG. From a practical standpoint, carrier FDG is not a major concern in the final product. Like I mentioned a second ago, chloride ion is a halogen, and lies just below fluoride on the periodic chart. Chloride ion undergoes reactions in very similar fashion to fluoride ion, and it produces chloro-deoxyglucose, or Cl-DG.

The first publications on the use of anion exchange resins in the production of [18F]FDG described a process that produced larger than expected quantities of Cl-DG. It turned out that the resin was in the chloride ion form. During the trap and release process that I discussed earlier, the chloride ion was stripped from the resin and resulted in the Cl-DG. So to avoid this, we convert the anion exchange resin from the chloride form to the carbonate form and the amount of Cl-DG produced is minimal. This is way it is important to understand the ionic form of the anion exchange resin.

[^{18}F]FDG Chemistry

Six steps—

- [^{18}F]Fluoride ion production
- Trap and release the [^{18}F]fluoride ion
- Preparation of the [^{18}F]fluoride ion
- Radiolabeling
- Hydrolysis
- Purification

Slide 19

That completes the discussion of the first two steps in the production of [^{18}F]FDG. Next, I'll discuss the preparation of the [^{18}F]fluoride ion. And when I say "preparation," I don't mean "making it," but instead I mean "how do we get it ready for use?"

Preparation of the [^{18}F]Fluoride Ion

What are the requirements of the [^{18}F]fluoride ion?

- Must be anhydrous (no water)
- Must be in solution
- Must be reactive

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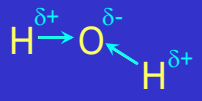
So what are the requirements of the [^{18}F]fluoride ion to make it an effective reagent in the subsequent production steps? First, the [^{18}F]fluoride ion must be anhydrous. Second, it must be in solution. And third, it must be reactive. Now, I'd like to discuss *why* the [^{18}F]fluoride ion must be anhydrous, in solution and reaction. I will also discuss *how* we accomplish these requirements.

Preparation of the [^{18}F]Fluoride Ion

Why must the [^{18}F]fluoride ion be anhydrous?

- Chemical bonds between oxygen and hydrogen are *polar covalent*
- Water forms hydrogen bonds with fluoride ion
- Hydrogen bonds give water it's solvating power



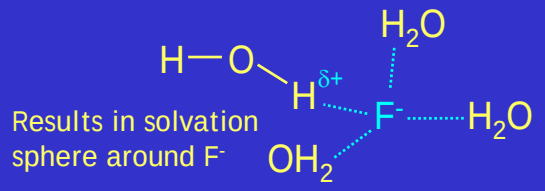
Slide 21

This slide talks about why the [^{18}F]fluoride ion must be anhydrous. Let's begin with water. The chemical bonds in water–the bonds between oxygen and hydrogen–are "polar covalent" bonds. This results from the fact that oxygen is more electronegative than hydrogen. These "polar covalent" bonds allow water to form "hydrogen bonds" with anions like [^{18}F]fluoride ion. You may recall from general chemistry class that water is called "universal solvent." This is because the hydrogen bonds give water superior solvating properties.

The bottom of this slide is animated to illustrate this point. The electronegative oxygen attracts the electrons in the O-H bond, which results in a partial negative charge on the oxygen and a partial positive charge on the hydrogen atoms. I have denoted the polar covalent bond with arrows and partial charges with the Greek letter "delta." What does this mean regarding [^{18}F]fluoride ion?

Preparation of the [^{18}F]Fluoride Ion

Why must the [^{18}F]fluoride ion be anhydrous?



Results in solvation sphere around F^-

Slide 22

This slide illustrates the concept of solvation for [^{18}F]fluoride ion. Here is a water molecule with a partial positive charge on hydrogen. The negative charge on the [^{18}F]fluoride ion strongly attracts the partial positive on the hydrogen; so much, in fact, that there are numerous water molecules surrounding the [^{18}F]fluoride ion to create a solvation sphere around the [^{18}F]fluoride ion.

The formation of the solvation sphere is strongly favored thermodynamically, and greatly reduces the reactivity of the [^{18}F]fluoride ion. Therefore, one requirement for reactivity is that the [^{18}F]fluoride ion is anhydrous to eliminate this solvation sphere. How do we do this?

Preparation of the [^{18}F]Fluoride Ion

How do we make the [^{18}F]fluoride anhydrous?

- Water boils at 100 C
- Water/acetonitrile forms azeotrope that boils at 80 C
- Add acetonitrile to solution of the [^{18}F]fluoride ion and evaporate

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One way to accomplish this is to remove the water by distillation at 100° C. A more effective approach is to mix the water with another liquid to produce an azeotrope. It turns out that water and acetonitrile form an azeotrope that boils at 80° C. Reducing the boiling point greatly facilitates the distillation process and yields an anhydrous solution of [^{18}F]fluoride ion.

Preparation of the [^{18}F]Fluoride Ion

What is an azeotrope?

- Mixture that boils like a pure liquid
- Cannot separate an azeotrope into its individual components by distillation
- Allows easy removal of water

Slide 24

To complete this thought, let me note that an azeotrope is a mixture of two liquids that behave like a single, pure liquid. Thus, an azeotrope is a mixture with a single boiling point, and it is impossible to separate the components of an azeotrope by distillation.

[^{18}F]FDG Chemistry

Six steps—

- [^{18}F]Fluoride ion production
- Trap and release the [^{18}F]fluoride ion
- Preparation of the [^{18}F]fluoride ion
- Radiolabeling
- Hydrolysis
- Purification

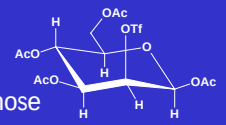
Slide 25

All right, that completes our discussion of why the [^{18}F]fluoride must be anhydrous. Next, we'll cover how to make the [^{18}F]fluoride ion soluble and reactive. Remember, the [^{18}F]fluoride ion is a salt, and we've just removed the water, so how is it soluble in a solution of acetonitrile? Before discussing that, let's move to the next step in the production of [^{18}F]FDG. Step four is the key step known as the radiolabeling step.

Radiolabeling with [^{18}F]Fluoride Ion

The precursor—

- Provides organic building block of [^{18}F]FDG
- 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethane-sulfonyl-β-D-mannopyranose



- Synonyms: mannose triflate, mannose, triflate

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During the radiolabeling step, [^{18}F]fluoride ion, which is now anhydrous, is reacted with an organic precursor to produce an [^{18}F]FDG intermediate. The name of the precursor is 1,3,4,6-Tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-β-D-mannopyranose. Thankfully, this awkward name is shortened to its common abbreviations, like mannose triflate, mannose, or triflate. This slide illustrates the chemical structure of mannose triflate, and you can see that it is a six-membered ring that exists predominantly in the chair form.

Radiolabeling with [^{18}F]Fluoride Ion

Key properties of mannose triflate—

- Six carbon atoms…we only want to label one of them with [^{18}F]fluoride ion
- Use "protecting groups" and "leaving groups" to steer [^{18}F]fluoride ion to the desired carbon (carbon atom #2)

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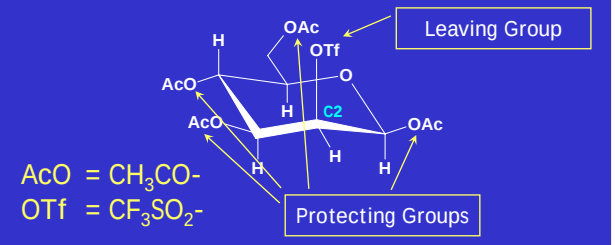
This slide notes some of the key properties of mannose triflate that make it suitable for use in the synthesis of [^{18}F]FDG. First, note that mannose triflate has six carbon atoms and we only want to label one of them. To accomplish this, we employ a combination of leaving groups and protecting groups to direct the [^{18}F]fluoride ion to the desired carbon atom, which is designated as carbon atom number 2, or simply "C-2."

Radiolabeling with [^{18}F]Fluoride Ion

Key properties of mannose triflate—



AcO = CH_3CO-

OTf = CF_3SO_2-

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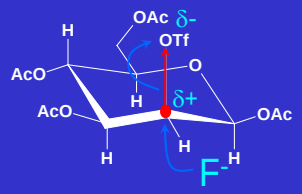
This slide describes the details of the leaving groups and the protecting groups. You see that the carbon atom of interest – C-2 – contains the triflate group, which is abbreviated "OTf" in this structure. The triflate group is a very effective leaving group due to its highly electronegative nature. We'll discuss this further in a moment. The remaining hydroxyl groups on mannose triflate are protected with acetate groups. On this slide, the acetate groups are abbreviated "AcO." The acetate groups effectively protect their associated carbon atoms to prevent reaction with the [^{18}F]fluoride ion. The fact that there are four acetyl protecting groups results in the "tetra-acetyl" in the full chemical name of mannose triflate. The chemical abbreviations for both protecting are shown in the bottom left corner of this slide.

Radiolabeling with [^{18}F]Fluoride Ion

Key properties of mannose triflate—



F$^-$ is a nucleophile ("nucleus" + "loving")
Reaction is a "nucleophilic substitution"

Slide 29

Next, I'd like to discuss the chemical properties imparted by these groups, and how they make mannose triflate such an effective precursor in the production of [^{18}F]FDG. The most important aspect from a chemistry standpoint is the polar covalent bond between carbon atom number two and the triflate group. This bond is polar due to the high electronegativity of the triflate group. This results in a partial positive charge on carbon atom number two, and a partial negative charge on the triflate. As before, the partial charges are denoted in this slide with a lower case Greek delta sign.

The negative charge of the [^{18}F]fluoride ion is attracted to the partial positive charge on C-2, and it displaces the triflate group to result in the formation of a carbon to [^{18}F]fluorine bond. Due to its propensity for the nucleus of atoms like C-2 of mannose triflate, chemists refer to [^{18}F]fluoride ion as a "nucleophile," which derives from the phrase "nucleus loving." The chemical reaction resulting from this action is known as a "nucleophilic substitution reaction."

One of the key implications of nucleophilic substitution reactions like this is that they result in the inversion of stereochemistry at the carbon atom of interest, in this case carbon atom number 2 of mannose triflate. From a practical standpoint, this converts the mannose architecture into a glucose structure. You may remember back to your days of organic chemistry that the difference between mannose and glucose is the configuration of the hydroxyl group on C-2, so the inversion of the mannose stereochemistry at C-2 results in glucose. Thanks to the reaction mechanism of a nucleophilic substitution reaction, we can start off with a mannose substrate and end up with a glucose product.

Radiolabeling with [^{18}F]Fluoride Ion

Some key terminology—

- F$^-$ is a "nucleophile"
- Nucleophilic substitution reaction causes "inversion of stereochemistry" at C-2 of mannose triflate
- Converts "mannose" substrate into "glucose" substrate

Slide 30

This slide summarizes the key points of this discussion. The [^{18}F]fluoride ion is a "nucleophile" that reacts at carbon atom number 2 of mannose triflate with "inversion of stereochemistry" thereby converting "mannose" substrate into a "glucose" product.

Radiolabeling with [^{18}F]Fluoride Ion

Requirements of [^{18}F]fluoride ion—

- Must be anhydrous (already discussed)
- Must be in solution
- Must be reactive

Slide 31

Now, I'd like to return to our discussion of the requirements of the [^{18}F]fluoride ion that are necessary for its effective use in the production of [^{18}F]FDG. We already discussed how and why the [^{18}F]fluoride ion must be anhydrous. Now, let's talk about how we get the [^{18}F]fluoride ion into the solution and how we maximize its reactivity in the nucleophilic substitution reaction.

Radiolabeling with [^{18}F]Fluoride Ion

The problem with [^{18}F]fluoride ion—

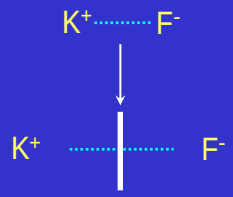
- F^- forms strong ion pair with potassium
- Reduces solubility in solvents
- Reduces reactivity in nucleophilic substitution reactions

Slide 32

You may recall that [^{18}F]fluoride ion forms a "strong ion pair" with a positively-charged potassium atom. This very strong ionic bond between [^{18}F]fluoride ion and potassium reduces the solubility of [^{18}F]fluoride ion in organic solvents and reduces the reactivity of [^{18}F]fluoride ion.

Radiolabeling with [^{18}F]Fluoride Ion

Increase reactivity/solubility of fluoride ion—



Build wall between K^+ and F^-

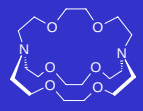
Slide 33

This slide describes how we overcome this problem. The strong ion pair interaction between the potassium and [^{18}F]fluoride ion is represented on this slide by a dotted line. What we have to do is literally build a wall between the potassium and the [^{18}F]fluoride ion to enhance the reactivity of the [^{18}F]fluoride.

Radiolabeling with [^{18}F]Fluoride Ion

How do you build the wall?

- Cryptands are a class of molecules that "encrypt" cations
- Cryptand of interest is Kryptofix® [2.2.2]
- K222 forms three dimensional cage around potassium



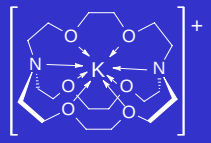
Slide 34

Chemically, this is accomplished on a molecular level with a class of compounds known as "cryptands." Due to their unique molecular properties, cryptands literally encrypt cations and bind them so tightly that they cannot form strong ion pairs with anions. The cryptand of interest here is known as Kryptofix®[2.2.2], which is a trademark name, and may be abbreviated "K222." The chemical structure of K222 appears at the bottom of this slide and you can see the three-dimensional cage. This cage perfectly surrounds potassium cations and prevents the formation of a strong ion pair with [^{18}F]fluoride ion.

Radiolabeling with [^{18}F]Fluoride Ion

How does K222 work?

- Cage is rich in electrons
- Attracts electron-poor K^+
- Goes after the K^+



Slide 35

This slide explains why cryptand works. It turns out that the three-dimensional cage consists of six oxygen atoms, each of which has two pairs of electrons. Each of these electron pairs, as well as those on the nitrogen, point toward the inside of the cage to create an electron-rich environment. This environment attracts the electron-poor potassium cation. The bottom line is that the cryptand enhances the reactivity of the [^{18}F]fluoride ion by sequestering the potassium cation. The cryptand is a cation prison that frees the [^{18}F]fluoride ion to do its job. In addition, sequestering the potassium inside the cryptand cage greatly increases the solubility of potassium [^{18}F]fluoride. Thus, the K222 is responsible for both the increased solubility and the enhanced reactivity of [^{18}F]fluoride ion.

Radiolabeling with [^{18}F]Fluoride Ion

Other details of radiolabeling reaction—

- Acetonitrile (anhydrous) solvent
- Volume = 1 to 2 ml
- Elevated temperature (90-100 C)
- Complete within 5 minutes
- Produces tetra-acetyl[^{18}F]FDG ([^{18}F]fluorinated intermediate)
- Remove acetonitrile afterwards

Slide 36

Now, I'd like to note a few details of the radiolabeling reaction to complete the picture. The reaction takes place in anhydrous acetonitrile, which readily dissolves the cryptand complex of potassium [^{18}F]fluoride. The volume of the solution is 1 to 2 milliliters. The reaction takes place at elevated temperature, and is complete within five minutes, possibly even quicker. The product of the radiolabeling step is tetra-acetyl[^{18}F]FDG, which may be referred to as the [^{18}F]fluorinated intermediate. After completing the reaction, the acetonitrile solvent must be removed, either by evaporation or by solid phase adsorption.

Radiolabeling with [^{18}F]Fluoride Ion

Summary—

K^+/K222/[^{18}F]Fluoride Ion

Mannose triflate | CH_3CN

↓

[^{18}F]Fluorinated Intermediate

↓

Remove acetonitrile

Slide 37

This slide summarizes the radiolabeling step. We start with a cryptand complex of potassium [^{18}F]fluoride and we heat it with mannose triflate in acetonitrile to produce an [^{18}F]fluorinated intermediate. We remove the acetonitrile and we are ready for the next step.

[^{18}F]FDG Chemistry

Six steps—

- [^{18}F]Fluoride ion production
- Trap and release the [^{18}F]fluoride ion
- Preparation of the [^{18}F]fluoride ion
- Radiolabeling
- Hydrolysis
- Purification

Slide 38

Here is our summary of the six steps in the preparation of [^{18}F]FDG. We have completed our discussion of the radiolabeling reaction. Let's discuss the hydrolysis reaction.

Hydrolysis Reaction

- Removes acetyl protecting groups
- Acid or base catalyzed addition of water across C-O bonds
- Releases acetic acid (acetate)
- Results in crude mixture of [^{18}F]FDG

Slide 39

The purpose of the hydrolysis step is to remove the acetyl protecting groups. This may be accomplished by the acid or the based-catalyzed addition of water across the carbon-oxygen bonds in the acetate linkage. This releases acetic acid in the case of an acid hydrolysis, or acetate in the case of a base hydrolysis. This results in a crude mixture of the [^{18}F]FDG that must be purified before use.

Hydrolysis Reaction

Acid catalyzed—

- First process historically
- Elevated temperature (100-110 C)
- HCl concentration 1 to 2 N
- Volume = 1 to 2 ml
- Reaction time 12-15 minutes

References: Hamacher, *et al.*, J. Nucl. Med. Isot., *27*, 235, 1986.
Padgett, *et al.*, Appl. Radiat. Isot., *49*, 433, 1989.
Mock, *et al.*, Nucl. Med. Biol., *23*, 497, 1996.

Slide 40

This slide discusses the acid-catalyzed process in more detail. Historically, the acid catalyzed process was the first process that was used to make [^{18}F]FDG. It occurs at an elevated temperature of 100 to 110 degrees centigrade, with a hydrochloric acid concentration of 1 to 2 normal. The volume is typically 1 to 2 milliliters and the reaction time is 10 to 15 minutes. This slide contains some of the pertinent references for the use of the acid hydrolysis in the production of [^{18}F]FDG.

Hydrolysis Reaction

Base catalyzed—

- Ambient temperature (30-35 C)
- NaOH concentration 0.3 N
- Volume = 1 to 2 ml
- Reaction time 2-3 minutes
- Free of [^{18}F]FDM

References: Fuchtner, *et al.*, Appl. Radiat. Isot., *47*, 61, 1996.
Meyer, *et al.*, Appl. Radiat. Isot., *51*, 37, 1999.

Slide 41

This slide discusses the details of the base-catalyzed process, which proceeds at ambient temperature with a sodium hydroxide concentration of 0.3 Normal. The volume for this process is 1 to 2 milliliters and the reaction time is 2 to 3 minutes. Under these conditions, the base-catalyzed process yields [^{18}F]FDG that is free of [^{18}F]fluoro-deoxy-mannose, or [^{18}F]FDM. The base-catalyzed process may also be performed on the [^{18}F]fluorinated intermediate after isolation of the intermediate on a solid support.

Hydrolysis Reaction

Summary—

[^{18}F]Fluorinated Intermediate

↓

Acid/Heat or Base

↓

Crude [^{18}F]FDG

Slide 42

This slide summarizes the hydrolysis process. We've taken our [^{18}F]fluorinated intermediate and either heated it with acid, or reacted it with base at room temperature, to produce a crude reaction mixture of [^{18}F]FDG.

[^{18}F]FDG Chemistry

Six steps—

- [^{18}F]Fluoride ion production
- Trap and release the [^{18}F]fluoride ion
- Preparation of the [^{18}F]fluoride ion
- Radiolabeling
- Hydrolysis
- Purification

Slide 43

Now, let's look at the purification process, which is the last of the six steps in the preparation of [^{18}F]FDG.

Purification Process

Impurities at end of hydrolysis—

- Unreacted [^{18}F]fluoride ion
- Unhydrolyzed [^{18}F]fluorinated intermediate (+)
- K222
- Acetonitrile, ethanol
- Acid (or base)
- Microbial
- Bacterial endotoxin

Slide 44

First, let's look at the impurities that may potentially be present in the crude reaction mixture of [^{18}F]FDG. These impurities include unreacted [^{18}F]fluoride ion and the unhydrolyzed [^{18}F]fluorinated intermediate. I have used a "plus" sign here to denote the fact that, in addition to the unhydrolyzed [^{18}F]fluorinated intermediate, it is possible to have small amounts of products of intermediate hydrolysis. Another impurity at the end of the hydrolysis is K222 that was used in the radiolabeling step. Remember that the K222 is complexed with a potassium cation, so it is positively charged.

Two solvents may be present at the end of the hydrolysis. One is acetonitrile from the radiolabeling step, and the other is residual ethanol from the activation of purification cartridges that we'll mention in a minute.

Since the hydrolysis was performed with either acid or base, the pH of the solution must be neutralized. Finally, we have to worry about microbial contamination and bacterial endotoxins since the radiopharmaceutical is administered intravenously.

Purification Process

Purification (Acid Catalyzed Hydrolysis)—

- K222
  - Cation resin
- Neutralization
  - Ion retardation resin
- Unhydrolyzed [^{18}F]FDG
  - C-18 SEP-PAK (activated with EtOH)
- [^{18}F]Fluoride ion
  - Alumina SEP-PAK
- Microbial Contamination
  - Membrane filter

Slide 45

This slide discusses purification methods for the acid-catalyzed process. Since the K222 is positively charged, it is trapped by a cation exchange resin. Similarly, we use an ion retardation resin to do neutralize the pH of the solution. The unhydrolyzed [^{18}F]fluorinated intermediate and its related species are removed by a C-18 purification cartridge. These cartridges must be activated with ethanol prior to use. Unreacted [^{18}F]fluoride ion is removed by an alumina purification cartridge. Finally, microbial contamination is removed by passage of the solution through a membrane sterilizing filter.

Purification Process

Purification (Base Catalyzed Hydrolysis)—

- K222, [^{18}F]Fluoride ion, acetonitrile
  - Solid phase product extraction; rinsing
- Neutralization
  - Buffering
- Unhydrolyzed [^{18}F]FDG
  - C-18 SEP-PAK (activated with EtOH)
- Microbial Contamination
  - Membrane filter

Slide 46

This slide discusses purification methods for the base-catalyzed hydrolysis. Like the acid-catalyzed process, K222 and unreacted [^{18}F]fluoride ion must be removed. In this case, instead of removing them by ion exchange, we remove them by immobilizing the [^{18}F]fluorinated intermediate on a C-18 cartridge prior to hydrolysis, then we rinse these impurities through the cartridge. This is also how the acetonitrile is removed. We neutralize the solution with buffers, and unhydrolyzed [^{18}F]FDG is removed by an activated C-18 purification cartridge. Microbial contamination is removed by passage of the solution through a membrane sterilizing filter.

Purification Process

Final Product Vial Assembly—

Slide 47

After purification, the product is collected in the final product vial. This slide illustrates the assembly used for the collection process. The membrane sterilizing filter is a disc that is directly inserted into an empty, pre-sterilized product vial. Two syringes are used to remove quality control samples: one for the sterility test sample, and the other for the remaining quality control tests.

The assembly also has a three-way stopcock at the bottom of a QC syringe. The side arm of the stopcock has a 0.2 micron membrane vent that allows gas to escape the vial during the filtration process.

Summary

$[^{18}O]H_2O \xrightarrow{\text{Accelerator}} [^{18}F]F^-$

$[^{18}F]F^- / [^{18}O]H_2O \longrightarrow K^+ / [^{18}F]F^- / [^{16}O]H_2O$

$K^+ / [^{18}F]F^- / [^{16}O]H_2O \longrightarrow K^+ / [^{18}F]F^-_{(anhydrous)}$

$K^+ / [^{18}F]F^-_{(anhydrous)} + \text{Mannose} \longrightarrow \text{Unhydrolyzed } [^{18}F]\text{FDG}$

$\text{Unhydrolyzed } [^{18}F]\text{FDG} \longrightarrow \text{Crude } [^{18}F]\text{FDG}$

$\text{Crude } [^{18}F]\text{FDG} \xrightarrow{\text{Purification}} [^{18}F]\text{FDG}$

Slide 48

Congratulations! That completes the chemistry portion of our discussion. This slide summarizes it all. The preparation of [^{18}F]FDG is a six-step process that starts with [^{18}O]water. A particle accelerator, or cyclotron, produces a proton beam that produces [^{18}F]fluoride ion in the enriched [^{18}O]water. The [^{18}F]fluoride ion is converted into a potassium salt in [^{16}O]water. The water is removed to yield an anhydrous solution of [^{18}F]fluoride ion, which is then reacted with mannose triflate to produce an unhydrolyzed [^{18}F]FDG intermediate. The [^{18}F]fluorinated intermediate next undergoes a hydrolysis to produce a crude mixture of [^{18}F]FDG, and then purification yields a sterile, injectable product.

QC Testing for [^{18}F]FDG

Overview—

- Quality Assurance vs. Quality Control
- *Execution* of the QC function vs. *oversight* of the QC function
- USP specifications for [^{18}F]FDG

Slide 49

Next, I'd like to discuss QC testing for [^{18}F]FDG. To begin, I want to discuss "Quality Assurance" and "Quality Control." I will also discuss one of the special regulatory needs in the production of PET radiopharmaceuticals, and how this relates to the *execution* of the QC function versus the *oversight* of the QC function. Finally, I'll complete this portion of the presentation with a summary of the USP specifications for [^{18}F]FDG.

QC Testing for [^{18}F]FDG

Quality Assurance (QA)—

- A wide-ranging concept covering all matters that influence a product's quality
- All organized efforts to ensure that a product possesses the quality required for its intended use
- QA incorporates QC
- Oversees the QC function

Slide 50

So, what is "Quality Assurance?" How is it different from "Quality Control?" There is persistent confusion in the nuclear medicine community about the difference between QA and QC, and I hope to clarify that here.

Quality Assurance, or QA, is a wide-ranging concept that covers all matters that influence a product's quality. It's an organized effort to ensure that a product possesses the quality that is required for its intended use. To accomplish this, QA incorporates QC. If you look at the universe of Quality Assurance, *one* portion of it is Quality Control. In a sense, QA oversees the QC function.

QC Testing for [^{18}F]FDG

Quality Control (QC)—

- A narrow-ranging concept that deals with sampling, testing and release of materials, components and finished products
- QC is a subset of QA
- Execution of the QC function occurs locally

Slide 51

Quality Control, or QC, is a narrow-ranging topic that deals with testing products after production. Think of QA as "building quality into your product," and think of QC as the testing you perform on the product to make sure it meets final product specifications. In other words, think of QC as the testing you perform to make sure you made what you intended to make. QC is a subset of QA.

Of course, due to their short half-life, the execution of that QC function must occur on a local level. Note, however, that the QA oversight of the QC function may take place remotely. This is an important concept in PET due to the small number of staff that operate some production facilities.

QC Testing for [^{18}F]FDG

USP Specifications—

- Appearance
- Specific Activity
- Radiochemical ID/purity
- Radionuclidic ID/purity
- Chemical purity
- pH
- Filter Integrity Test
- Bacterial endotoxins
- Sterility

Slide 52

This slide introduces the USP specifications for [^{18}F]FDG, which consist of nine categories, including: appearance, specific activity, radiochemical identity and purity, radionuclidic identity and purity, chemical purity, pH, filter integrity test, bacterial endotoxins and sterility.

QC Testing for [^{18}F]FDG

Appearance—

- USP Specification:
  - Clear, colorless, particulate-free
- Visual inspection through leaded glass

Specific Activity—

- USP Specification:
  - No-carrier added
- No testing required

Slide 53

Let's talk about appearance first. The USP specification is for the product to be clear, colorless and particulate free. We determine this through visual inspection through leaded glass.

The USP specification for specific activity is that the product be "no carrier added." In effect, the USP recognizes the extremely high safety margin for no carrier added [^{18}F]FDG and does not require any testing.

QC Testing for [^{18}F]FDG

Radiochemical ID/purity—

- Thin layer chromatography (TLC)
- Separates components based on polarity
- Separates [^{18}F]FDG, [^{18}F]fluoride ion, [^{18}F]fluorinated intermediate(s)

Slide 54

Radiochemical identity and purity are the next specifications that I'll discuss. This test requires the use of thin-layer chromatography, or TLC, which separates the components of a mixture based on their polarity. TLC separates [^{18}F]fluoride ion from [^{18}F]FDG and the unhydrolyzed [^{18}F]fluorinated intermediates.

Radiochemical ID/Purity

USP Specification—

- Identity:
  - R_f of [^{18}F]FDG corresponds to that of non-radioactive standard
- Purity:
  - ≥ 90% [^{18}F]FDG

Slide 55

The USP specification for radiochemical identity is that retention factor for the radioactive spot of interest be the same as that for a non-radioactive FDG standard. The radiochemical purity must to be greater than or equal to 90%.

Radiochemical ID/Purity

Thin layer chromatography (TLC)—

- Silica gel stationary phase
- Liquid mobile phase (95:5 CH_3CN: water)
- Two lanes: Cold FDG standard and [^{18}F]FDG
- Measure retention factors (R_f)

Slide 56

The USP requires that stationary phase for the TLC method is silica gel, and the mobile phase is a 95:5 mixture of acetonitrile and water. The method requires the development of two lanes: one lane for the non-radioactive FDG standard and the other for the product. The retention factor, or R_f, of each lane is then measured and compared for the radiochemical identity determination.

Radiochemical ID/Purity

Typical R_f Values—

	R_f
• [^{18}F]FDG	0.4
• [^{18}F]Fluoride ion	0.0
• [^{18}F]Fluorinated Intermediate(s)	0.6-0.9

Slide 57

Typical R_f values for [^{18}F]FDG and its impurities are summarized on this slide. [^{18}F]FDG has an R_f value of about 0.4. [^{18}F]Fluoride ion has an R_f value of zero – it does not move from the origin. The [^{18}F]fluorinated intermediates have R_f values between 0.6 and 0.9. Pure tetra-acetyl[^{18}F]FDG has an R_f of 0.9.

Radionuclidic ID/Purity

Identity—

- USP Specification:
  - Half-life is between 105 and 115 minutes
- Dose calibrator

Purity—

- USP Specification:
  - Not less than 99.5% of observed gamma emissions correspond to the 0.511 MeV, 1.022 MeV, or Compton scatter peaks of ^{18}F
- Multi-channel analyzer

Slide 58

The next slide discusses radionuclidic identity and purity. The USP specification for radionuclidic identity is that the product have a half-life between 105 and 115 minutes. This is easily done in a dose calibrator. For radionuclidic purity, the USP specification is not less than 99.5% of the observed gamma emissions correspond to 511 keV photons, the sum photons at 1.02 MeV, or Compton scattering that may occur at lower energies for these photopeaks. This, of course, requires a Multi-Channel Analyzer (MCA). The most useful application of this test is after the ^{18}F has decayed and you can analyze for low levels of potential activation products.

QC Testing for [^{18}F]FDG

Chemical purity—

- Kryptofix®
- Chloro-deoxyglucose
- Residual Solvents

Slide 59

This slide summarizes the three required tests for chemical purity. One test addresses the presence of residual Kryptofix®, a second addresses the presence of chloro-deoxyglucose, and a third test addresses residual solvents.

QC Testing for [^{18}F]FDG

Chemical purity—

- K222 TLC Test
  - See: Chaly and Dahl, Nucl. Med. Biol., *16*, 385, 1989
- K222 Spot test
  - See: Mock, *et al.*, Nucl. Med. Biol., *24*, 193, 1997
- Detect Kryptofix® with iodine vapors
- USP Specification:
  - Less than 50 ppm (μg/ml) K222

Slide 60

The USP test for Kryptofix® was published in 1989 by Dahl, and consists of a TLC test. Mock and his colleagues later developed a spot test for Kryptofix® that is basically a TLC test without a mobile phase elution. In each case, the Kryptofix® is visualized with iodine vapors. The USP specification for Kryptofix® is less than 50 ppm, or micrograms per milliliter.

QC Testing for [^{18}F]FDG

Chemical purity—

- Chloro-deoxyglucose (Cl-DG)
  - HPLC with electrochemical detection
  - See: Alexoff, *et al.*, Appl. Radiat. Isot., *43,* 1313, 1992
- USP Specification:
  - Less than 1 ppm Cl-DG in total batch

Slide 61

The USP chemical purity test for chloro-deoxyglucose, or Cl-DG, requires an HPLC system with an electrochemical detector. The original work for this method was published by Alexoff in 1992. The USP specification is less than 1 ppm or 1 microgram per milliliter of Cl-DG in the total batch. This test is typically not performed on every batch of [^{18}F]FDG. Instead, we rely on validation studies to show what ranges of Cl-DG are possible to obtain in the final product, then use these results as a basis for not testing every batch of [^{18}F]FDG. I'll talk more about the frequency of tests that the USP specifies at the end of the talk.

QC Testing for [^{18}F]FDG

Chemical purity—

- Residual Solvents
  - Gas chromatography (GC) to determine concentration of acetonitrile, ethanol
- USP Specification:
  - Must be less than 0.04% CH_3CN
  - Must be less than 0.5% EtOH

Slide 62

The USP requires a gas chromatographic method to determine the amount of residual acetonitrile and ethanol in the final product. Ethanol can be used as a stabilizer, so it's also useful for determining stabilizer content. The USP specification for acetonitrile is less than 0.04%, while that for ethanol is less than 0.5%.

QC Testing for [^{18}F]FDG

pH—

- USP Specification:
  - between 4.5 and 7.5
- Measured by pH strips

Slide 63

The USP specification for pH is between 4.5 and 7.5. This test is performed with narrow-range pH strips.

QC Testing for [^{18}F]FDG

Bacterial Endotoxin—

- Limulus Amebocyte Lysate
- USP Specification:
  - Must be less than 175 EU/batch
- In event of failed test, can retest at maximum valid dilution (MVD)

Slide 64

The USP requires the use of the Limulus Amebocyte Lysate (LAL) test for bacterial endotoxins. The specification is less than 175 EU per batch. Typically, this test is performed on a product dilution that is well below this limit, but in the event of a failed test, the product may be retested at the maximum valid dilution.

QC Testing for [^{18}F]FDG

Sterilizing Filter Integrity Test—

- Bubble point
- Must meet supplier's specification

Slide 65

Regarding the membrane sterilizing filter integrity test, this test isn't discussed in the USP monograph for [^{18}F]FDG, but is instead discussed in Chapter <823>. This test is necessary to ensure that the filter maintained its integrity during the filtration process. The test is performed after the filtration, so you must take appropriate precautions in terms of radiation safety. The integrity test used in the preparation of [^{18}F]FDG is the bubble point test. Depending upon the sterilizing filter, each manufacturer has a different specification for the bubble point test. So the filter must meet the manufacturer's specification.

QC Testing for [^{18}F]FDG

Sterility Testing—

- Two growth media
- Inoculate day following production
- Smaller inoculation volume
- Incubation time: 14 days
- USP Specification:
  - Sterile

Slide 66

The USP Sterility Test requires the use of two growth media, and allows for the product inoculation to occur the day following production. This consideration is taken in light of radiation safety constraints. In addition, the USP allows for the use of smaller inoculation volume to account for the small volume of product. The required incubation time for both media is 14 days, so the results of the test are not known until well after product release. Of course, the product must be sterile.

Frequency of Testing

USP General Notices—

"Every compendial article in commerce shall be so constituted that when examined..., it meets all of the requirements in the monograph defining it. However, it is not to be inferred that application of every analytical procedure in the monograph to samples from every production batch is necessarily a prerequisite for assuring compliance with Pharmacopeial standards before the batch is released for distribution. Data derived from manufacturing process validation studies and from in-process controls may provide greater assurance that a batch meets a particular monograph requirement than analytical data derived from an examination of finished units drawn from that batch. On the basis of such assurances, the analytical procedures in the monograph may be omitted by the manufacturer in judging compliance of the batch with the Pharmacopeial standards."

Slide 67

In addition to information contained in individual product monographs, the USP also addresses the frequency of QC tests. The "General Notices" section of the USP at the beginning states "it is not inferred that the application of every analytical procedure in a monograph is necessarily a prerequisite for assuring compliance with the standards in that monograph prior to release for distribution." Therefore, it is possible to perform validation studies to reduce the frequency of end-product QC tests, and to provide a margin of safety when tests cannot be complete due to half-life considerations.

Closing

- Introduction to chemistry behind the production of [^{18}F]FDG
  - The six steps
- Quality control testing used in the production of [^{18}F]FDG
  - Summary of QC tests
  - USP requirements

Slide 68

That completes our discussion of the Production and Quality Control of [^{18}F]FDG. I hope you have learned something about the six steps behind the production of [^{18}F]FDG. I also hope our discussion of the Quality Control testing used in the production of [^{18}F]FDG was useful. PET is an exciting and challenging area of nuclear pharmacy. I hope you have the chance to experience the excitement for yourself someday. Thank you.